Environmental Quality:
Monitoring and Analysis
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Lecture No. 24
Analysis Methods – Chromatography Fundamentals

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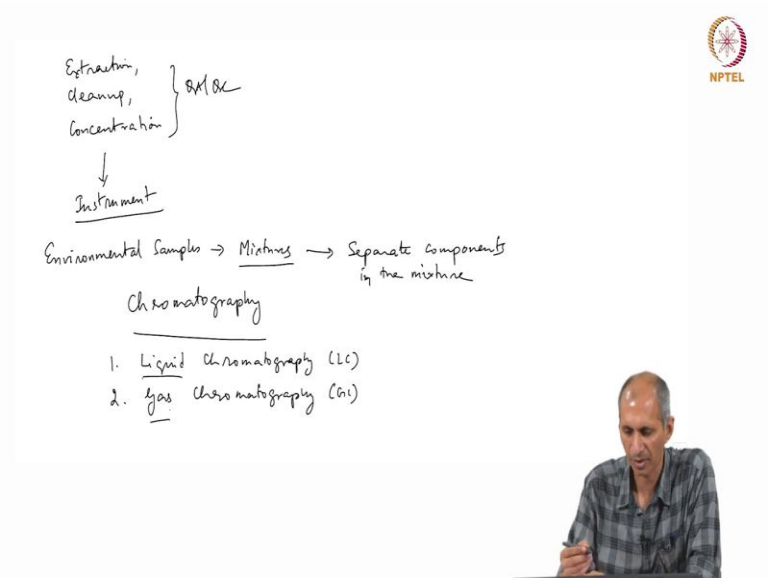

So we have talked about extraction we have talked about cleanup, we talked about concentration is usually in this sequence. And there are a lot of QA / QC issues. In this you can imagine that you are taking a sample and doing a lot of processing with a sample can go can get lost somewhere. And you can also add sample in which where you did not have a solvent that is not clean and you can get sample addition on these things. So now we go down to the instrument itself, analysis.

So we look at the different possibilities for analyzing organic chemicals. So, this is just analytical chemistry. Getting into that the reasons why most of the methods if you have environmental samples in every inevitably environmental samples, you take A sample solid some soil samples, water samples, the analyzed are usually mixtures. You do not find 1 specific compound, only 1 compound sitting in a sample of water or air it is very unlikely it is very rare.

I am not even sure if it is possible. So, they usually mixtures and you are even if you are interested, typically we are interested in multiple analyte. But even if you are interested in 1 analyte you are interested in 1 particular compound whether it is there or how much of it is there in water or air, you still have to resolve all of it. So, you have 2 separate components of the mixture and this is usually done using chromatography process of chromatography is used for doing this.

So, the chromatography itself is separation, it is not analysis it separation, or common you still need something to analyze the compound and the end of it. So we will talk about that also. So, there are different kinds of chromatography one, the oldest type of chromatography the older type of chromatography called as liquid chromatography older type is called LC, the more the later development called gas chromatography GC the word liquid or gas is very specific to one thing. So, we look at chromatography itself the process of chromatography what it is? Then we will appreciate what it means by this.

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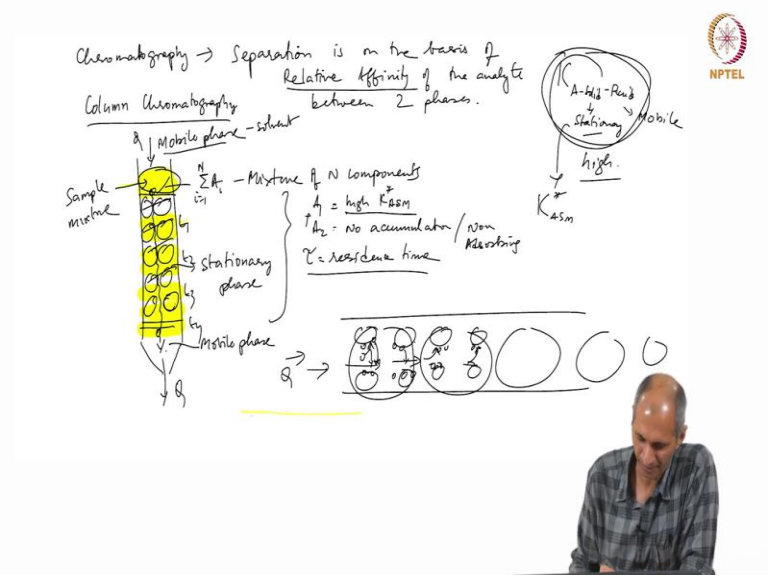

