Environmental Quality:
Monitoring and Analysis
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Lecture No. 25
Analysis Methods – Gas Chromatography

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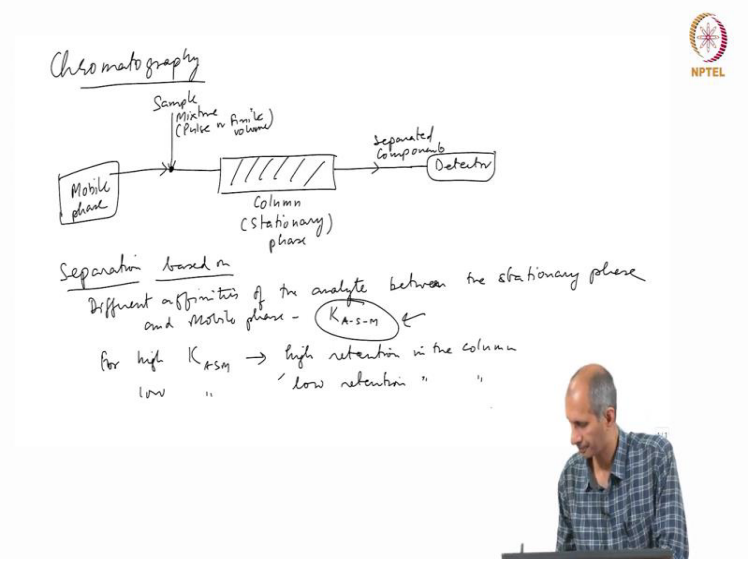

Talking about chromatography in the previous class. So here the main part of the chromatography system is the column which is also called as a stationary phase and there is also what is called as a mobile phase. So, the purpose of the mobile phase here is, you introduce the sample, a mixture which is usually a pulse or finite volume just before the column and then you have the separated components coming out of the column which are then detected. The main purpose of the column is the separation.

So the separation occurs mainly because it takes advantage of different affinities of the analyte between the stationary phase and mobile phase. So, in other words, we are talking about some partition constant between the stationary phase and the mobile phase. So, the extent of the separation depends on the type of the affinity.

So, just to give you an example, higher the value of K the higher retention in the column, lower K means low retention in the column. It means if you are able to somehow manipulate the retention time in the column, then you can possibly separate some of the components that are in the mixture based on the way in which we manipulate them. So, to control separation you can manipulate 2 factors :one is the retention and the other is partitioning constant.

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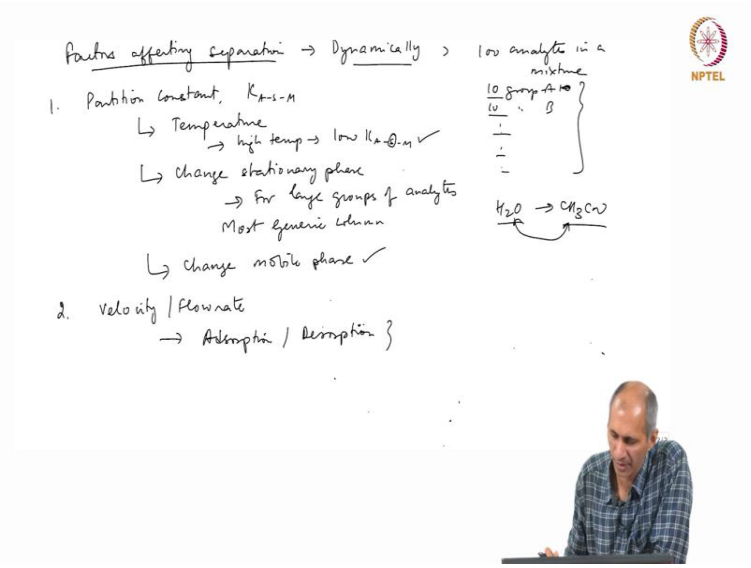

We summarize the factors affecting separation one is the partition constant K_{A-S-M} whatever we are talking about ok, so how do we adjust? How do we manipulate the partition constant to increase it or decrease it? One way is the temperature, Adjust temperature factor so typically that a high temperature leads to low K_{A-S-M} means low retention so higher compound comes off quickly. Second is to change the stationary phase this is far more difficult to do because stationary phases sometimes are very expensive.

