Quantitative Methods in Chemistry
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Lecture-44
Practice of Chromatography-Gas Chromatography

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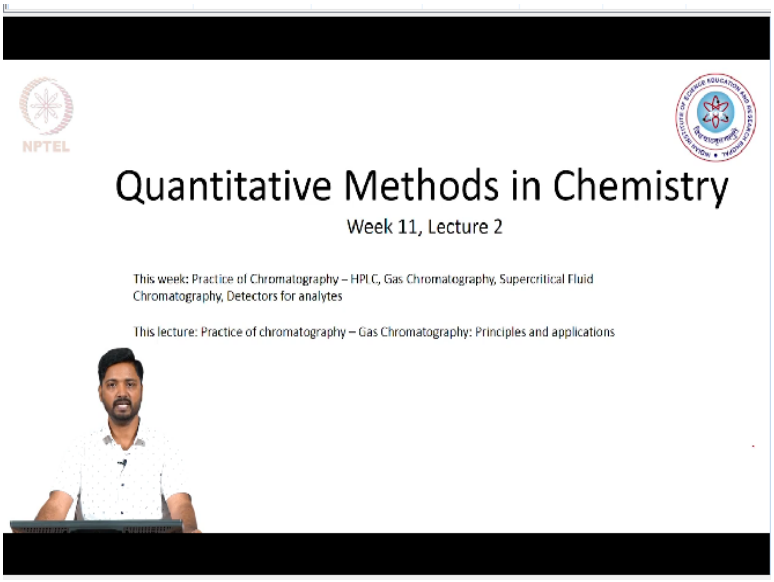

Hello and welcome back to this course quantitative methods in chemistry, we are in week 11. And this week our emphasis has been on understanding practical implications of undertaking chromatographic separations, previous lecture of this week talked about the high performance liquid chromatography and this lecture will be mainly dedicated to the practice of gas chromatography.

We will be understanding the principles and applications of it and we will also be contrasting it to the high performance liquid chromatography to understand what are the key differences between these 2 techniques and for which kind of systems will gas chromatography be applied. So, let us get started with this week's lecture 2.

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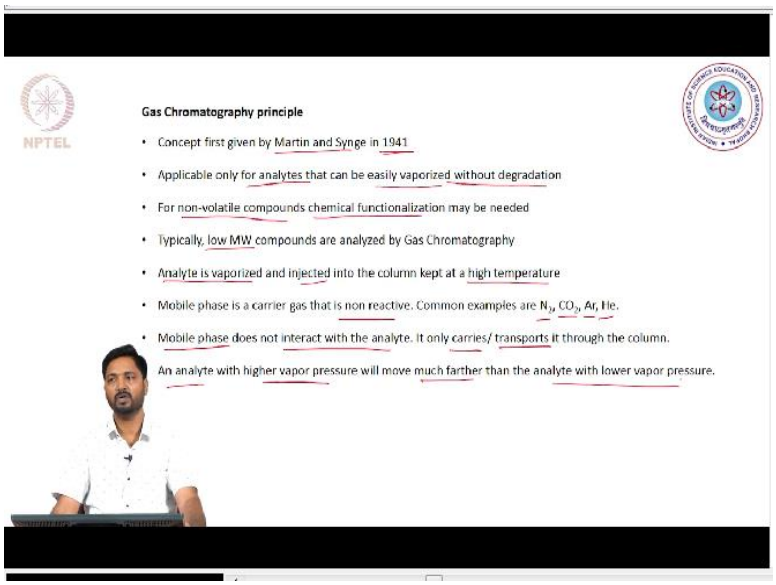

And let us first have sort of a brief introduction about gas chromatography. So, chromatographic techniques as we have been studying are mainly developed through the efforts and the theoretical framework that were developed by Martin and Synge. So, they propose the concept of gas chromatography in 1941. It was figured out was that gas chromatography will require the analyte to be vaporized.

So, it will be applicable only for analytes that can be easily vaporized without undergoing degradation. So, essentially we will be having to heat our solute or analyte converted into a gaseous phase and move it through chromatographic column. So, what needs to be done for non volatile compounds and we will see a very interesting example of this subsequently in this lecture is that we need to undertake some sort of chemical functionalization or derivatization.

So, that the final product of this derivatization has a higher vapor pressure or is easy to vaporize. Now, given this requirement for gas chromatography, it is not surprising that gas chromatography will be applicable only to molecules that have low molecular weight and these are the main systems or analytes that are analyzed in gas chromatography. So, indeed gas chromatography is applied to large number of small molecules that are synthesized in organic chemistry laboratories.

And here what we need to do is to vaporize the analyte and inject it into a column that is kept at high temperature. So, this is again an important requirement here in terms of the practical consideration that our analyte will be passing through a column that is kept at a high temperature and to push the analyte through the chromatographic column and the chromatographic system, we use and we require certain carrier gases, the gases that will act as the mobile phase in gas chromatography.