So, the chromatography the separation on the basis of like what we do what we have discussed what we have been discussing in our course right from the beginning based on relative affinity of the analyte between 2 phases. What does this mean relative affinity of an analyte in 2 phases? 2 phases means here, there are 2 phases, we are trying to separate the analyte that is there in 1 phase we are trying to separate it from that phase.

So, we have to use another phase which is not miscible with this phase, the simplest thing that we can do is you can bring it in contact with a solid and do separated like that you can also bring in contact with a liquid also do it we have done it in liquid - liquid extraction or in the SP case like that. So, if you if you bring an analyte to unseparated we are talking about later affinity. What are we really talking about? When you say relative affinity, what is a quantitative measure of that you have seen that earlier in the course.

Solubility is very specific however it is from pure substance to this thing, but in this case it is in a solution or it is in a vapor phase. So, it is the distribution of the partitioning of chemical between two phases search is a partition constant really is a partitioning is between you are really talking about KA between the solid phase between make it simple between phase 1 and phase 2. So, why do we need this relative affinity following.

So, chromatography, as we saw in the case of liquid chromatography or what is called as column chromatography, which is the older method, it was the oldest form and you have studied chromatography one way or the other from school, so, you need something called as this stationary phase. So, for convenience, so, the 2 phases it is convenient 1 phase is solid or a stationary phase and the other phase is a mobile phase is a fluid.

So, this is a solid phase it is sitting here, the easiest way a solid phase can we arrange this you have to take some solid make a backing of it there I say I am taking some solid particles putting it here. So, I can make soil itself like soil solid particle then I need a mobile phase. Mobile phase is a liquid that is flowing through this continuously. Then if I introduce a mixture of call it as Ai where it goes from 1 to N mixture of the components.

And number of analysts are there and this is a mixture here, give it color this one and we continue the mobile phase mobile phase is continuing to go through so what will happen is this this will go through this thing and appear up here to the mobile phase comes out will come out if the partitioning of a particular analyte is very high on the solid phase if K if this is K high what will happen to the analyte what has happened.

And let it will absorb onto the solid phase and it will stay there. So, let us say that it takes though the residence time of a flow mobile phase is being sent at some flow rate it is coming through. If I add mobile phase is here it will appear here at the end in some residents time tau let us not worry about how tau is always calculated for the time being or what it is we it is not important for this discussion, there are waste calculated.

The residence time is the liquid as a liquid starts here and goes through it there is a residence the liquid is not doing and liquidity is going through nicely. But say there is an analyte A1 which has high K let us call it as K ASM solid phase and mobile phase which is called as this K ASM, which star high K ASM star and there is another chemical A2 which has very low K ASM star which 1 will appear out of the column quickly.

Let us say a chemical has no accumulation it will not absorb at all no absorbing. In other words, we say that this particular analyze A2 does not cannot absorb on this stationary phase if that happens, what will be the time at which it appears at the end of the column it will be very similar to the residence time of the liquid and in the contrast side if you have a chemical which has very high this thing it will not come out for a long time.

Will it come out at all? Why will it come out? Exhaustion, very hard message completely saturated with A, but that is not useful to us so, now you have to go back to your analysis method. What are we talking about analysis in your analysis what is the sample is your sample and infinite sample or a finite sample it is a finite volume which means I only have this much this is my sample done 1 ml or 20 ml or 10 ml or whatever is you add this.

What is going to happen? Will it ever come up? It will absorb, say, say there are 2 compounds A1 and A2 or A2 comes out, it does not absorb A2 comes out, everyone is sitting inside the column in this column will it come out. So, there is a continuous flow of mobile phase, which is a solvent. Continuously this queue is going through continuously. And in the middle of that you add this little amount of your thing and you continue the flow.

So, let us look at this process again. So, you have this solid here. I am saying I am sending in some particles these are the same molecules of A that enter in the flow, the moment they enter they absorb here beginning and then it is all gone. All the A is all here now there is no A here. What is coming here it is a pure mobile phase. When it crosses this one what will happen it will leave it will dissolve.

