Analytical Technologies in Biotechnology
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Module - 3
Chromatographic Methods
Lecture - 7
Gas-Liquid Chromatography

Till now we have discussed 3 chromatographic techniques, that is iron exchange chromatography, gel filtration chromatography, and affinity chromatography. All these three techniques, the mobile phase is liquid or a solvent. Today, in this lecture, we are going to discuss a technique, which is where mobile, phase is gas rather than a liquid.

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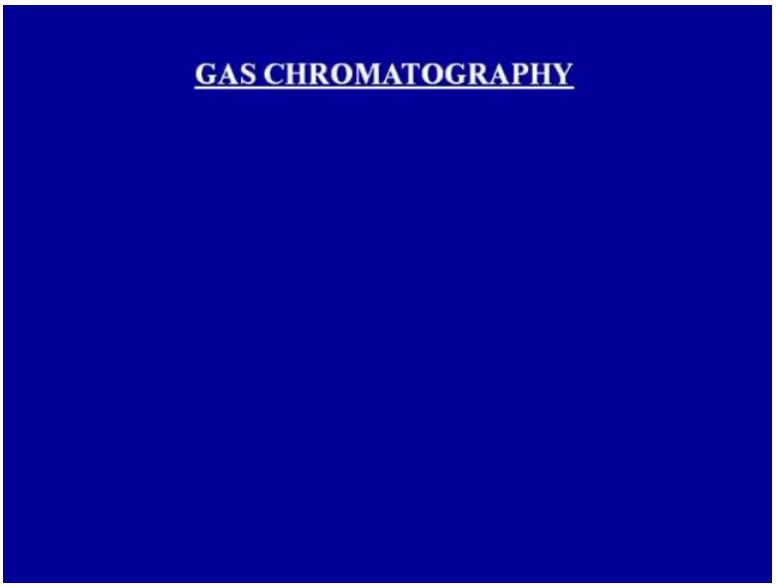

So, this particular technique is gas chromatography. So, we are going to discuss about in particular gas liquid chromatography. Now, gas chromatography is the technique used for separation of thermally stable and volatile organic and inorganic compounds. So, gas chromatography, if a particular analyte has to separate or has to partition between a liquid phase and a mobile gaseous phase, then it has to be in vaporised state. So, only those analyse, which are thermally stable and volatile could be utilised and could be separated in here.

Now, there could be two kinds of gas chromatography. One is gas liquid chromatography, which accomplishes the separation by partitioning the components of a chemical mixer between a mobile gas phase and stationary liquid phase held on a solid support. Then there could be another chromatography that is gas solid chromatography, which uses a solid adsorbent as the stationary phase. So, in this lecture, we are going to focus on gas liquid chromatography technique.

Now, if we compare gas chromatography with normal or other liquid chromatography technique, which is in terms of either low pressure or high performance liquid chromatography techniques, all the general principles and the performance parameter applies here also. For example, writ tension volume or writ tension time is the corrector stick of an analytic and that will correct rise am, its particular characteristics stick of a particular analytic.

If you have to find know about or identify a particular analytic, then if you know the retention time or retention or volume resolution volume, you can identify that particular component from the mixer. Then other parameters like resolution, capacity ratio are also shown. All those things will be applying here also. It is a similar kind. The difference says that here only volatile and thermally stable components of a mixer could be separated. Now, gas chromatograph or the gas chromatography equipment is little different from the normal chromatography equipment.

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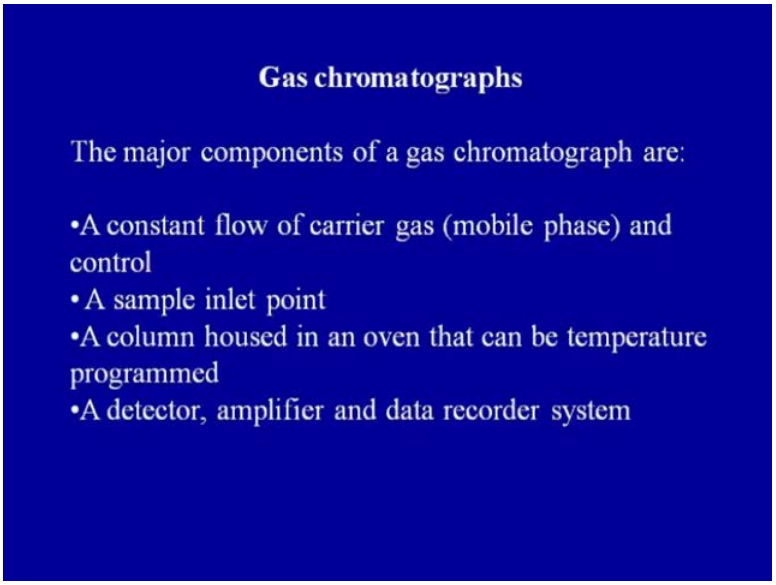

The major components of a gas chromatograph are a constant flow of carrier gas that is the mobile phase. We have to control the flow fluoride of that carrier gas through different walls and the flow meters. There should be a simple system of introducing the sample into the gas flow, and then into the column. So, there is a sample inlet point, which is very important. Then after that, a column has to be housed in an oven. That is because you are doing all the experiment or separation procedure at a very high temperature. The oven should be temperature programmed.

So, column a column in gas chromatograph is based in an oven, which is temperature control. Then when analytic comes out of the column, then a detector amplifier, a data recorder system needs to be there to analyze the chromatography of the particular analytic. So, these are the important components of a gas chromatograph. We are going to discuss all different components in detail.

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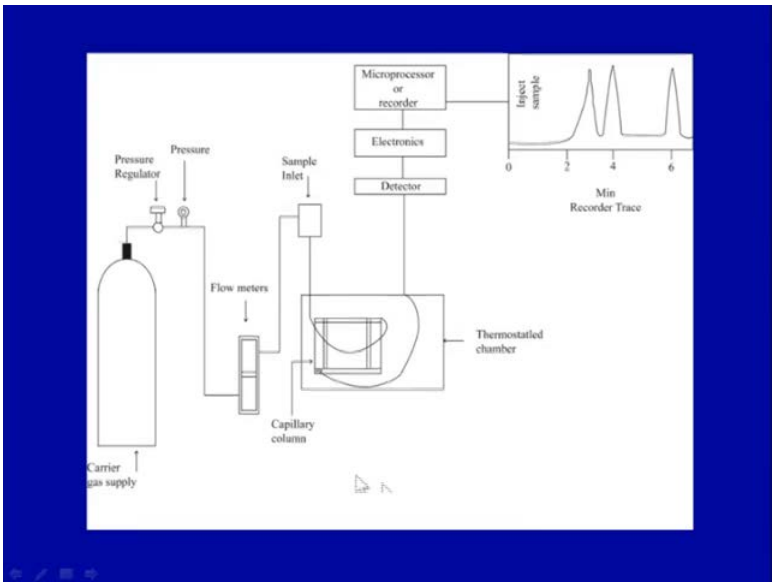

Now, here in this diagram, if you can see on your screen, it is a schematic of a gas chromatograph. It very nicely detects the different components of the gas chromatograph. Now, if you see from starting here, this is the carrier gas supply in a particular tank or cylinder. This carrier gas is supplied from here from this tank. As it goes through the tubing, there are pressure regulators and pressure meters.

