Environmental Quality:
Monitoring and Analysis
Prof. Ravi Krishna
Department of Chemical Engineering
Indian Institute of Technology-Madras

Lecture No. 25
Analysis Methods – Gas Chromatography

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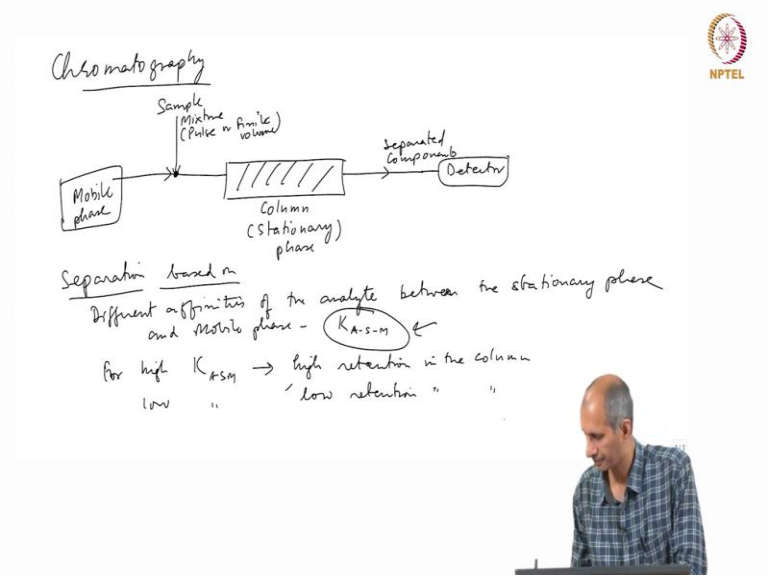

Talking about chromatography here the previous class before. So here chromatography has the main part of the chromatography system is the here is the column is also called as a stationary phase and there is a mobile phase. So, the purpose of the mobile phase here is and then you, you introduce the sample,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

So the separation occurs mainly because of the, affinity different affinity have it takes advantage of different based on 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 thing.

So, for just give you an example, the higher the value of this thing for high K this is high retention in the column, low K and which means low retention in the column means, if you are just if you are able to somehow manipulate the retention time. The column you are possibly able to separate some of the components that are in the mixture versus the way we which we manipulate written. So, if you manipulate, so, there are 2 factors that the control separation 1 is the retention, 1 is partitioning constant.

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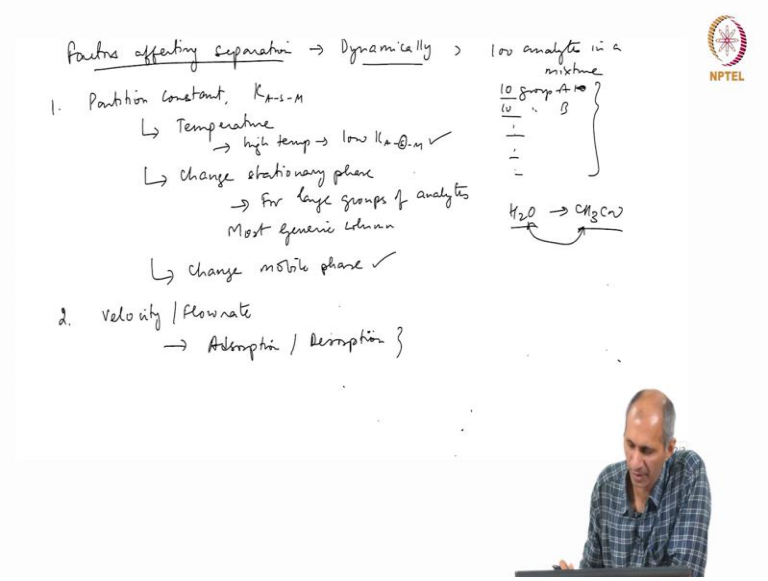

We summarize factors affecting separation 1 is the partition constant is what we are talking about how do we adjust? How do we manipulate the position concept to increase it or decrease it 1 raise the temperature, the just a temperature 1 factor typically high temperature leads to low case means low retention for higher the compound comes off quickly. This is 1 second as you change the stationary phase this is far more difficult to do because stationary phases sometimes very expensive.