So theoretically it is difficult to have a large number of stationary phases for a given set of analysis. So, commercially available chromatography columns are typically fairly expensive. Also, one of the things we would like to do when we are talking about effecting separation is we want to do this dynamically. We want the opportunity to do this dynamically in a given sample.

So, in a given sample means suppose you have you have 100 analytes in a mixture, So in 100 analytes 10 belong to one group i.e,10 belong to Group A, 10 belong to Group B and so on.

There are different types of compounds in this and you do not want to use the same set of conditions for all of them. So, you are like for one of them at one time, 1 set of conditions such as partition constant, for another one another partition constant and so on. So, you would like to operate this at differing conditions of partitioning within a single run.

So normally when you inject a sample, it starts the beginning of what is called as a sample run i.e, sample running through the column. So within that time your goal is to do all in one analysis in short, if you cannot, then you might have to run it again with a different set of conditions, but the goal typically is to optimize the analysis because an analysis takes time and analysis costs money, and therefore, we are trying to minimize all of that in in a commercial scale.

So, usually stationary phase changing is not done, but it is done for large groups of analysts. For example, if you know that, you will need to do analysis for a set of compounds alkanes, or pH or PCBs or pesticides, something you know you are only interested in this and the column that you have is not suitable to give you a very unreasonable separation and not good then you go and select a stationary phase that will be very suitable for this, otherwise, we will stick to the most generic columns.

And last class we talked a little bit about this, the most generic column are some samples columns that would do a very wide range of separations. So, the third thing that you can do is change mobile phase. These 3 things can only be changed in the system. To change the partition constant when you are changing stationary phase it means you are introducing a component that has a greater affinity to an analyte or lesser of affinity to an analyte.

Similarly, you can also change the mobile phase, and introduce a compound that has greater affinity or less affinity. For example, mobile phase could be water or it could be something like acetonitrile. So if you look at water and acetonitrile. Water is more polar than acetonitrile obviously. So you can increase or decrease the polarity in this case by changing it from water to acetonitrile. Acetonitrile and water are miscible which means that you can gradually change the polarity by adjusting the ratios of water and acetonitrile you can make a mixture of these 2 and 1 is to 10 or 20 is to 1 is to 2 is to 8 and so on. So, you can gradually change this thing. So, last

class, we discussed that you can also change the temperature and the composition gradually. So these are the 3 things that you can do to change partition constant.

So, you can do this dynamically. Stationary phase is selected based on the kind of separation you want to get .The second factor that you can play around is the velocity. So, this velocity and flow rate are components that will influence because you to realize that both components that is partition constant and velocity flow rate influence adsorption, desorption cycles rates.

Chromatography is a series of adsorption and desorption cycles. The overall separation in a given system is influenced by these parameters. So, you can play around with these parameters and say so, so there is a there is something called as the rate of adsorption desorption which depends on what is called as a mass transfer coefficient. We will we will talk about it later for now. Generally, the efficiency is the rate at which mass transfer occurs faster when you have large amount of flow.

But there is a compensation here because if you have high flow rates, the compound does not have enough opportunity to do mass transfer, hence it is out of the system before it target is completed, and may not even come to equilibrium or achieve the objective of the mass transfer. Therefore, there is a payoff, the effect that is not just trade simply. It does not mean that the separation is greater or lesser but overall effect of velocity is there.

But if I increase the velocity, the compound is going to come out faster, the retention is going to be lower. Separation here, is a different aspect. Because separation also involves partition constant. So, it is a complicated equation to solve. Adsorption is an unsteady state problem, it changes across the length of the column and as it is going through, so we would not discuss that too much here. But from a realistic point of view, you need to know that if you are looking at separation, then you would like more time for the analyte to be spent in the column.