There are flow meters, which can regulate the flow. Then there is a sample inlet in here, which lets the sample in a vaporised form. That will be around some where here.

Vaporisation will take place. So, there is a sample inlet from where sample will be injected. Now, this could be a direct injection as we will see later on. It could be automatic sampler could also be used in here.

Then, there is a column here. This column is a very long column. Since, it is a long column; it is coiled actually here, which is not seen. So, in this oven, there is a coiled a long column, but very thin. The diameter is very less. They are very narrow board columns. Then this columns length is required for proper separations. Then this column comes out here.

The analytic from the column will pass through detector. Then it will pass the electronic microprocessor amplifiers. Finally, it will pass through the data recorders. You will see the peaks in here as other chromatography techniques. So, the system or principles are same as per the partitioning between the gas and the liquid phase. These analcites will a lute at different times as per their attention times or there distribution co efficient here.

So, the separation will take place at every analytic on a particular standard condition. It will have a corrector stick retention time or an evolution volume and through which, you can identify the particular analytic even for an unknown sample. You can make kind of standard curve or you can run standard compounds. Then you can plot it like we have seen in other chromatography techniques. Then unknown sample retention time could be calculated from that. So, this is how you see detect. There is a very simple way to detect how gas chromatograph will look like. What are the different components of gas chromatograph?

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So, let us start with the first thing. We have seen in the gas chromatograph effect, carrier gas supply is the most important at the beginning. So, we have to choose a mobile phase and that is the catch phase. So, a constant supply of a carrier gas with proper control is very important necessary aspect of gas chromatograph. Normally, helium, nitrogen, carbon dioxide or hydrogen can be used.

These are the most commonly used carrier gases or mobile phases. Obviously, hydrogen has the disadvantage of explosion danger. There are suitable flow regulator walls and means of reproducing the fluoride. I think that all of you understand that fluoride has to be reproduced to reproduce chromatographic separation or to maintain a particular retention time, revolution volume, a fluoride change, the retention time. The revolution volume also changes. Then you cannot compare that to experiments. A typical meter like soap bubble meter can be used for this particular purpose.

So, the carrier gas is one part, where you have to choose particular carrier gas as a mobile phase as carrier gas goes on. The next thing is the sample injection. Now, like I showed you a sample injection like sample has to be there many times. Sudden chemistry is involved to prepare the sample. May be derivatization and other things might be required to vaporise the sample because not all the compounds could be vaporised.

So, this is the limitation of gas chromatograph. So, sample injection when we have to do or it has to be done is that the sample has to be vaporised. So, it can partition between the liquid mobile, liquid phase that is stationary and mobile gas phase. So, there are two ways in which you can do it. One is direct injection. So, sample could be directly injected. It is the standard more suitable for 95 percent of pegged columns. What is pegged column?

Then, a sample is injected by a hypodermic syringe through a self sealing silicon rubber septum on to a glass inner, a glass liner with in a metal block, where it is vaporised and swept into the column. So, what is done? There is a heat block, metal block which is heated to a certain temperature higher than an oven temperature may be. Instantly, as the sample is put on there, it is vaporised and swept into the columns. Now, the block is heated at a fixed temperature, which is sufficiently high to convert liquid sample into a plug of vapour.

What you are doing is you are injecting a liquid sample, which could be vaporised. It is done very fast. Samples can be measured with a calibrated loop. Then they are introduced into the flowing gas extreme by means of wall for capillary columns. We will see what capillary columns are as we go along. Reduction sample volume is necessary because capillary columns' capacity is very low.

This will be accomplished by an injector splitter where 1 micro as a litre can be injected. But, only 0.01 micro litre and close capillary is chosen. So, what is done is as you are injecting the 1 micro litre samples. Other is a injector splitter actually, which will split the sample where only 0.01 micro litre will go to the capillary column and rest of went to the waste. A split less injection is required for very small amounts of sample. The entire sample is injected to the open tubular column through a modified heated flesh vaporises. So, there could be many ways in which you can do it.

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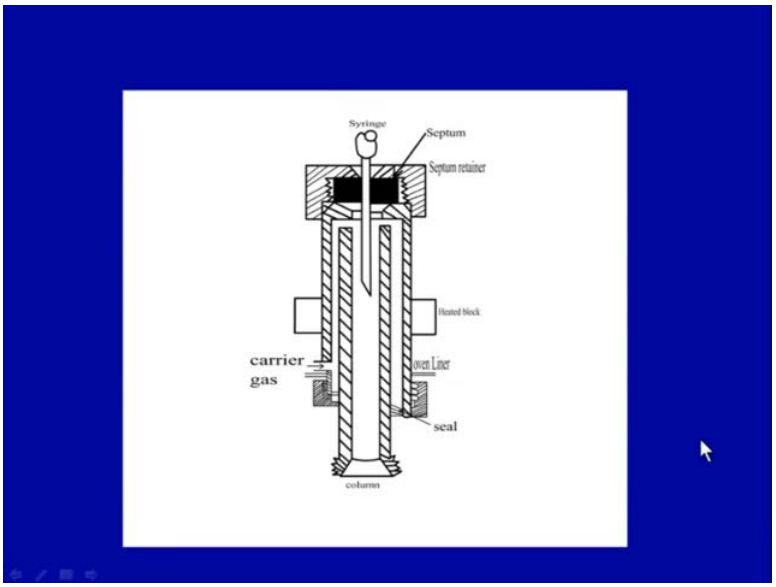

Now, this is a very typical representation of typical vaporiser, where you can inject the sample in here. As you can see on a screen, there is a syringe, which injects the sample in here through a rubber septum. There is a heat block and this is heated to very high temperature. So, the liquid could be converted to vapour instantly. Then once it is done, this will flush into the column, where it can be separated. So, this is the typical vaporiser, you can say a flesh vaporiser.

Then, you can do injection by or load a sample by automatic sampler, sample wise glass, vapour like septum caps, vapour tight septum caps. So, these vapour type septum caps could be taken and now the procedure injection is carried out. If you see what is done is the sample fleshes the syringe with a new sample to remove traces of the previous sample. That is very important that you do not do kind of a contamination of previous run in the sample injector pumps.

Then, what is done is a new sample will be pumped into the syringe to eliminate any bubbles. Then it would be taken; a precisely measured amount of sample is taken. Then it will be injected into the gas chromatograph. So, you have to be very careful when you are utilising an automatic sampler. The automatic sampler provides highly reproducible regales. They are much more precise. So, you can very nicely load a sample through automatic samplers. So, this was about sampler injection that could be done on the gas chromatography column.