So some we do not want to do it is possible theoretically difficult to have a large number of stationary phases for a given set of analysis. So, chromatography columns are typically fairly expensive. Now commercially available columns. Also, 1 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 what we mean by dynamically is in a given sample.

So, in a given sample means suppose you have you have 100 analyst in a mixture, 100 analyze 10 belong to one group, 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 1 of them at 1 time, 1 set of conditions partition conference, partition control. So, you would like to operate this at differing conditions of partitioning within single come through.

Normally when you inject a sample, it is called it starts the beginning of what is called sample run sample running through the column etc. So we did not the goal is to do it all in 1 analysis 1 short, if you cannot, then you run it again with a different set of conditions that is, but the goal typically is to optimize the analysis because analysis takes time analysis costs money, and therefore, we are trying to minimize all of that in in a commercial scale.

So, stationary phase, usually systemic phase changing is not done, but it is done for large groups of analysts. For example, if you know that, your analysis you will need to do analysis for 1 set of compounds alkanes, or pH or PCBs or pesticides, something you already know you are only interested in this and the column that you have is not just suitable give you a very unreasonable separation and not good then you go and select a stationary phase that will that was very suitable for us 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 subsamples columns that are that will do a very wide range of separations. So, the third thing that you can do is change mobile phase this is a possibility these 2 things are the only things that are there in the system. To change partition constant, because now you are changing. When you are changing stationary phase you are changing this, which means you are introducing a component as a greater affinity to an analyte or lesser of native to analyte.

Similarly, you can also change the mobile phase, you can introduce a compound that is greater affinity or less 70. For example, mobile phase could be water or mobile phase could be something like citronitrile. So we have looked at water and acetone nitride. Water is more polar

than a senator obviously. So you are increasing the decreasing the polarity in this case by changing it from what it was from a trade by a little bit.

So, again, what are our miscible which means that you can gradually change the polarity by adjusting the ratios of what are an estimated you can make a mixture of these 2 and 1 is to 10 or 2 is to 1 is the rate and so on. So, you can gradually change this thing. So, last class, we discussed that you can change the temperature gradually, you can also change the composition gradually. So these are the 3 things that you can do to change partition concert.

So dynamically, you can do this dynamically. This you have to do this before the analysis is done stationary phase you completely want to get 1 kind of separation you do that the second factor that you can play around it is the velocity. So, this velocity and flow rate are components that will influence because you realize that both these components both partition constant and velocity flow rate influence adsorption, desorption cycles rates.

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

But it is also there is a compensation because if you have high flow rates, the compound does not have enough opportunity to do mass transfer, it is out of the system before you for it completed, may not even come to equilibrium, it will not finish what it is want so what is the objective of the mass transfer and therefore, there is a payoff, the effect is not just trade for simply. It does not mean that the separation is greater or lesser but the overall effect of velocity is there.

But you can guess if I increase the velocity, the compound is going to come out faster, the retention is going to be lower. But separation is a different aspect. We have a separation also was partition content. So it is a complicated equation to solve. So it is what impact but adoption is a

steady state problem, it changes the length of the column and as it is going through, so we would not discuss that too much here. But from a very rule of thumb a point of view, you need to know if you are looking at separation, you would like more time to be spent in the column.

That is the only way you are going to separate you are going to stretch the separation that is only you have the components come out, they would not separate they will they 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 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 all the 100 components in each sample and 200 samples in a reasonable amount of time.

What we call a standard on time to for the analysis. So chromatography people have worked a lot on it. If you look at the literature on photography, even if you look at to take a catalog of any chromatography system, they are all companies that are designing chromatography systems are all working at currently, this is already old chromatography is fairly old, but now current systems into they will say ultra-rapid chromatography separation, which means they do not want to spend even this much time.