You are going to stretch the separation to hurry components to come out, if not, they would not separate and are likely to get club together. But on the other hand, if you have 100 samples, let us say I have collected 100 samples and I want to analyze them in a system I cannot wait for a long

time to for analysis to finish. So I am also trying to optimize my analysis methods so that I can finish up as much as quickly as possible I can analyze to all the 100 compounds in each sample in a reasonable amount of time which also referred to aa the standard time for the analysis. So, chromatography people have worked a lot on it. If you look at the literature on chromatography, or at the catalog of any chromatography system, all companies that are designing chromatography systems are all working currently. Chromatography is fairly old and in current systems ultra-rapid chromatography separation is seen , which means they do not want to spend much time.

They want to do it very quickly in a matter of something which was for example take 25 minutes, but now they want to do it in 2 minutes, which means it will increase the number of sample throughput and all sample becomes more efficient in analysis. So that is the engineering part of it, which relies on some of these basic factors.

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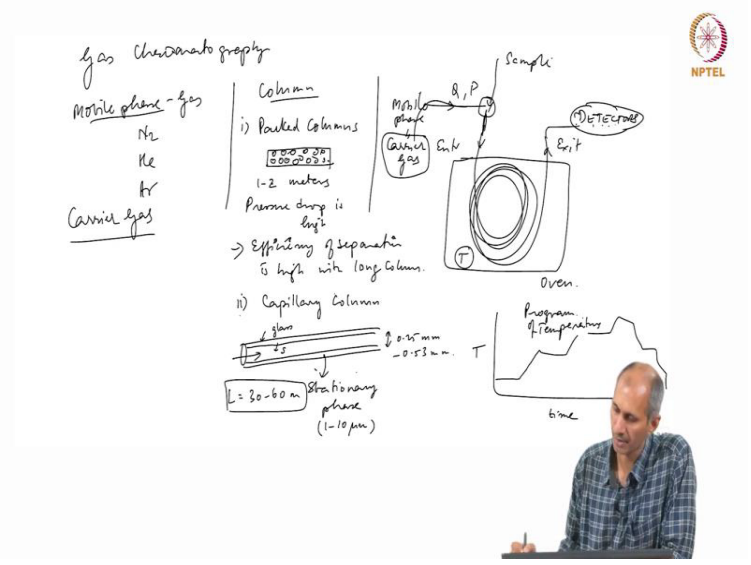

So, last class, we were discussing mainly the gas chromatography. So, in this context, chromatography analysis, the mobile phase is a gas typically nitrogen, helium or argon or inert gases. But sometimes you also use other gases such as hydrogen and this depends on what your analysis is specifically. There is also a column, packed columns or capillary columns as we discussed earlier.

Packed columns are columns which contain a packing length anywhere between 1 to 2 meters or even slightly longer. One big disadvantage in a packed column is that your pressure drop is high. The efficiency of separation is high if you give an opportunity for compound separation by providing the length of the column long enough that it will separate nicely.

So, if columns are long it provides a large opportunity because it is related to adsorption desorption cycles. Even if there is a very small difference in adsorption desorption cycles, if you provide a large distance for the adsorption desorption to occur the separation will be effective. Therefore, it is good to have the efficiency of separation high with long columns. Unfortunately, if you have long columns in a packed column pressure drop is very high you can't have the column beyond a certain length.

So, separation is a kind of restricted. So, to remove that, the people developed what is called as a capillary column. Capillary column is a column that is made of glass, which has a coating of some plastic. So, the outer core is glass silica. Thus, what we are talking about the stationary phase is coated in here. So, it is a small column hence its dimensions IDs is anywhere from 0.25 mm, 0.53 mm.

The stationary phase film is in the order of few 10s of microns, 10 microns or 1 to 10 microns very small. So, the length of these columns can go up to 30 to 60 meters or even longer. So if they are thin, and can be wound like a packing. Packed column stationery phases usually consists of spherical beads or some beads, which are commercially made and they may have some coating or they may have the entire bulk.

For packed column, larger the particles, greater will be the mass transfer issues and if the particle size is small then, bigger will be the pressure drop. So, there is a design aspect here. But capillary columns remove that restriction. Because capillary columns are very small, you do not use very high flow rate through capillary column nor you can run it at very high velocity, since pressure drop will again increase. You cannot have it at high pressure because it is basic last column, even though it is flexible. It is like an optical fiber.