And typical examples of such carrier gases which need to be nonreactive are nitrogen, helium, argon or even carbon dioxide. So, these gases, they are essential role is to sort of push the analyte through the chromatographic system and during this process the separation of the analyte from the impurities or other analytes happens. Now, it is very important to realize a key difference between the liquid chromatography that we discussed in the previous lecture and gas chromatography.

Indeed gas chromatography in this sense is quite different from all the other chromatographic techniques, in that the mobile phase in gas chromatography does not react or interact with the analyte. Since, the mobile phase itself is gaseous; it is not dense enough to have a particular interaction with analyte of our interest. Moreover, the gaseous mobile phase is also non reactive and often non polar.

So, it usually will have no particular interaction with the analyte and this is a very, very important the key difference between the gas chromatography and the other chromatographic techniques, the function of mobile phase is to simply carry the analyte through the column or transported from one end of the column to the other end, sort of pushing it through the column and eluting it out of the column and moving it to the detector.

That is the only role that the mobile phase in gas chromatography has. So, as we can think that we require our analyte to be in a gaseous phase, so, an analyte that has higher vapor pressure will move further than analyte with lower vapor pressure and this becomes a sort of discriminating factor for us, that helps us purify or separate analytes based on the vapor pressure that they have

at a particular temperature or the conditions in which the gas chromatography is being undertaken.

So, I hope that this gives you a very clear flare of or the analytes that you can use for gas chromatography and how gas chromatography is indeed quite different from other chromatographic techniques.

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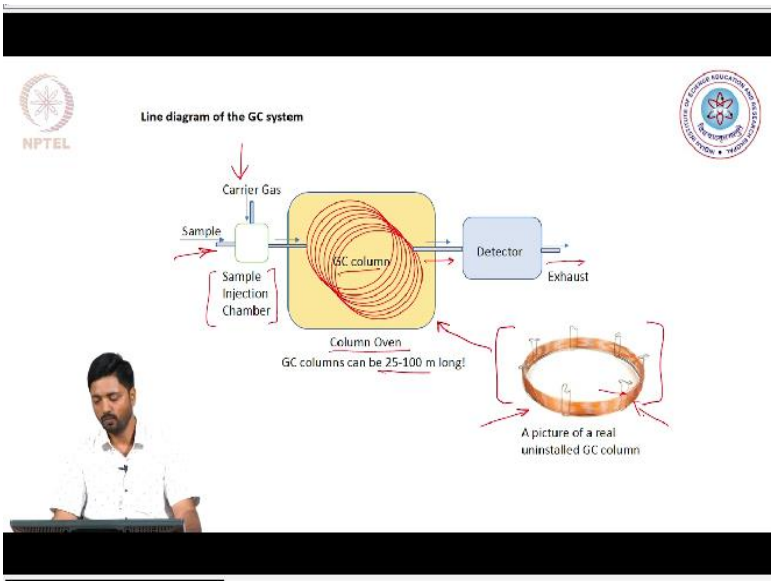

Now, let us move forward and see a very basic line diagram of a gas chromatography system. So, we inject our sample from one side of this chromatographic system and we also pass in the carrier gas into chromatographic system, these 2 mix in what is known as the sample injection chamber. So, the vaporize sample or the sample vapors and the carrier gas mix together in sample injection chamber.

They are then carried to a gas chromatography column which is kept inside a column oven and this is where the separation will happen and finally, the analytes will come out of this column and move to the detector where their presence will be detected and a chromatographic profile generated. Finally, all these gases and vapors will come out of the gas chromatography system in form of an exhaust.

Now, it is interesting and important to note that the gas chromatography columns can be really, really long. So, for example, it is quite common to have chromatographic columns that are 25 to 100 meters long. So, this is for example, a picture of a real uninstalled gas chromatography column. And as you can see that it is this fine tube, which is wrapped into a circular fashion and is really, really long and thin.

And this whole structure is now kept inside the column oven, where its temperature is maintained or we can also have a change in the temperature as the chromatography proceeds.

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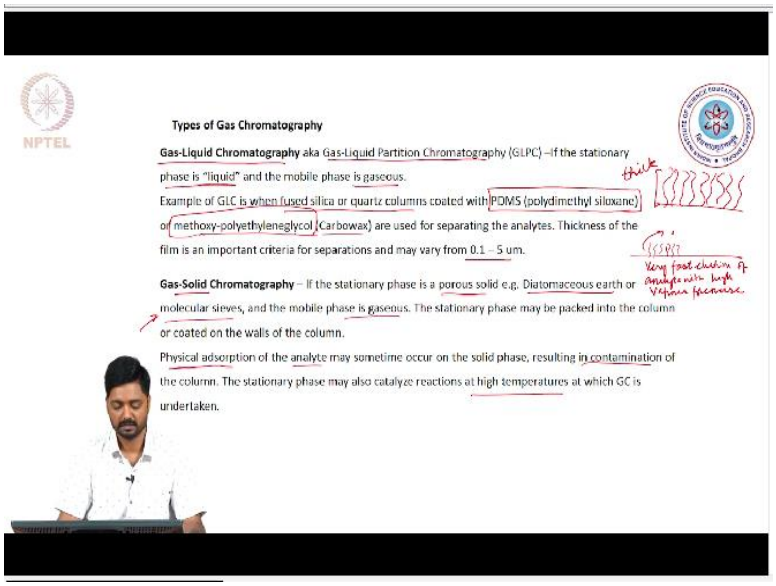

So, let us look at the 2 types of gas chromatography that have been developed and this differentiation in the types of gas chromatography are based on what is our stationary phase going to be in the column that we just saw. So, in this column, we would have filled a stationary phase and that will dictate what type of chromatography we will call it.