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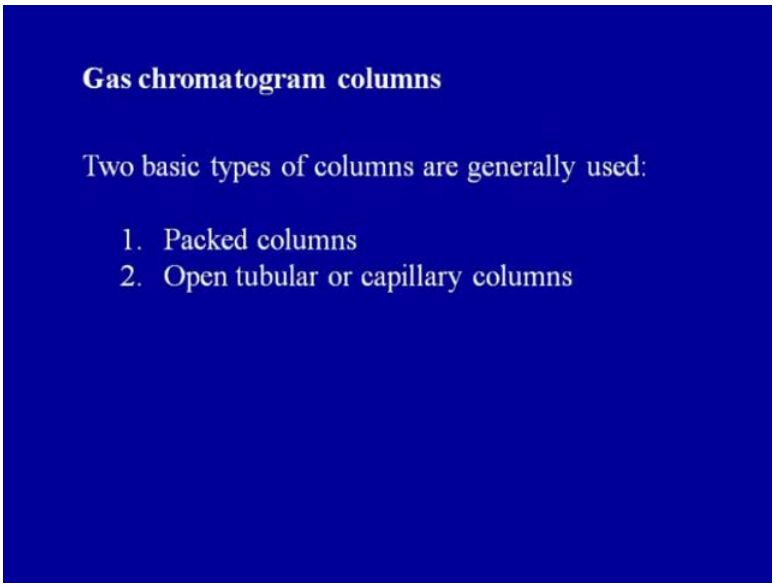

Now, let us come on to the next part that is gas chromatogram column. The two basic types of columns, which are generally used gas chromatogram, are called packed columns. Another one is open tubular are called capillary columns. Now, let us discuss each of them one by one. Now, packed columns are constructed from stainless steel or nickel or may be glass. Now, remember all of these things have to be heat resistant because they will be kept in an oven at high temperature.

So, they are heat resistant. The inner diameter of these columns ranges somewhere around 2 to 4 millimetres. They are very long up to 1 to 3. Now, these columns are packed with stationary phase, which is coated on to an inner silica support. Now, they are packed means they are completely packed. They are fully packed and support should not take part in the separation like in agar chromatogram technique. It should be inert. It must be thoroughly inactivated when polar compounds are to be analysed. So, a liquid phase or a stationary phase is on support and support has to be inert.

There are some of the commonly used stationary phases, which could be polyethylene, glycols, metal, phenyl and metal, vinyl, silicon, gums, esters of autistics and succinct, atelic acids. They are available actually. There could be beta cyclo Dutch based phases for carrying all separations. The most commonly support material is silica or Earth. So, that hydroxide group is modified to avoid support sample interaction. This could be achieved by scintillation of support with compounds like hexa methyl dye salad gene.

So, you can use this. This is most commonly used support. Columns are dried, packed under a slide positive gaseous pressure. After packing, there must be condition for 24 to 48 hours for heating to near upper temperature limit. The carrier gas at normal flow rates pass through the column. Now, during this conditioning, the column is disconnected from the detector because you do not want to fold the detector to prevent and you do not want to condemn it.

Now, what happens is when you condition its columns there might be a situation. Here, column bleeding is taking place. You are losing stationery phase or support material due to heating. In that case, it will certainly contaminate the detectors. So, that has to be consoled. So, before the column is commercially provided or you make it for use, a test should be conditioned of low bleeding. It is minimised. Special packing materials may be needed for particular applications.

For example, very tightly loaded gas bleeds are used for very rapid analysis well below the boiling point of the sample components. There might be c calzone supports used where corrosive substances are handled. Then in gas solid chromatography, the packing material is an absorbent like silica gel or a bonded fish support or a molecular sea flow. So, you can have different types of supports for different types of applications

So, packed columns are very commonly used columns. There are capillary columns different from packed columns. They have an internal diameter say 1 millimetre or less. So, they are very narrow board as compared to packed columns are they are constructed of fused silica having higher degree of crossed licking with in the silicon oxygen matrix. There is a high tensile strength of silica cubic. It permits the construction of thin volt flexible columns for protection of thin wall against scratches coating of polyimide is applied to the outer wall.

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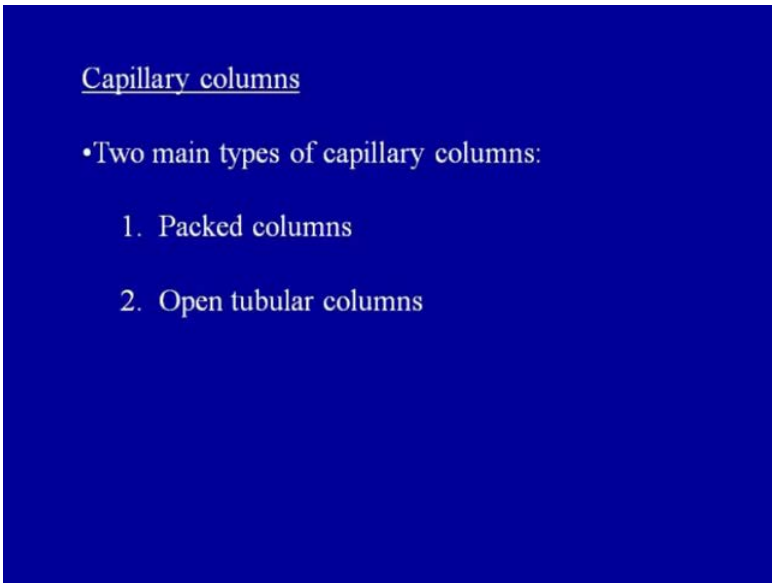

Now, there are two types of capillary columns. These are could be packed columns or open tubular columns. Now, when you say packed columns, this is having solid particles over the whole diameter of the column like we have seen in packed columns. Open tubular columns are different. What you have is an open and unrestricted flow part through the middle of the column divided. For example, let me show you this on your screen. So, what you have is a packed column.

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If I have a very thin column of or very small diameter column when you say it is a packed column, then it is filled. All over, you have bleeds, which is small stationary support, which is filling, which is all over completely over the diameter. So, it is like filling the whole area in here. There is no open space in middle actually. When you are saying open tubular column, then in open tubular column, you will have bleeds on the walls. So, you will have bleeds here. You will have bleeds here. You have an open area in middle. So, this open area lets the gas flow without any problem. So, there is a clear difference between packed column and capillary column. So, I hope you are able to understand this.

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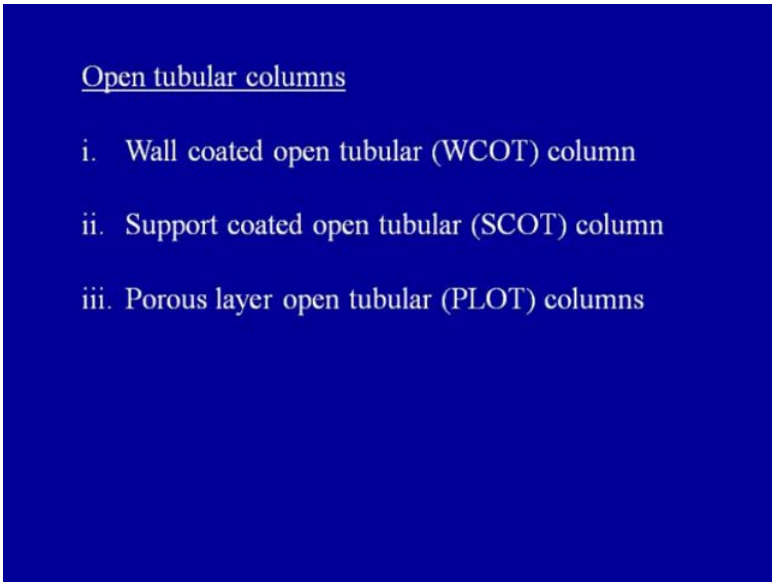

So, let us return to our discussion with an open and unrestricted flow and open tubular columns and capillary columns. There is a flow part, which is quite unrestricted here. The gas can flow very easily here. Now, there are different kinds of open tubular column. One is called wall coated open tubular column. In short form, you can say WCOT, wall coated open tubular column. Now, there here the stationary phase is thinly coated directly on to the walls of the capillary.