So, normally the capillary columns are wound like this . One end this is the entrance to the column. And this is the exit of the column. So, the 60 meters of column looks like it is tied up. So, write here there is an entrance zone here. Your mobile phase which we also call it in the gas chromatography as a carrier gas. This carrier gas is stored in some cylinder or some such listings commercially available and is then sent in at some flow rate, some pressure. Here, I have to introduce my sample. This is where we discussed this and stopped. And right here we have a detector at the exit of this column. This is the schematic view of gas chromatography. This is the general arrangement of the capillary columns. So, in this section, the column is kept in an oven.

In the case of gas chromatography, you cannot change the mobile phase too much, mobile phase is a cylinder, and you cannot adjust the composition of cylinders. If you want to influence separation, you can only adjust velocity by changing the pressure, the pressure of the inlet gas or you can change the temperature. So, in GC generally, temperature is the most flexible factor or parameter that influences separation.

So, you can have the entire analysis run as temperature profiles. You can start at one temperature go up and you can keep doing whatever you want you want by going up and down, you can also program the temperature in a particular analysis. Now we quickly look at some of the detectors that we have for GC.

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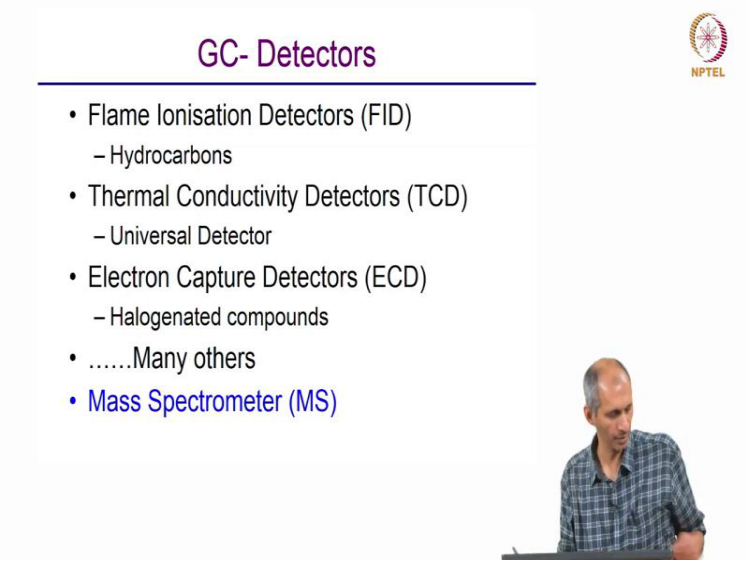

There are a few commonly used detectors. The first one is called as FID; the flame ionization detector. It is mainly used for hydrocarbon analysis. We would not go very much into details of this as the name suggests. What the flame ionization detector does in general is based on some kind of electrical measurement. The signals are all electrical measurement so in this case, there are 2 contact points. In between this whatever sample is coming out will burn.

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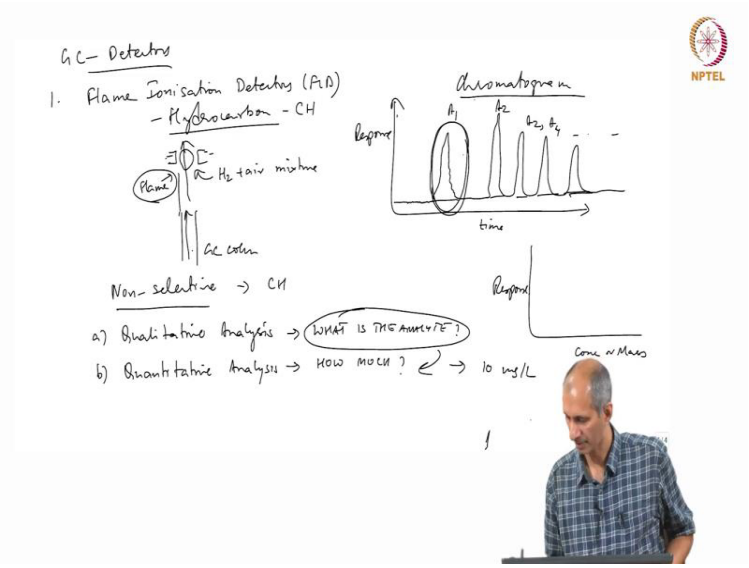

This is the GC column from the exit. Gas is coming out and it goes in. There is a reason there is an element here and these are two contact points. Gas is going through this, right here it burns in this region. There is a flame here all the time. If there is a hydrocarbon in your gas for it to burn you need to provide a fuel hydrogen and an air mixture is added at this point here. There is a flame here and this flame is burning constantly.