They want to do it very quickly in a matter of something which was take 25 minutes, but they want to do it in 2 minutes, which means it will increase the number of sample throughout 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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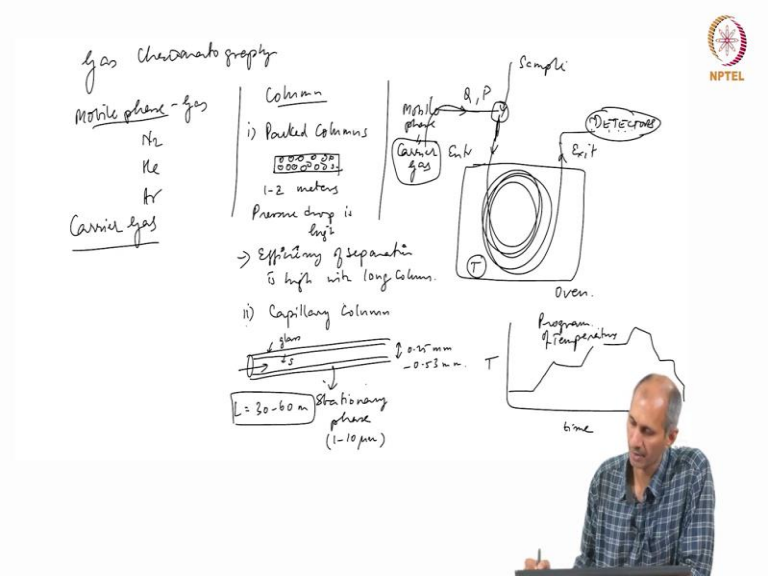

So, last class, we also discussed we were discussing mainly the gas chromatography. So in this constant in this context, chromatography the analysis, the mobile phase is a gas typically it is nitrogen, helium or argon or inert gases. But sometimes you also use other gases you can also use hydrogen and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

Pack columns very simply the columns are columns which contain a packing their length is anywhere maybe 1 to 2 meters or even slightly longer. One big disadvantage in pack column is that you pressure drop is high the efficiency of separation is high if you give it a large opportunity for compound separate when you give the length of the column long enough that it will separate into separate nicely.

So, column of columns are long it provides a large opportunity because it is adsorption desorption even if there is a very small difference in adsorption desorption, if you provide a large distance for definitely after the separation will be effective. So therefore it is good to have efficiency of separation is high with large, long columns. Unfortunately, if you have long columns in a packed column pressure of a very high you can have gone beyond a certain length.

So, separation is kind of restricted. So, to remove that the people developed what is called as a capillary column. Capillary column is where we say it is a column were made of glass, which has

a coating of some plastic. So, this is outer core is glass silica and there is a coating of this stationary material. Thus what we are talking about the stationary phase is coated in here. So, it is a small column so it is dimensions IDs is anywhere from 0.25 mm, 0.53 mm.

And the film the stationary phase is of the order of few 10s of microns, 1 to 10 microns very small. So, this length of these columns can go up to 30 to 60 meters or even longer 10, they can be warmed is this packing. This is a pack belt, simple pack belt, pack column in the pack column stationery phases usually beads spherical beads or some beads, which are commercially made with some, they may have some coating or they may have entire bulk, because everything is happening on the surface is inside, the more.

The larger the particles you have, the more the greater mass transfer issues and all that so, the particle size is also small, but the smaller the particle size, bigger is pressure drop and all that so, there is a design aspect there. But capillary columns remove that restriction. But because capillary columns are very small, you do not use very high flow rate through capillary column velocity, you cannot run it at very high velocity, pressure, drop it again increase, and you cannot have it a high pressure also because it is basic last column, even though it is flexible. It is like an optical fiber. It is flexible.

So, normally the capillary columns are wrong like this they round like this. One, and this is the entrance to the column. And this is the exit of the column so there is 60 meters of column looks like this it is tied up. So write here there is an entrance zone here. So, your mobile phase is which is we also call it as C in the gas chromatography we call this as a carrier gas chromatography the mobile phase is called as a carrier gas.

This carrier gas is stored in some cylinder or some such listings commercially available and it is it was sent in at some flow rate, some pressure. Here, I have to introduce my sample. This is where we stopped we discussed this and we stopped it here. And here we have a detector at the exit of this column. This is the schematic of this so variety of this is the general arrangement of the capillary columns. So this this section, this column is kept in an oven.