Then, there could be another one called support coated open tubular column. Now, here is support coated like SCOT support matrix is bonded to the walls of the capillary columns. So, here the stationary phase is not directly linked to wall of the column, rather

it is link through a support supporting matrix. So, the stationary phase is coated on to the support and support is coated on to the wall.

Then, they could be another one called porous layer open tubular columns, which is kind of support coated used for adoption work. So, the true means are wall coated and support coated open tubular column. In terms of capacity, certainly the amount material, which a support coated column can open; tubular columns can theory will be higher than wall coated columns. So, commonly used stationary phases are polythene, glycol, methyl, philly, and polyclone. Since, another material are utilised, they are coated on to the supporting matrix to give 1 to 25 percent loading depending upon the analysis.

So, the capacity of support coated columns is very high, considerably high. Then the wall coated columns have certain advantages of capillary columns. The capillary columns with particularly open tubular columns are having shorter tension time. They have greater inertness. They are longer life, lower bleed, which is higher in packed columns. They have higher efficiencies and greater reproducibility.

So, there are certain advantages of capillary columns. The capacity of these columns is very low. So, many times, they could be for only analytical purpose rather than the preparative purposes. We have discussed about the column. Now, column is contained in an oven, which is temperature control. So, chromatographic columns, they are coiled and held in a basket that is mounted inside oven. Now, column must be able to be rapidly heated and cold in this oven.

So, this requires the well designed and very adequate system of ear flow. Ovals are usually constructed of flow mass stainless steel. The working temperature range is chosen to give a balance between a peek retention time and resolution. Now, a column temperature is controlled to say plus minus 0.1 degree Celsius and analytic partition co efficient is particularly sensitive to temperature. So, that is very important. Analysis time may be regulated by the adjustment of the column oven, which can be operated in different modes.

It could be operated either as an isothermally analysis, where a constant temperature is applied and should be maintained here. There could be temperature programming as well. So, you can have isothermal as well as temperature programming, where the temperature gradually increases to facilitate the separation of compound. So, widely

different polarity or molecular weight temperature should be maintained within plus minus 2 degree Celsius. You can have both where you can operate the oven that is as thermally or by the programming the temperature.

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Now, liquid phase stationary phase of coated on inside the column or on the support material, it is like this. Here, partitioning takes place. It separates the samples compounds, components into discrete peeks. It should be chemically and thermally very stable because otherwise column bleeds and other problems would occur, which certainly damage. It will result into not so good chromatography separation on gas chromatography operations. They are always kept 10 to 15 degree Celsius below the upper temperature limit of the phase because again column bleeding is much higher.

If you go at the upper limit, then the mount of column bleed must be minimized. It will certainly prolong the column life, prevent any falling of the detector and maintain best line stability on the chromatogram. So, that is a very important part that the column bleed could be minimized at the enhancement, the quality of the separation.

Now, bonding the stationary partitioning liquid to the enough surface of capillary columns or the column packing permanently occurs. The stationary phase to the surface commonly used phases includes, which we have already discussed includes a polyethylene, glycol, methyl, phenyl and methyl, veline, silicon, gums, asters of ad pick, succinic, tantalic acid, phenol and so on.

So, they could be different kind of depending on temperature what we are actually going to use. We will use different kinds of liquid phases. Once the analyte has passed through the column, it has partition. The mixture has different analytes. The mixture has separated. They will come out of the oven. They will be passing through the detector.

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They will lute from the column and pass through the detector, so that they could be identified or that chromatogram could be obtained. Now, detectors are of many types. There are many different types of detectors. We will see as the detector is located at the exit of the separation column, what it does is it will; since the presence of individual components as they live the column, there could be different ways methods. This is because a particular analytic is in a vaporised phase. Now, volume must be small to prevent the re mixing of the components separated. So, that should reach detector fast.

The electrical analogy output of the detector is amplified and then sent to the script chart recorder or converted to the digital signal and sent to a computer system. Then the computer system can process the data, store them and display the chromatograph or chromatogram with analytical result on your screen or a recorder. There are several types of detectors. Let us see.

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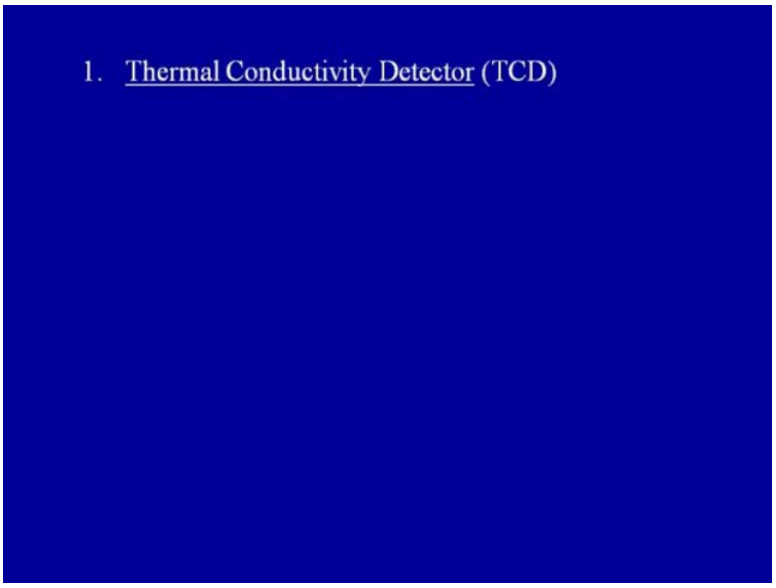

Let us discuss some of these detectors here. There is a very simple type of detector called thermal conductivity detector TCD. We call it a thermal conductivity detector. It is one of the earliest detectors actually, which was developed for the use with gas chromatography. Now, what it does is it utilises heated filament placed in an emerging gaseous trip. So, the amount of heat lost from the filament by the conduction to the detector walls will depend on the thermal conductivity gas phase. I will show you that in a little while. Now, with in cavity in the metal block, there are existents tightly called filament. This is a filament.

The filament is heated to a constant temperature, but less than dull rot condition by a regulated current supply. Now, heat loss from the filament to the metal block is a constant when only carrier gas is flowing through the detector. So, when only like sudden carrier gas and there is no analyte in it, then the heat loss will be a particular amount to the walls to the detector.

Now, the thermal conductivities of say hydrogen and helium are roughly 6 to 10 times greater than those of the most organic compounds. So, what will happen if there is an analyte organic compound present? The presence of even a small material, organic material will cause an electively large decrease in thermal conductivity of the column influent. So, what will happen is the amount of heat lost or which is conducted to the

walls of the selector will be less. The filament will retain more heat and its temperature will rise.

