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

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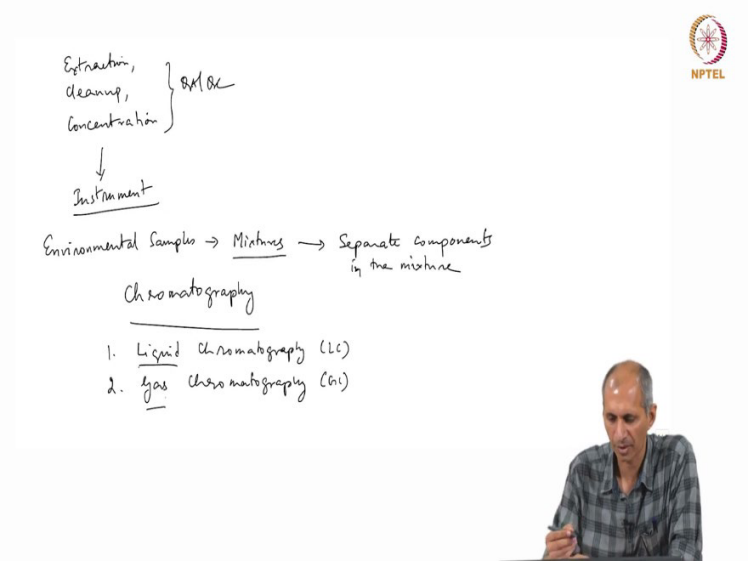


So we have talked about extraction we have talked about cleanup, we talked about concentration is usually in this sequence. And there are a lot of QA / QC issues. In this you can imagine that you are taking a sample and doing a lot of processing with it sample can go can get lost somewhere. And you can also add sample in which where you don't have we have a solvent that is not clean then you can get sample addition all 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 we are getting into that, the reasons why most of the methods if when you have environmental samples inevitably environmental samples, you take a sample solid soil samples, water samples, the analytes are usually mixtures. You do not find one specific compound, only one 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 analytes, yeah. But even if you are interested in one analyte you are interested in one 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, ok. So, you have to 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's separation, separation of compounds you still need something to analyze the compound at the end of it, ok. 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 is called as liquid chromatography older type is called as LC, the more the later development is called gas chromatography or 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 thing.

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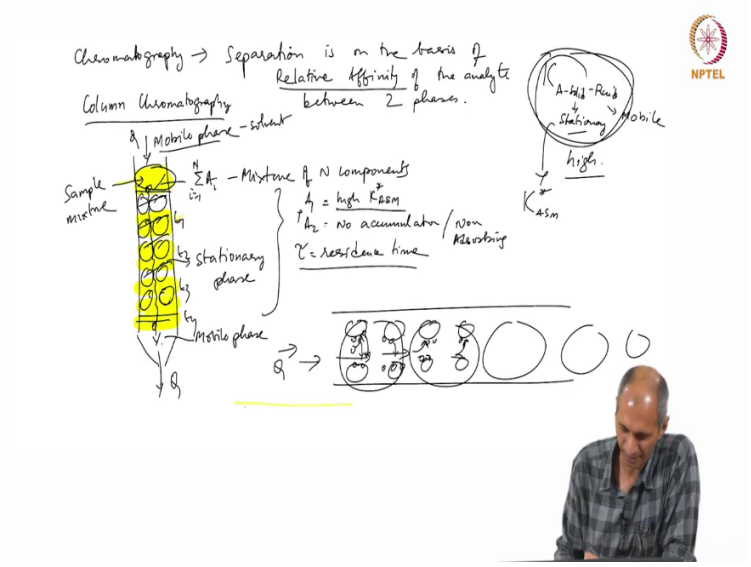


So, the chromatography the separation is on the basis of like what we do what we have discussed what we have been discussing in our course right from the beginning based on relative affinity of the analyte between 2 phases. What does this mean relative affinity of an analyte in 2 phases? 2 phases means 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 bring it in contact with a solid and do separate it 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 SPE case like that. So, if you if you bring an analyte to and separate it so we are talking about relative 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. Ah? Solubility. Solubility, can you? Is very specific solubility is from pure substance to this thing, but in this case it is it's in a solution or it is in a vapor phase. So, it is the distribution of the partitioning of chemical between two phases so it is a partition constant really is a partitioning is between we are really talking about $K_A$ between the solid phase between let's even make it simple between phase 1 and phase 2. So, why do we need this relative affinity is the 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 be arranged is you take some solid make a packing of it let us say I am taking some solid particles putting it here. So, I can make soil itself like like soil solid particle then I need a mobile phase. The mobile phase is a liquid that is flowing through this continuously, ok. Then if I introduce a mixture will call it as $A_i$ where 'i' goes from 1 to N, mixture of the components.