So in the case of gas chromatography, you cannot change the mobile phase too much, but when phase is a cylinder, you cannot adjust competition of cylinders, so you cannot do much with the mobile phase. If you want to influence separation, you can only adjust velocity by changing the pressure, the pressure of the gas or you can change the temperature. So, in GC generally, temperature is the most flexible factor or parameter that influences oppression.

So, you can have you can have you run the entire analysis as temperature profiles you can start at one temperature go up, you can keep doing whatever you want you can keep going up and down, you can 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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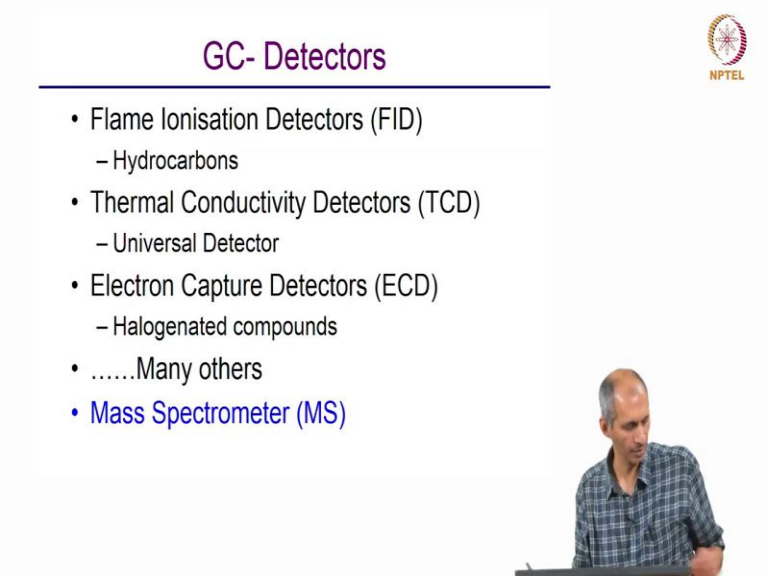

There are a few commonly used detectors. The first one is called us FID, it is called the flame ionization detector. It is mainly used for hydrocarbon analysis. We would not go very much into details of this as the name suggests. What the flame ionization detector does in general is that it is all they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

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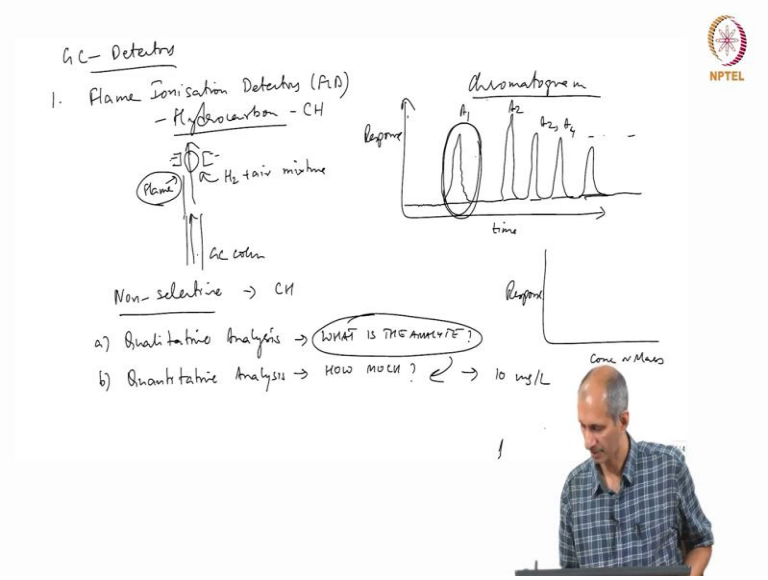

So if you are looking at the very simply speaking there is this is from the GC column. This is the GC column from the exit. Yes, it is coming out and it goes in. There is a reason there is an element here is an element here is just to contact points. Gas is going through this right here it burns in this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

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

And the detector detects it when it detects it there is continuously is happening here is directing the real time changing the intensity of this flame. So, you may have what is called a baseline some this may be the baseline only the carrier gas is going in, when you have a sample, the signal may go up like this and it may come down the signal so, this is a signal for 1 particular component.