And in this flame whatever is coming in also burns and when it burns it changes the resistivity between these 2 electrical points. So, it is measured as a signal that comes up and if you have more hydrocarbons more burning happens and therefore, the signal goes up and comes down. So, in the output you are measuring signal. You have injected the sample in the beginning of the column and the sample is going at some flow rate and it is going out .It take some time for it to come out.

And the detector detects it. When it detects it, this is continuously happening and brings a real time change in the intensity of this flame. So, you may have what is called as a baseline. Some of this may be the baseline, only the carrier gas is going in and when you have a sample, the signal may go up like this and it may come down, so, this is a signal for 1 particular compound.

So, you may have multiple compounds, each 1 coming one after the other. Because it is chromatography, your hope is that this peak that you are looking at is only 1 compound. It is not a mixture of compounds. So, in the resolution separation you need to be sure that this is separated. That is a big challenge in the analysis of mixtures. So anyway, this is the way the detector represents the signal.

So this is called as a response so in the case of FID, it will give you some response. It is raw data in terms of millivolts or some such unit and you have to convert it to your units or units that you are comfortable with which can be concentration or area or some such thing. So, we do not do anything here, this is the response and this data as it appears, is called as a chromatogram. This is our raw data chromatogram and it is the record of the signal as a function of time as a compound come out of the detector.

So now in this the FID does not care what the compound is as long as there is something burning. This is what we call as a non-selective detector. The only selection it does is, if it is a hydrocarbon or not. What is the definition of hydrocarbon? You need to have C and H in your compound. If you only have C nothing will happen it will not detect anything, H has to be there for it to detect it.

But in non-selective which means anything that has C and H it will detect it does not know whether it is benzene, acetone, alkane, methane hexane nothing it will just detect as it is. So, we have 2 things that we do in chromatography analysis one is called as a qualitative analysis. Qualitative analysis answers the question what is the analyte and quantitative you are asking the question how much.

What does analyte mean, what is the identity of analyte if I have an unknown sample I injected? I need to know what is what and why do we need to know what is what and what is an analyte?. We need to know the answer to this question in order to understand. What do we need to know before we do quantification? Quantification means the answer to this question is some number or some concentration I have that is 10 milligrams per liter of something.

This is a number that I am going to report to somebody hence to get this what do I need? But why do we need to get quantification? Now in an instrument, how do I get data concentration? Why it is necessary to do calibration? I can do calibration for general hydrocarbons but all hydrocarbons may not give me the same calibration. That is a problem.

So, calibration has to be done very specifically for 1 compound, which means I need to know what the compound is and very specifically, I need to know if there are different analytes here A1, A2, A3, A4 so on. I do not know which peak is what? So, the next part of it is how do I do calibration? So, calibration is a chart between concentration or mass and some response. Now, what is the index or what is the quantity of response I am using? What can I use here in the response? So, if this is the response I am getting, how can I use this to get a calibration information.

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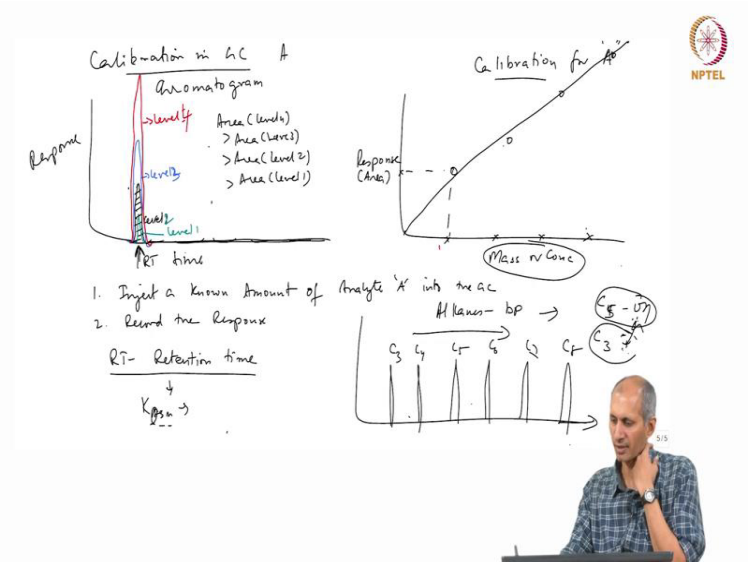