So, the particular electrical resistance will go up. The advantage of thermal conductivity detector is simplicity its large linear dynamic range, which is around 10, is to 5. There is a general response to both organic and inorganic species. It does not differentiate between them. It is a non destructive method that is destroying the sample. So, you can collect the sample after detection. One limitation could be it has low sensitivity. So, that could be one limitation.

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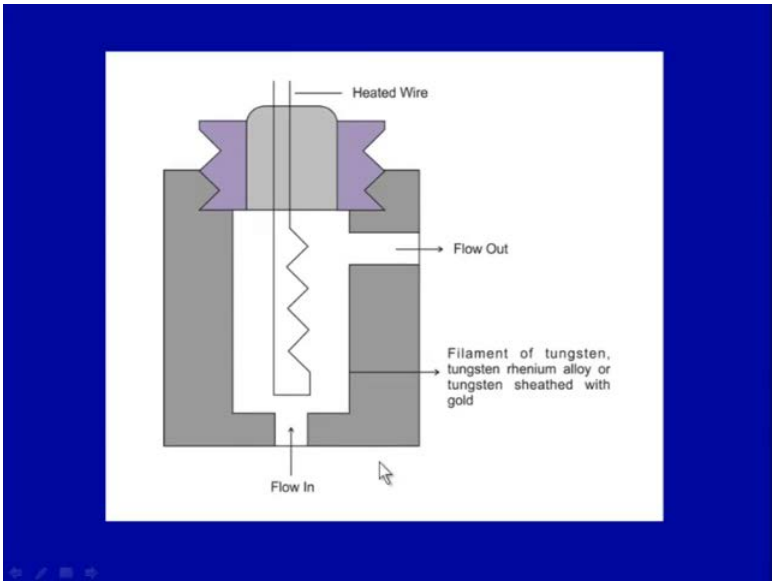

Now, this is very simple schematic of this thermal conductivity detector. You can see there is a filament here. This filament is heated. When it is heated, there is a gas flow here, which is only carrier gas. The heat generated from here will go to the walls of the detector. Now, when there is an analyte organic compound mixed with the carrier gas, then the conduction of the heat will be lower. That could be measured actually because resistance will go up here. That is how you will get the signal like we said non distractive. So, flow out here you can collect your sample after it has been deducted. So, thermal conductivity is a type of detector. It has the low senility.

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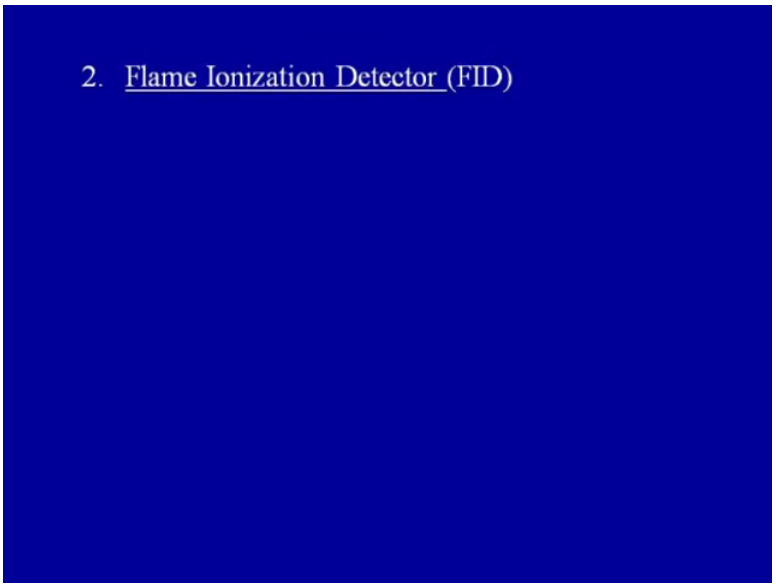

Now, second most commonly used detector is flame ionization detector or we call it FID. Now, FIDs are the most generally applicable. It is the most widely detector. A mixture of hydrogen and air is introduced onto the detector to give a flame. So, flame is an important part of this detector. So, flames form one elect rot. So, what is done is mixture of hydrogen and air is introduced into detector to give a flame and adjust, which forms one elector.

Other elector will be a brass of platinum wire mounted near keep of the flame. So, this detector will have 1 elect rot will in form of flame, another in form of the wire. So, what happens is when sample components emerge from column, they will be ionised in the flame. When they are ionised, they will result in increase single will passed to the recorder. That will be measured. Upper temperature limit here is 4 degree Celsius and minimum detection quantity is the order of 5 into 10 is to power minus 12 gram per second.

If you compare with 10 is to minus 8, actually this is lower than this much higher sensitivity. Here, one thing has to be remembered that this flame ionisation detector respond approximately to the number of cs 2 groups. So, if you have 2 cs 2 groups, then signal will be a particular amount. If you have 4 cs 2 groups, it would double of that. It is like directly proportional to the number of cs 2 groups. There is a problem that no response from the fully oxidised carbons will be obtained.

So, there is response from carbon attached to say certain groups like they have rocks and groups. AMD mines group is lower and oxidised carbon will not give any signal. So, that is a disadvantage. Now, it is an advantage is to use FID that is flame ionisation detector. This is because the detector is affected by the florid. It is non combustible gaseous. It is not affected by fluoride or non combustible gaseous or water. The properties allow FID high sensitivity and low noise. The unit is both very reliable and very easy to use, but you require flame, here a flammable gas. Also, the sample is destroyed. So, unlike thermal conductive detector, it was not distractible. This destroys the sample.

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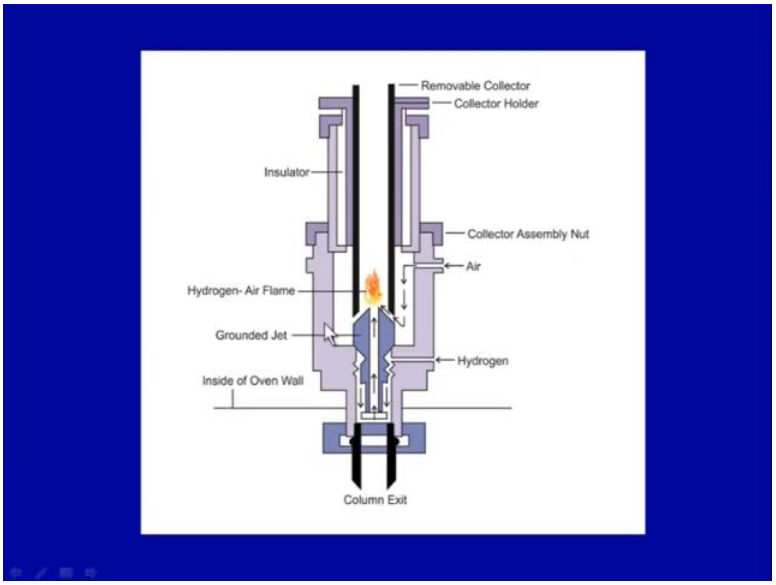

If you see the very simple schematic, here a flame ionisation detector, when you can see here, there is a flame here. There is a collector. You can say collector assembly wire. So, what happens is the exit or material is put in this flame. It ionises and the current is passed. The signal is recorded and destructible like grounded jet. There are a lot of hydrogen air flame is here. Hydrogen is supplied from here and air is supplied for the flame.