So, you may have multiple compounds, each 1 coming after the other you may have multiple things coming 1 after the other. So hopeless 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, the resolution separation and you need to be sure that this is separated. That is a big challenge in in analysis of mixtures. So anyway so this is the way this the detector represents the signal.

So this is called as this is a response so in the case of a FID we will give you some response it is it is raw data is in terms of millivolts or some such unit and you have to convert it to you or units that you are comfortable with for you whatever is comfortable is 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 is the record of the signal as a function of time as in as a compound come out of a detective.

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 for the hydrocarbon what is the definition of hydrocarbon you need to have C and H in your compound that is all you only have seen nothing will happen it will not detect anything head just 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 nothing it will just detect as it is so, what is it that you know if you are doing an analysis that you need to know. So, we have 2 things that we do in chromatography analysis one is called us a qualitative analysis. Qualitative analysis answers the question what is the analyte and quantitative you are asking the question how much.

What does analyte means, 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 is that like? We need to know this question answer to this question. We need to know the answer to this question in order to understand answer this question, why is that? What do we need to know before we do quantification? Quantification means the answer to this question is some number from concentration I have 10 milligrams per liter of something.

This is a number that I am going to report to somebody some concentration to get this what do I need? But what do we need to get quantification? Now in an instrument, how do I get concentration data concentration? What is necessary calibration, unique calibration to do calibration? You need to have you cannot do calibration for you know, I can do calibration or general hydrocarbons but all hydrocarbons may not give me the same calibration sure, true. 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 there are different analysts here A1, A2, A3, A4. I do not know if which speak is what? So, the next part of it is how do I do calibration? So, the calibration is done calibration is a chart between concentration or mass and some response. Now, what is the index or is the quantity of response I am using what can I use here in the response. So, if I am using a response in this particular this is the response. This is the signal I am getting, how I can use this to get a calibration information.

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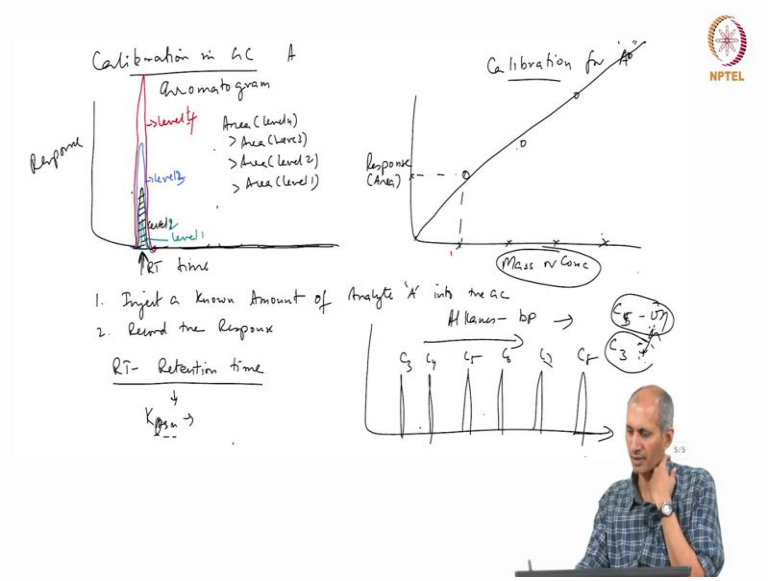

And calibration and gas chromatography and this I have the chromatogram. Now let us say I have some peaks like this and then I need a calibration. This is a chromatogram and this is a calibration in the calibration I will have mass or concentration versus some response. So, how do I get a calibration information I am doing the calibration for a particular type of compound for I

need to do calibration for a particular for some 1 compound for which I need signal mass or concentration.

So, I have to inject a known amount of analyte A into the GC. I record the response now so I inject A so I record the response this is say, M 1 row 2, row A 2 let us call it as M 1 or call it as mass 1 or concentration 1, level 1, 1 concentration. Now, this response here in chromatography typically the current state is recalculate the area under the speak to this signal, hopefully corresponds to this particular compound A injected there is 1 signal that comes.