In gas chromatography for calibration I have the chromatogram. Now let us say I have some peaks like this and then I need to do a calibration. This is a chromatogram and this is a calibration. In the calibration I will have mass or concentration versus some response. So, how do I get a calibration information? I am doing for a particular type of compound. For example, I need to do calibration for a particular compound that is for some A or 1 compound for which I need signal mass or concentration.

So, I have to inject a known amount of an analyte A into the GC. So I inject A and I record the response let say this is, M 1 or call it as mass 1 or concentration 1. Now, this response here in chromatography typically is the current state. To recalculate the area under this peak to this signal, hopefully corresponding to this particular compound A as I injected A. So corresponding to this 1 mass, I get 1 response and the response here is the area. I have one point in my calibration, and I inject another one. So I get another chromatogram. So let us let us draw this, this is level 1. And I will draw other chromatogram same place and I inject another sample, I will get another one. Let us call it as this blue thing. This is level 2 then inject the third one, level 3 and I can inject fourth one which is smaller than this.

So, we, have different areas of this, as you can see the area of level 1 is smaller than 2, 3, and 4. So level 4 is greater than level 3, 3 is greater than level 2 and 2 greater than level 1. So, using the area of the 4 points, I can draw a calibration chart. So, this is a calibration which means I need to know what is coming. So one of the features of the calibration is the information I am getting here from a chromatogram.

If I inject compound A with a certain set of conditions with 1 column and 1 temperature profile conditions, I can expect that compound A will always come out at a particular time, as long as you do not change anything. Every time I inject it should come around the same time. Compound B which is which has slightly different properties than A will come before or after compound compound A so on. So, the retention time is the time at which a particular compound comes out. Retention time is a very important characteristic of an analyte for a given system. So you have to understand that the retention time will change if I change temperature or if I change the properties of my mobile phase or if I change column. So, if I know the system and I am

introducing a sample at a retention time, 1 or 2 or 3 minutes, some peak comes and I know with a certain amount of confidence that it is the characteristic of the particular analyte arriving at that particular time.

So, retention time is the characteristic if you have compounds that are in a series. So, if I have a series for example, you have series of alkanes with boiling points in the increasing order. I can get series of peaks let's say this is C 3, C 4, C 5, C 6, C 7, C8, and the boiling point are increasing in this order, as well as molecular weights are also increasing in this order. So, you can predict that as these boiling points are increasing, by seeing the order of the peaks with which it will come and so on.

This will change if you have functional groups that are added on to this. So, if for example, if you have compounds where one has C 5 and one has C 3. But one has an OH group and this one does not have OH group. So, the polarity of this one has an OH group and this one does not have an OH group, but the polarity of this maybe greater than this so despite being higher molecular boiling point, it may still come out faster.

Because it does not like the mobile stationary phase it is more polar. If the stationary phase is very nonpolar, it does not like partition constants. So, in this case, this analyte and this analyte are not similar. They do not follow a trend simply based on molecular weight or boiling point. It is based on the functionality of the functional groups attached to it.

So essentially, the retention time we know is a function of the partition constant. Now there are 3 things here; there is an A, S and M, the A, the polarity of A with reference to S and M is important for compound that has a polar group. The hydrophobic nature of this compound determines also when and where the compound will come out and what is its retention in these? So, all these are their points to be considered. So, there is 1 very important question if you analyze a mixture and you have a calibration that is fine.