'N' number of analysts are there and this is a mixture here, we will give it a 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 will go through this thing and appear out here so 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 will happen to the analyte?

It will absorb onto the solid phase and it will stay there. So, let us say that it takes 'τ' residence time 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 residence time 'τ' let us not worry about how 'τ' is calculated for the time being or what it is it's not important for this discussion, there are ways to calculate it.

The residence time is the liquid as the as a liquid starts here and goes through it there is a residence time the liquid is not doing it liquid is just 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 we call this $K_{ASM}$ star with kigh high $K_{ASM}$ star and there is another chemical A2 which has very low $K_{ASM}$ star which one will appear out of the column quickly.

Let us say a chemical has no accumulation it will not adsorb at all non-adsorbing. In other words, we say that this particular analyte A2 does not it 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, ok 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?
Student: Complete exhaustion.
Professor: Exhaustion?
Student: Exhaustion of solid.
Professor: Yeah but if the exhaustion of solid means it's completely saturated with A, but that is not useful to us no 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 this you add this.

What is going to happen? Will it ever come out? It will adsorb, say, there are 2 compounds A1 and A2, all the A2 comes out, it does not adsorb A2 comes out, A1 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. There

is a Q that 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 sending in some particles, these are the same molecules of A that enter in a flow, the moment they enter, they adsorb, they adsorb here beginning and then it is all gone. All the A is all here(stationary phase) now, there is no A here(mobile phase). What is coming here, it is a pure mobile phase. When it crosses this one what will happen it will desorb it will desorb, then go on to the next one it will adsorb again here, this band a few of these chemicals fill these up and go again here because it is not in an equilibrium when a pure chemical solvent comes. This is not an equilibrium with the solvent anymore. See, you still have to follow this partitioning. 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 zero. So 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 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 band of yellow moves here and then after some time after some time after some time, so, this is at say at some time, t1, t2, t3, t4 it moves through. One of the things you will notice is that when it moves through 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 don't have the same partition constant they have they go in and come out. So give you an analogy to this say 15 a group of people go into a shopping mall an arcade. You know what an arcade is?

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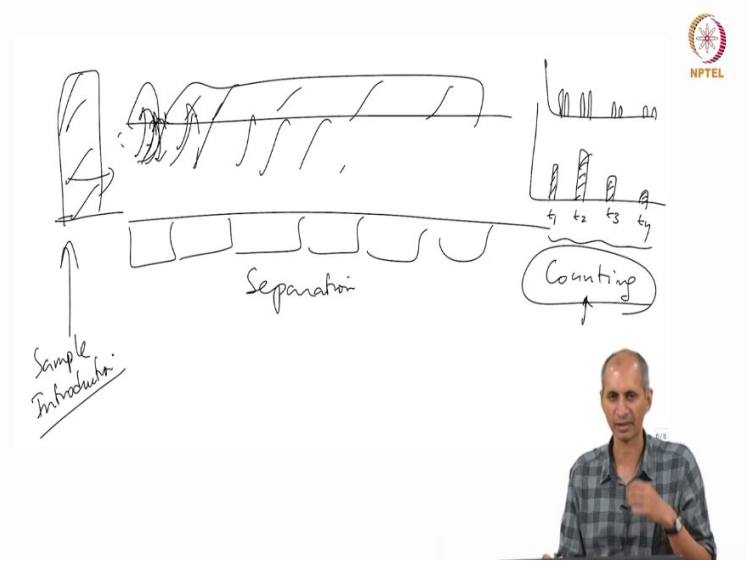


Arcade is an older system, where there are no malls, there used to be a long list of shops, they are all shops. Let us say this is entry 10 people get in here. Who will come out first? You only can go in one direction who will come out first. Somebody who has no interest in shopping will come out first, which means there is no affinity towards the shopping they like to get out. Somebody who likes 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, different groups will appear at different times. There will be one group that will appear at a particular time. If I am 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 t4 all of them go in at the same time here.

The big chunk that are going in here, yeah, but there is a distribution at which 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, ok. So we have to count how do we count that is our analytical, this is really the analytical instrument that is this 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 chromotagraphy 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 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 more. So we talk about this in the next class.