So corresponding to this 1 mass, I get 1 response in the response here is area. One point of my calibration, and I inject another one. So I get another chromatogram. So let us draw this, this is level 1. And I will draw other chromatogram same place 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 smaller than this.

So we will call this as level 1 this is level 2 and this is level 3. This is level 4 for completion. So this is level 1 level 2, level 3, level 4. So we, have different areas as you can see the area of level 1 is smaller than 2, 3, and 4. So level 4 is greater than level 2 is greater than level 2 rather than level 1. So, the area of I have 4 points and I can draw a calibration chart through this. 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 is one of the things information I get from a chromatogram this is on a time axis.

If I inject compound a with a certain set of conditions with 1 column and 1 temperature profile conditions, I can expect that this will come out at 1 particular time compound a will always come out at that particular time, as long as you do not change anything every time I injected should come around the same time compound be which is a slightly different properties and they will come before or after compound it so on. So, the retention time the time at which a particular compound comes here scholars retention time.

Retention time is a very important characteristic often analyte for a given system. So you have to understand that the retention time will change if I change temperature if I change the properties of my mobile phase if I change column. So, if I know the system if I say I am introducing a sample at retention time, 1 at a 2 minutes or 3 minutes, I am always for some becomes I know of a certain amount of confidence that it is this particular compound that is 1 characteristic of the particular analyte arriving at a particular time.

So, it is the retention time characteristic of this so, if you have compounds that are in a series. So, if I have series for example, you have alkanes series of alkanes with boiling points in the increasing order I can get series of peaks this is C 3 this is C 4, C 5, C 6, C 7 for example, and this boiling point are increasing in this order, and is the molecular rates are increasing in order. So, you can predict that as this boiling points are increasing, I can see something in this all of peaks will come in this order and so on.

This will change if you have functional groups that are added on to this. So, if for example, if you have a compound that has both of they have one has C 4 and one has C 5 and C 3. But this one has an OH group and this one does not have group. So, this polarity of this one has the OH group and this one does not have an OH group, 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 very different of this one. So, in this case, what this analyte and this analyte are not similar. And they cannot be, they do not follow a trend simply based on molecular rate or boiling point. It is based on functionality of the functionality or that is functional groups attached to it.

So essentially, the retention time we know is a function of the partition constant, which now there are 3 things here there is a ASM, the A, the polarity of a reference to ASM is important for component as a polar group. The hydrophobic nature of this compound determines also when where the compound will come out 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 so you have a calibration that is fine.

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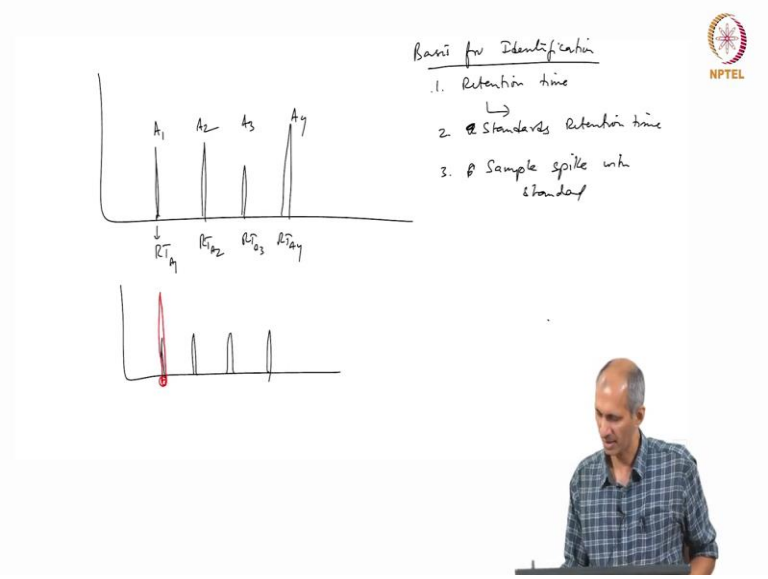