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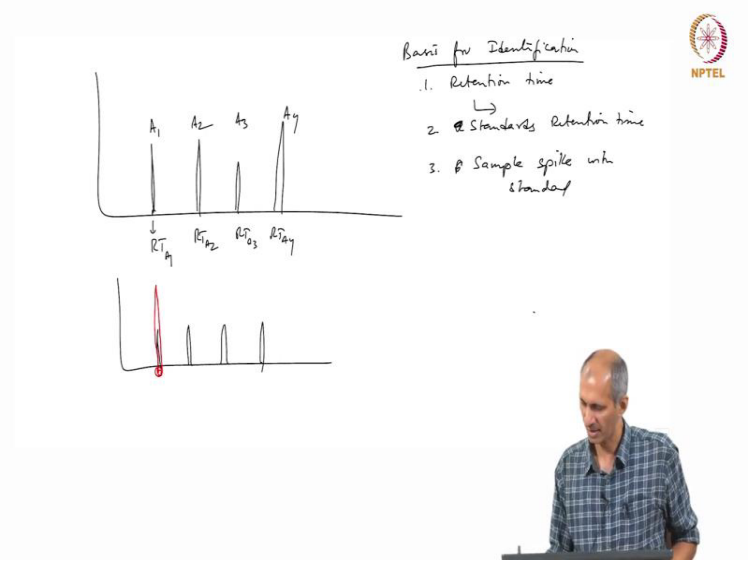

If I have a chromatogram that is I run a chromatographic analysis of a sample and I get 4 peaks. How do I know which 1 is what? So to know which 1 is what, the basis for identification is retention time. But we also know that retention time for 2 compounds can be the same. They can have different boiling points. But if they have a set of functional groups that make their partition constant same, they may both come at the same time.

So, retention time alone cannot give you anything, but that is the only information we have here. So one of the things we have to make sure that it is a component you are looking at is by injecting standards. So you inject standards for A 1, A 2, A 3 and A 4 you inject A 1, only A 1, and then you know where it is coming. This is a retention time for A 1. Then you inject A 2, you know the retention time for A 2 and so on.

You also verified it because now you have checked it with that particular compound. Therefore, you have a fairly good confidence that the A 1 is a compound will come at that retention time. But beyond this you have no identification this thing, so you have to rely on that alone. So it is quite possible that there may be another compound which is exactly waving the same way as your standard could be a compound that you are not aware of is there in the sample that is possible.

So one of the things people do is the only thing that you can check verify cross check this is if this is really A 1 in your sample is that you can spike your sample. So normally if you have a standard sample you run the sample you will get a peak like this, but if you spike your sample, one of the things that may happen is this if you spike the sample with A you will see that in the next run if you run the sample again with the spike, you will see an increase in the peak area, which means that it is it is verification that at the same retention time there is an increase.

So you can have some amount of confidence that it may be this compound, but you still are not sure that it is this compound you cannot go and tell anybody that this is this compound. So you have to guess. And it is a very complicated game of guessing because you need to know where your sample is what your sample is. So we need to have a lot of information about what is your sample. Suppose I am collecting exhaust from an automobile. I have a fairly good guess that what my sample will contain, it should contain some products of the combustion that is going on or it could contain the fuel itself. So, there is not a whole lot of compounds that are there. If I go and collect sample in the middle of the city in the air somewhere. I have no idea what is there because the air is well mixed, it could be coming from 100 different sources of all kinds of things. So, analysis is not very easy because if I just run an FID program, I will get some peaks.

I do not know what is it, it may match with alkanes. It may also match with some benzene or something like that. So, it is very difficult to do this. So, this is a problem with non-selective detectors. But FID is a very sensitive detector, it can go to very low concentrations, and it is very widely used in petrochemical industries, because they are interested in separations. So, it is a very commonly used detector. It is a cheap detector. Gas chromatography with an FID is a reasonably inexpensive detector.

