Environmental Quality:
Monitoring and Analysis
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Lecture No. 27
Analysis Methods – Liquid Chromatography

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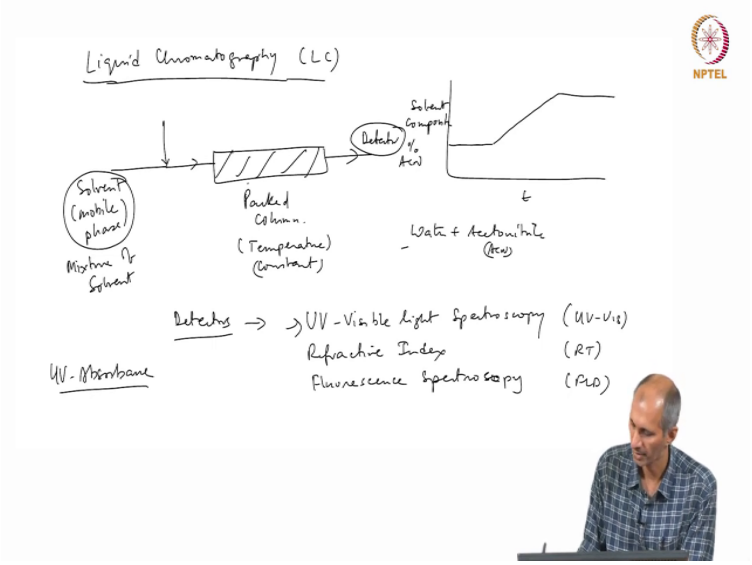

So, now is this what GC liquid chromatography is much more much simpler in operation it is just liquid there is no problem of in the class when you talked about gas chromatography one of the things is if you want to inject a gas sample gas has to be converted to vapor in that injector and the injector has to accommodate the phase transfer and all that so, you have some limitations there. Your sample has to be extracted using a compound solvent that will vaporize in the injectors to become and go along with the gas liquid chromatography the problem does not exist.

So, especially LC is used for samples where you would not want to do extraction and all that and for various reasons, 1 is the compound will get denatured or something will get point will react second, this concentration is very low. So you did not really want to do this third is some compound you cannot analyze using GCMS. GCMS is if you have it is preferred because it will remove a lot of uncertainty straightaway. But if you did not have GCMS we use FID with a lot of quality control verification and all that.

Liquid chromatography is, used in some cases, you see a lot of standard methods, many of them did not use liquid chromatography, they are all GC based methods, because the range of GC is much higher, but for some compound LC is used for you can use it so, what I am telling you is you can use liquid chromatography if the compound can be analyzed using that same system, but it is a packed column no capillary columns in liquid chromatography and the same things apply here.

The mobile phase is a solvent it can be a single solvent or a mixture of solvent. So this back column you can change the temperature but you cannot change it in the range of a GC temperature control is usually not why did not the reason we did not do it is there is a liquid inside and if one of the liquid if you increase temporarily so they will start boiling and it will vaporize and that will create a bubble and that bubbles stuck in the column is a problem flow will not occur properly.

So you want flow to occur properly because you are basing everything on retention time and retention time changes because of low non uniformity which will occur because of bubbles, you did not want all that for temperature people did not usually play around with liquid chromatography. So temperature is there, usually to maintain constant temperature that is all the oven is there as a controller for ambient temperatures may change widely.

And therefore partition constant will change that for but in order to prevent that they have a constant temperature compartment for usually around ambient, 25 to 40 degrees that is the range in which they are maintaining. What you can change is the solvent carrier gas you cannot change in gas chromatography on over here. You can change the solvent because you are pumping you have freedom to change the solvent. So you can mix mixture of solvent.

So we are in the GC you can do temperature programming here I can do solvent composition. I can change the I can start solvent composition at 1 and then I can go I can increase it dynamically this is possible solvent composition is for example, if I have 2 solvent there are

solvent A let us have water plus acetonitrile I can start this is percentage of proceeds this percentage of acetonitrile I consider a very low percentage of acetonitrile.