Then go on to the next one until I absorb again here just banned a few of these chemicals fill these up and go again here because it is not an equilibrium when a pure chemical solvent comes. This is not an equilibrium with the solvent anymore. See, you have to you actually you still have to follow this partitioning. When you have when it is mixed initially it has some concentration and it partitions in and it goes in and it keeps partitioning.

Until the concentration is 0 very high partitioning here, but when you send in pure solvent, the equilibrium is reverse the drying force is now in this direction from solid to the mobile phase it will come out it will come out and then go to the next layer of the solid, but next layer of solid is empty so it will absorb there and so on. So, this process continues, it goes from here to here to here to here to here. So, this this band of yellow moves it here.

And after some time, so, this is at say at some time, T 1, T 2, T 3, T 4, it moves through. One of the things you will notice is that when it moves through it is not the width of this thing is not the same. You will see slightly expanded. It is expanded because the there are multiple chemicals that are sitting in all of them did not have the same partition constant they have they go in and come out. So give you an analogy to this say 15 of a group of people go into a shopping mall and arcade. You know what an arcade is?

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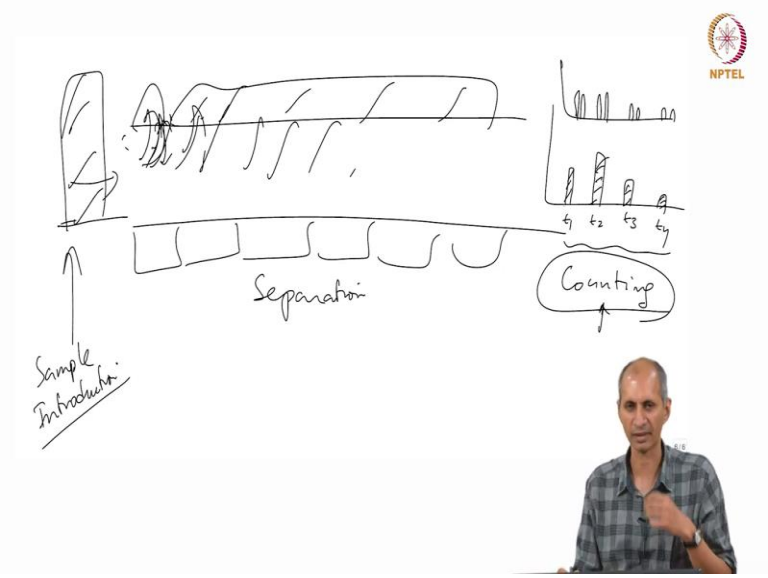

Arcade is it is an older systems where there are no malls. There used to be a long list of shops they are all shops. Let us say the entry 10 people get in here. Who will come out first? You only can go in 1 direction who will come out first. Somebody who has no interest in shopping will come out first, which means there is no affinity towards shopping they like to cut out somewhere you like to shop a lot will get in and get out of every shop and will take the longest to come out.

Some of them will have relatively lower affinity but they will still go in and come out. So you will see that based on this affinity for whatever is there on this side on the walls, you will different groups will appear at different times will be 1 group that will appear at the same standing outside, I am counting and I am counting I have a distribution. Group number 1 comes out at time T1, group number 2 comes out at time T2, group number 3 comes out at time T3 and so on before all of them go in at the same time here.

The big chunk that are going in here but there is a distribution and they come out. This is your chromatogram essentially. So what are we doing here? You are sending in a bulk and we are counting as they are coming out at different times. So we have to come how do we count there is our analytical, this is really the analytical instrument that is doing separation and this is doing the counting this is actual thing that is counting.

So, you have to find an instrument that will count whatever is coming from the counting then it will count the instrument that you use for counting is based on how the sample is it is gas sample liquid sample, what kind of organic compounds are coming based on that you can select different types of counters. So, this is different from this and there is a saw and there is a sample introduction phase that.

So, what can you how can you influence the separation here in a real sense not in this example. So, if I want to separate let us say that this group includes 2 or 3 different chemicals. If I want to further refine it, I have to find out a way in which you can separate this chromatogram can be made into smaller groups so that you have more resolution. So we talk about this in the next class.