So, this is very useful and most widely used detector. Certain limitations that oxidised carbons cannot be sensed here and other groups like hard rock will have lower detection limits. It is directly pronominal to the cs 2 groups. So, it is very useful for seal environmental sample and lot of seen in pollution studies and lot of other studies, it is utilised.

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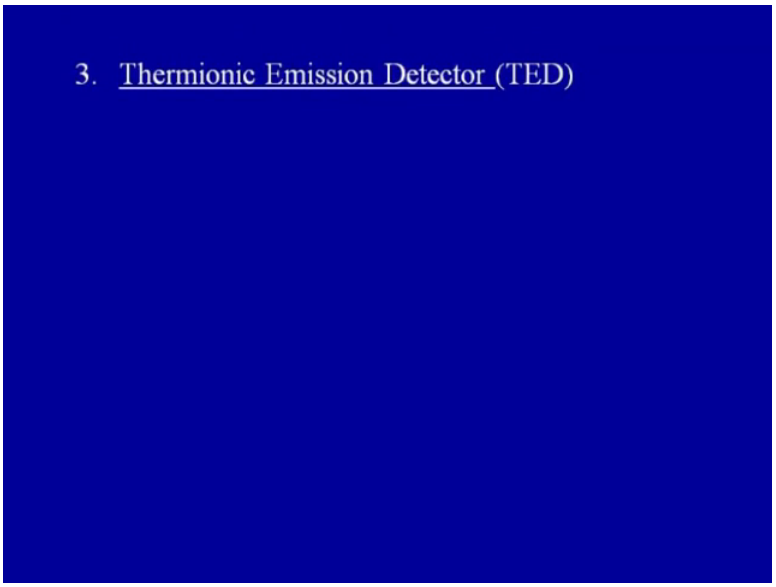

Then, another version of flame ionised is detector is thermionic emission detector or TED. This is also called nitrogen phosphorous detector. It seems as FID, but it has you can say much cooler as compared to the FID. It has the crystal of sodium salt fused on to the electrode system or burns a tip embedded in a ceramic tube containing a sodium salt or a medium chloride tip.

It has an excellent selectivity towards nitrogen and phosphorus. It has been modified for compounds containing nitrogen and phosphorus containing analyte. The upper limit range would go up to 300 Celsius like kind of cooler. This flame is cooler than the flame ionisation detector. Detection limit is very high say up to terrestris from minus 40 grams per second. It is wildly used organic phosphorus pesticide residue analysis.

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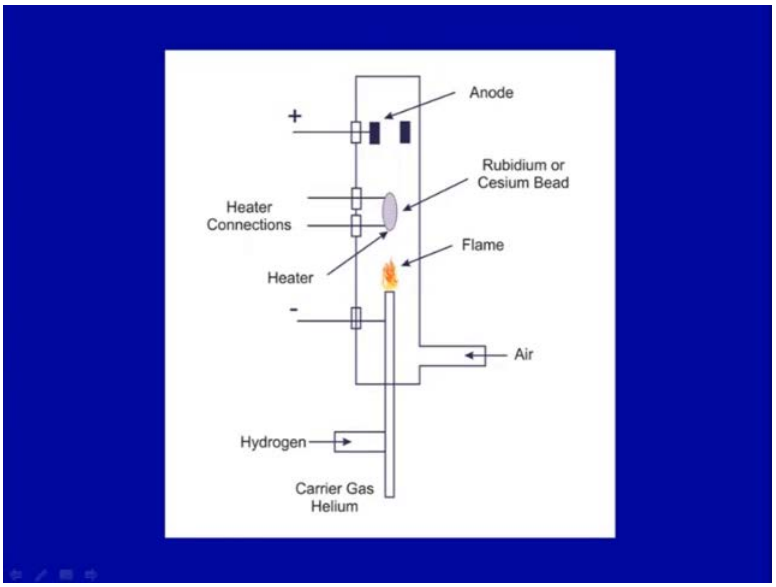

Another thing is here, there is a simple schematic like FID. Only detect here is a flame, which is a cooler flame. There are heater connections. There is a medium or caesium bead here and anode, which is collector here. As the comp analysts are ionised here than mostly nitrogen and phosphorous compounds, then the signal could be or could be recorded in here. Another detector, which is for particular kind of compounds is called electron capture detector.

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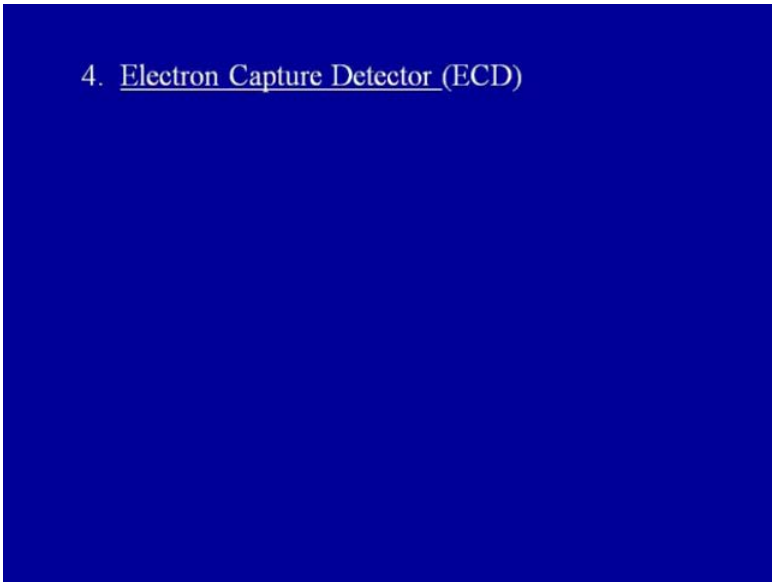

Electron capture detector only responses to analysts that can capture electrons, as the name suggests, particularly halogen containing compounds. It is widely used in analysis of sy polychlorinated compounds like pesticides DDT, aldrin, dieldrin and all those things. It has a very high sensitivity of around 10 is to minus 13 grams per second. The upper temperature could go up to 300 degree Celsius. Now, this detector works by means of a radioactive source present on surface of 1 electrode, which will emit high energy electron that is beta particles.

If you could recall in radio isotope, we have discussed about that radioactive substance will emit certain particles of a radiation. Now, these electrons bombard the carrier gas as we have seen in gas ionization based detectors. They are realising electron and that gives the current across the electrodes when a suitable voltage is applied. If you could recall gm counters is something like that.

Now, what happens is when electron capturing analyte emerges from, so when you taking carrier gas, different current is recorded. But, when you have an electron capturing analyte in the carrier gas, then what will happen is the ionised electrons will capture by these analytes. There will be a drop in a current. This change could be recorded.