And I can increase the percentage of acetonitrile and then I can keep it high. So, this by doing this I can change the polarity of the mobile phase and therefore, the partition concentration will change this is possible to do typically this because you are sending a fluid flow rates are very small, it was also packed column pressure drops are very high in the system. Pressure is very high. You did not operate a very high flows because of that pressure is very high.

There is a detector here liquid chromatography is straightforward because everything goes through into the colony, nothing no, vaporization no possible losses. It is a fairly straightforward. So the detectors, what kind of detectors? Because it is a liquid you have to use something that will can analyze liquid. So the common detectors used in liquid chromatography are UV, visible spectroscopy, refractive index and its fluorescence.

So, this is normally call it UV - Vis, RI fluorescence spectroscopy. UV - Vis and fluorescence spectroscopy are to some extent they have some limited capabilities of identifying the compounds. The bases is again, just like we have mass spectra, you also have UV visible absorption spectrum. So absorbance these are all this is based on absorbance, so, the UV absorbance I am going to the next slide.

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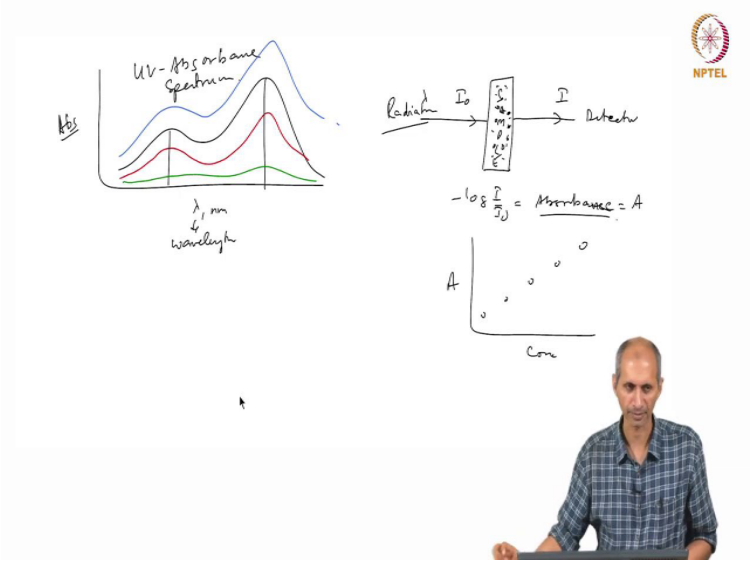

So UV absorbance like this absorbance this is a perfect combo now absorbance UV spectroscopy as a word suggests, this is your sample there is light at some wavelength, there is a light source this radiation source spectroscopy is a general basis of spectroscopy and you are you are sending it at some intensity I0 there is a molecule inside here which will absorb some fraction of the radiation and therefore, what is transmitted will be less that fraction and there is a detector here.

So, based on his minus log I / I0 is the absorbance. Basically, you calculate what is the amount absorbed by what is what is transmitted and what is the incident. And there is absorbance is a function of the concentration or amount of you have high concentration of a particular compound it means that there are a large number of molecules in this fixed volume and therefore, a large amount of absorption.

So, if you calibrate the absorption will be a function of the concentration, higher the number of higher concentration higher will be absorbed, this is absorbance this is molecular spectroscopy UV absorbance there is no fragmentation or anything it the entire molecule absorbs some if you go deep into it some sections of the molecule is actually absorbing more than the other outlet is there, but we are not looking at that.

So, if you look at the absorbance spectra this is absorbance spectra you can see some spectrum like this which means that it is absorbing at certain wavelengths is absorbing more so, this is a

UV absorbance spectrum lambda in nanometers is a wavelength. So at 1 particular composition, 1 particular concentration, this molecule will ensure this up, it will show the same spectrum is a concentrations smaller or higher.

So, there is a point where it can go all the way here. You cannot do anything. I will stop here. We will continue tomorrow morning. We will try to finish up this and talk about other methods of analysis of the metals and then we will get one more topic after that.