If I have a chromatogram that is I run a chromatographic analysis of a sample I get 4 peaks. How do I know which 1 is what? From so to know which 1 is what basis for identification this one is attention time. But we also know that retention time for 2 compounds can be the same. They can have different 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 any racing, 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 injecting 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 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 that you have no identification listing, 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 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 is in your sample, you can spike your sample. So normally if you have a standard sample you are on the sample you will get a peak like this if you spike your sample, one of the things that may happen is this spike a sample here you will see that the next run you are under 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 what it is this compound you cannot go and tell anybody that this is this compound. So you can guess 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 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 as against if I go and collect sample in the middle of the city in the air somewhere. I have no idea what is there because air is well mixed, it could be coming from 100 different sources all kinds of things are there. So analysis is not very easy because if I just run 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 the FID is a very sensitive detector, it can go to very low concentrations, it is very widely used in petrochemical industries, because we are interested in separations and all that. So it is a very commonly used detector. It is a cheap detector. It is not very expensive to get good gas chromatography with an FID is a reasonably inexpensive detector.

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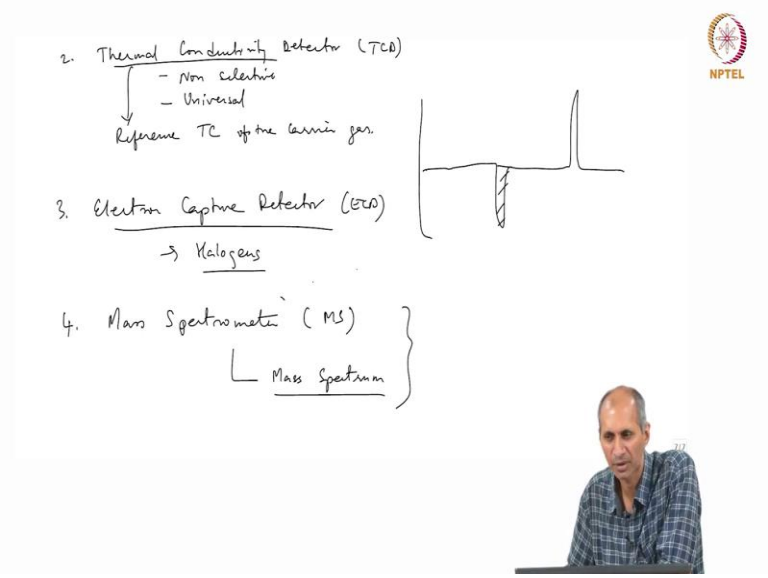

But it has limitations that it cannot it can only it can only analyze hydrocarbons, that is 1. The second type of detector that you would have seen is that what is called as thermal conductivity detector TCD this one is also non-selective which means that it does not give you any specific information about the call about the compound analyte. But this is a very this is what is called as a universal detector it will detect anything you can detect you can measure oxygen, hydrogen carbon, carbon dioxide, carbon monoxide anything that you want.

Because it is it is measuring thermal conductivity with a reference. So, if you want to measure thermal conductivity if you want to measure oxygen content in the sample you have to the carrier gas will change so, it will is 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 in the carrier gas in the sample.

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

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

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

And this becomes a problem throughout analysis depending on even if you have more tools at your disposal, you really need to know what is it 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 in the sample, which is usually a very bad unreasonable an unachievable target.

So, there is a fourth kind of detective that the disadvantages of all of this and it can give you a little more information just call as a mass spectrometer or a mass electric detector. So, it is MS scholars in MS mass spectrometer so, this gives you additional information what it gives you is it gives you in addition to a chromatogram it is the gives you another dimension of data every time every bit of sample is going through it undergoes a fragmentation and it gives you a spectrum gives you a mass spectrum.

And mass spectrum are used as a signature of that particular compound to identify. So we will talk about this next class a little bit more about this and how we can use it and nanometer and so, this is an if you go and look at the standard methods, few classes that we look at your methods for GC, FID or method for GC, ECD, TCD, ECD and for the MS this method is 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 take the fraction of you do a pre chromatographic separation using the separation 1 of those column chromatography methods and you take only pH or only oil alkanes separating and then put that into the GC, FID the peak will only have that particular fraction.

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