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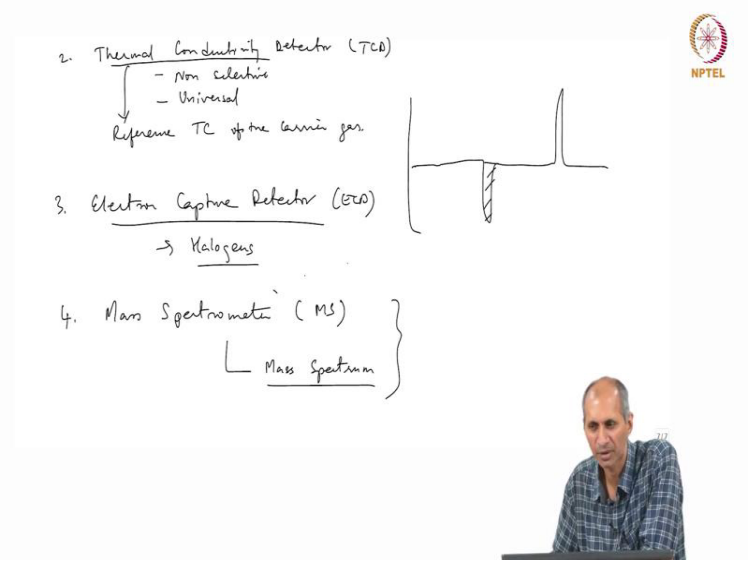

But it has limitations, it can only analyze hydrocarbons, that is one. The second type of detector that you would have seen is what is called as thermal conductivity detector or a TCD. This one is also non-selective which means that it does not give you any specific information about the compound analyte. But this is what is called as a universal detector and it will detect anything. You can detect oxygen, hydrogen, carbon, carbon dioxide, carbon monoxide or anything that you want.

Because it is measuring thermal conductivity with a reference. If you want to measure thermal conductivity for example if you want to measure oxygen content in the sample you have to change the carrier gas also. There will be a reference thermal conductivity of the carrier gas. So, it measures the difference between thermal conductivity of the carrier gas and whatever is there in the carrier gas.

So if you want to measure the amount of helium, you cannot use helium as gas you have to use something else, it could be a negative thermal conductivity difference or a positive. So the signal in a thermal conductivity detector, can go both ways. This is the baseline, then you can go and even get this kind of signal. Also you can get this kind of signal and this kind of signal. So, it is the same thing, it measures the difference between this signal and that one.

And you have to do calibration in the same manner as we talked about. Everything is the same except that this can do a little more, but it is not as sensitive. Because it is a little more universal detector the sensitivity is not very high. So you cannot go to very low concentrations in the thermal conductivity detector. The third kind of detector is called as an electron capture detector or an ECD this is very specific to halogens and has very high sensitivity to halogens, specially chlorinated compounds.

By high sensitivity it means you can go to very low concentrations with chlorinated compounds. This was developed because a very large class of compounds that are chlorinated are of interest to us from environmental point of view. So, this becomes very critical, but in all of these we have same problem, we really do not know what is the compound is, so you have to do a lot of preprocessing in the sense, you have to know, what is it that we are interested in.

And this becomes a problem throughout an analysis depending on even if you have more tools at your disposal, you really need to know what is what that you are looking for? Which means that you have to have some information about system and you cannot just go blindly and say I want to know what is everything that is there in the sample, which is usually a very bad unreasonable an unachievable target.

So, there is a fourth kind of detector that takes the disadvantages of all of this and it can give you a little more information called as a mass spectrometer or a mass selective detector. So, it is MS or it is called as an MS mass spectrometer. So, this gives you information in addition to a chromatogram. It gives you another dimension of data every time when every bit of sample that is going through it undergoes a fragmentation and it gives you a mass spectrum.

Mass spectrum are used as a signature of that particular compound to identify. So we will talk about this in the next class a little bit more and how we can use it in environmental analysis so, if you go and look at the standard methods, few classes ago we looked at methods for GC, FID or method for GC, TCD, ECD and for MS. They are all different because sample preparation and the way in which you do calibration, calibration is the same, but how do you identify compounds.

And therefore, you have to be very careful about preprocessing which means, you do a cleanup procedure where you only take 1 fraction, you do a pre chromatographic separation using 1 of those column chromatography methods and you take only pH or only oil alkanes separating and then put that into the GC with FID, the peak will only have that particular fraction.

Then you can you have a better chance of guessing what the compounds are. You have to be again be very smart about using the standards to get qualitative as well as quantitative information that you want, we will stop here tomorrow and we will pick it up.