So, the carrier gas so here that is the method. The cobalt could be utilised as radioactive material and other things could be also utilised. The carrier gas is most commonly used in conjunction with ACD nitrogen or organ plus 50nitrogen or an organ plus 5 percent methane mixture. So, that could be either nitrogen could be used or organ mixed with methane that is 5 percent methane could be mixture could be utilised.

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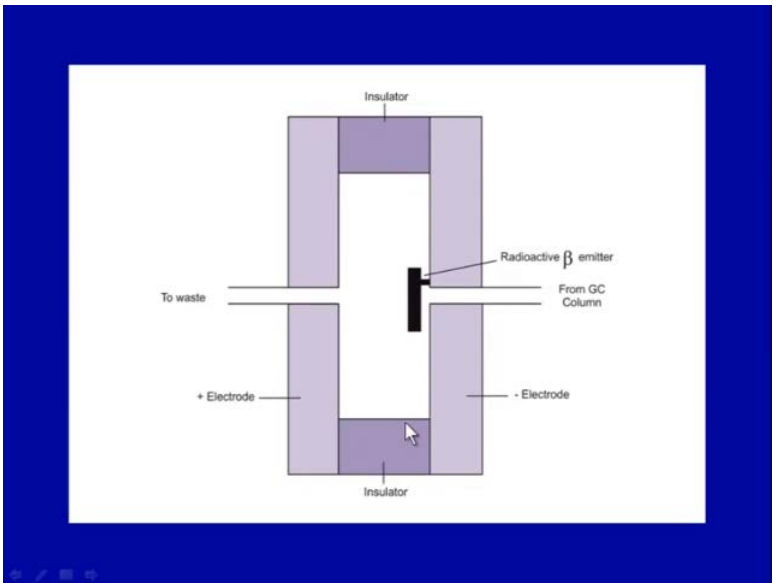

So, it is a very simple schematic of electron capture detector. There is a radioactive beta emitter. There is a gas here and there are electrodes. Here, a radioactive material will be emitted; beta particles which will ionise the gas. You will have a current as you apply the suitable voltage. So, when there is no analyte in the carrier gas, there will be current. When halogen containing or electron capturing analytes are there, there will be drop in current measure. So, electron capture detector also is very useful for analysts, which can capture electron. Now, there are other detectors also. These were what we have discussed were the most common types of detectors.

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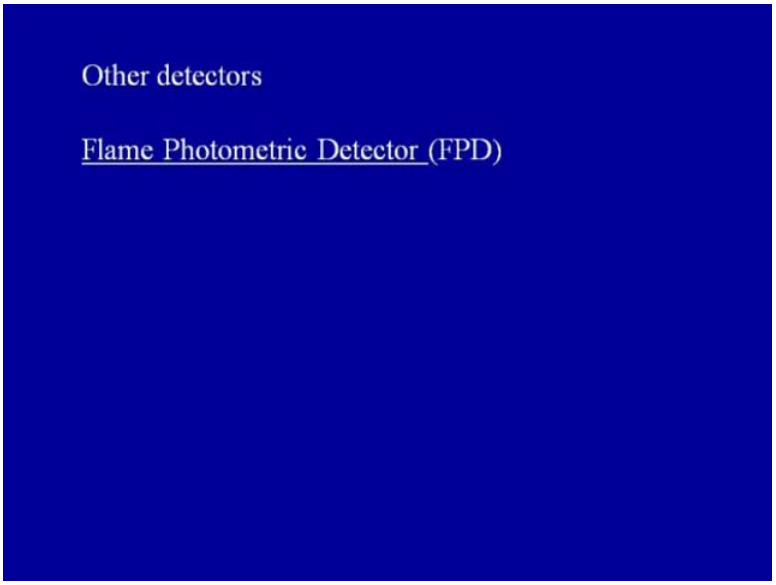

But, there are other detectors also like say flame photometric detector, which exploit the fact that phosphorous and sulphate containing analytes emitted light when they are burnt in a fib type detector. This slight is detected and quantified. Detection limit can go up to one pico gram for phosphorous containing compound and 20 pico gram for sulphur containing compound.

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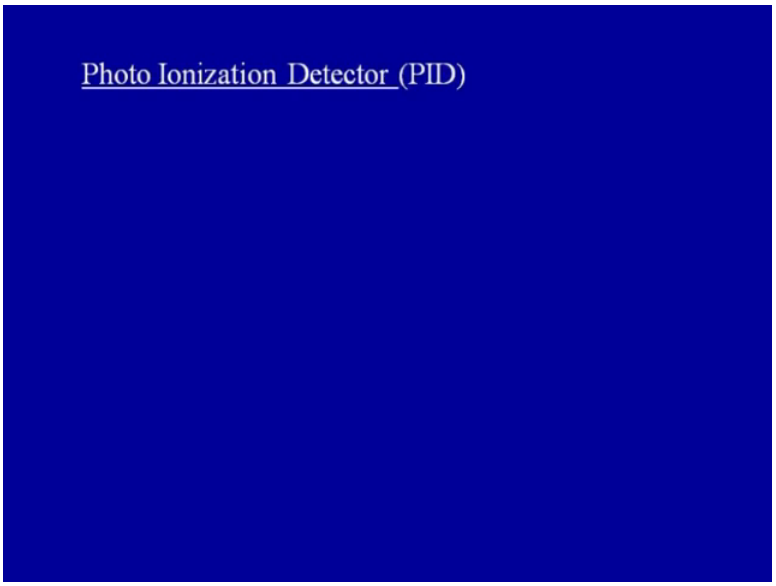

They could be like photo ionization detector, which uses ultra violet radiation from lamps with energies running from 9.5 to level electro volt to produce ionization of solute molecules. The ions are collected at positively charged electro volt. The current is measured. A compound ionization potential load than the lamp ionizing energy will give a response.

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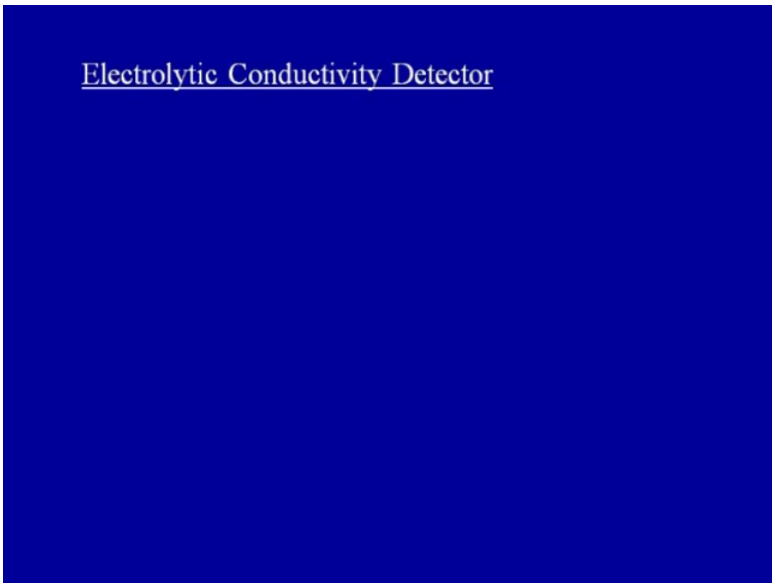

There could be another like electrolytic conductivity detector, which operates on electrolytic conductivity principle. So, organic compounds illuming from the column that is gas chromatography column will be burnt in say miniature furnace to form simple molecular species. Those thread anises and contribute to the conductivity of de ionised water. This change in electrolytic conductivity will be monitored.

So, we have discussed these different types of detectors, which could be utilised. They could have applications for different kinds of word, different kinds of scientific techniques. It could be environmental science or medical or clinical research, where gas chromatography is utilised. In basic research, the thermal conductive detector is very simple and very useful detector. The most widely used is flame ionisation detector.

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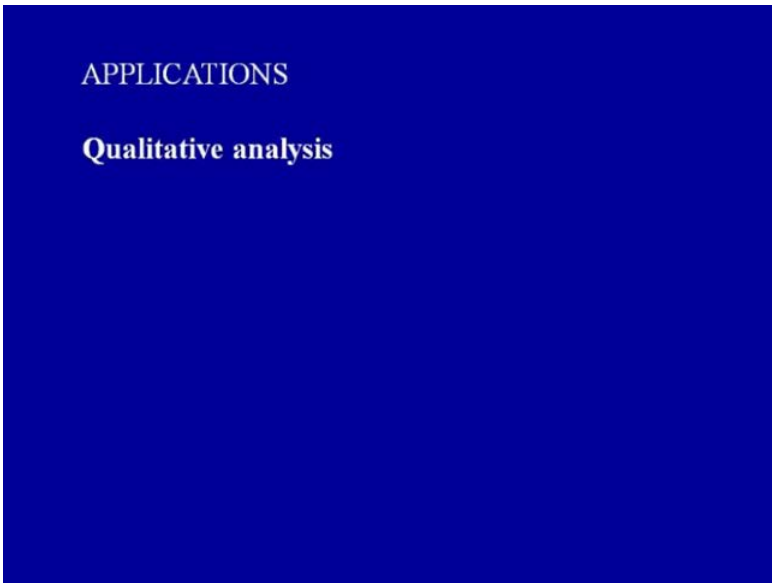

There could be many applications of gas liquid chromatography. They could be qualitative and quantitative. For a qualitative analysis, generally chromatogram data is presented as a graph of detector response against the retention time. You can make a standard curve or you can bring them. It is called a gas chromatogram. So, this provides a spectrum of a peeks for a sample representing the analytes present in a sample illuming from the column at different times.

Retention time can be used to identify analytes. If the methods contain the conditions on constant, by comparing retention time of sample as fill as the standard, I have shown you when I have shown gas chromatogram, the schematic of the gas chromatogram. Now, these pattern of the peeks obtained will be constant for a sample under constant, under standard condition. Then it could be identified from a very complex mixture of analytes. Then you can also test the purity of compound here by comparing standard as sample. If additional peeks are obtained, then impurities are present and otherwise the compound is pure.

So, if there is any additional peek compound not pure, so the purity of purified compound could be tested in gas chromatography. In most modern applications, the gas chromatography is connected to a mass spectrometer or a similar kind of detector that is capable of identifying the analytes presented by the peeks. The sample could be passed to mars spectro meter where there is structural information through molecular mars

information. You can identify these samples and many more are things could be done by mars spectro meter, where a lot of information can be obtained in quantitative analysis.

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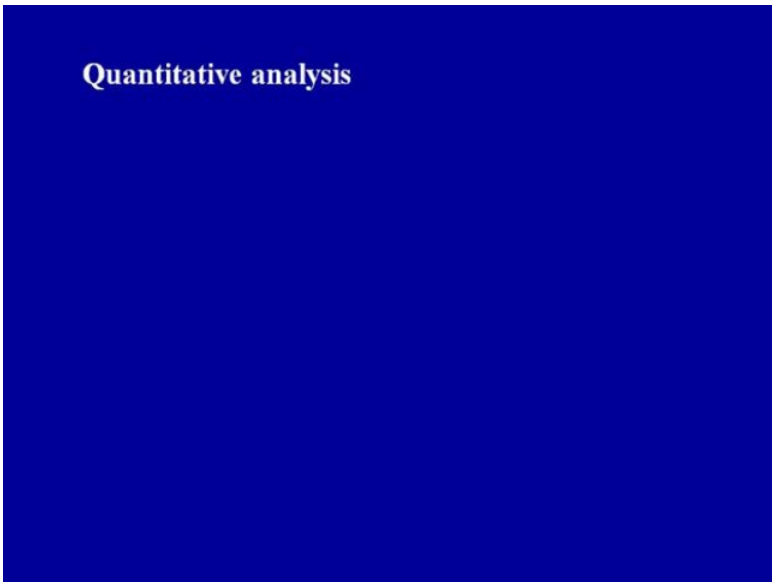

For example, area under peek we have done. You can do it for other chromatograph technique, where quantities analysis could be done by measuring the area under the peek. So, here also it is proportional to the amount of the analyte present in the chromatogram. By calculating the area of the peek using the mathematical function of integration, the concentration of analyte in the original sample can be calculated. The concentration can be calculated.

Concentration can be calculated being a calibrated calibration curve created by finding a response for a crease of concentration of an analyte or by terming the relative response factor of an analyte. So, this is like you can do quantitative analysis. Relative response factor is the expected ratio of an analyte to an internal as standard as to external standard.

It is calculated by finding the response of a loan amount of analyte and a constant amount of internal standard added to the sample of the constant concentration. They have a distant potential time like you have distinct retention time analyte. So, these are few methods for quantitative analysis. They are quite similar. As for other chromatography techniques, you can use internal or external standards and various methods. You can do that.

Gas chromatography is utilised for lot of different applications like elemental analysis in different fields of biotechnology. You can determine say elements like carbon, hydrogen, oxygen, sulphur and nitrogen. You can use it for analysing mixture of cracks. Isolation and identification of drugs could be done. You can have isolations and identification and the mixtures of components. For example, plants extracts, volatile oils and amine acids and lot of other could be tested and identified and even isolated. So, there are whole lot of applications for gas chromatography. With lot of applications, there are limitations.

As we were talking here, samples which are analysed are limited. This is because not all the compounds can be volatile and thermally stable, but many times can be made volatile derivatization, where a lot of chemistry is involved. Sample must be thermally stable to prevent to get irritation when heated. This is because that if it is d grade, then it is no use to purify or analysis. The case chromatography cannot be used to prepare sample for further analysis once separated because you do not get lot of amount. So, there are certain limitations also.

Gas chromatography nowadays has been replaced by high performances chromatography for many applications. But, still gas chromatography is used by many different streams of biotechnology. For example, clinical analysis environmental science, lot of like testing for drug pharmaceutical industry, like food industry, lot of analysis particularly of same certain analytes or certain element is done here.

With this lecture, we come to an end of chromatographic section. So, in chromatography, we discussed lot of different techniques. We have discussed about liquid chromatography, where low pressure and high performance liquid chromatography and we have discuss these 4 techniques. So, this completes our chromatographic section. In the next lecture, we will start a new topic. That is electrophoreses, which is another very important analytical method. It is routinely used in various laboratories for both research and for application purposes.

Thank you.