So, for example, in the gas liquid chromatography, whose most appropriate name should be gas liquid partition chromatography, because our solute gets partitioned between the liquid phase which is acting as the stationary phase and the gas which is the mobile phase or the carrier gas which is drives the analyte through the chromatographic column. So, this gas liquid partition chromatography term will be used in case the stationary phase is liquid and the mobile phase is gaseous.

So, an example of this gas liquid partition chromatography is when we have few silica or quartz columns, which are coated inside with polydimethyl siloxane or methoxy-polyethylene glycols of high molecular weight whose trade name is Carbowax. And these are compounds or coatings which have sort of semisolid or fluid nature; and that is why they are known as the liquid stationary phases.

And also please note that this is again a key difference between our HPLC system and the gas chromatography system in that in the GC or the gas chromatography, we can have a stationary phase which is more liquid like. So, the thickness of this film is a very important point to be kept in mind while undertaking the separations and indeed commercial columns with thickness variation of 0.1 to 5 microns are available.

So, how do we or how do we understand the impact of the thickness of the liquid stationary film present in the gas liquid chromatography, let us try to spend a little bit time on that. Suppose we have a column which has a thin layer of the liquid stationary phase and that is contrasted with a column that has a large or thick stationary phase thickness. So, if we partition our analyte through these 2 columns, the analyte with high vapor pressure will keep coming out of the column which has a thin layer of the stationary phase and hence will elute out fast.

So, very fast elution of analyte with high vapor pressure will be observed in a chromatographic column, which has a thin liquid stationary phase for such compounds, which have very high vapor pressure columns with thicker stationary phase are more preferred, because they will allow the analyte to get partitioned into the liquid stationary phase more often and the analyze will spend more time on the stationary phase compared to the previous column with a thinner stationary phase.

And as a result, we will achieve a more effective separation of such analytes. Now, a contrasting technique is when we have a solid stationary phase and of course, it is quite obvious and common to have stationary phases that will be solid in nature. They are typically porous solids

for example, diatomaceous earth or molecular slaves are coated inside the capillary columns and the mobile phase as expected is gaseous.

So, here the partitioning of the solute or analyte is happening between the solid support or the solid phase which is compromised of either molecular slaves or diatomaceous earth or alumina or silica or any such sort of a porous material and the mobile phase which is the carrier gas. So, a major challenge or limitation while using gas solid chromatography has been that often these porous materials act to physically absorb the analyte onto them.

And as a result, the columns undergo contamination after repeated use, and they will no longer be able to give the requisite resolutions as time progresses. Another key consideration with regards to having the solid stationary phase is that many of these compounds are inherently catalytic, especially at the high temperatures at which gas chromatography is undertaken. So, we need to keep this aspect in mind when we are choosing a column for our chromatographic separations.

Please note that the previous examples of having polydimethyl siloxane or methoxy polyethylene dye call for gas liquid separations carefully chose these stationary materials because of their inherent inertness.

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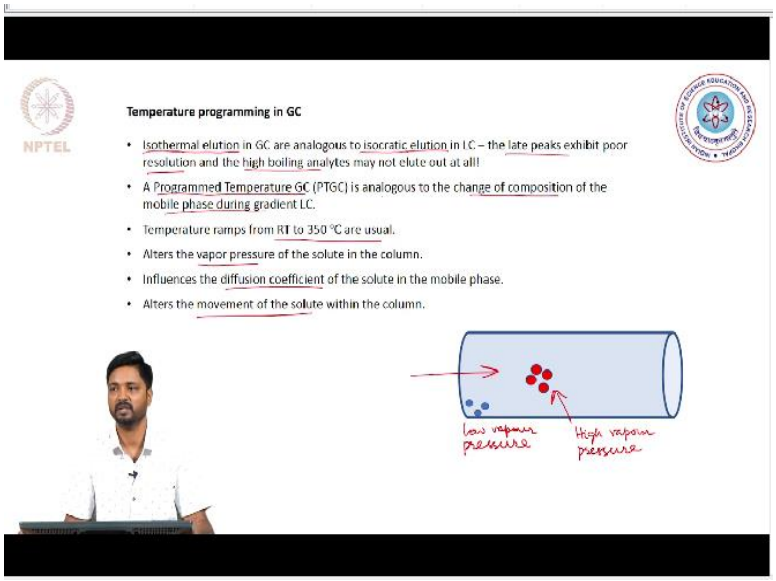

Now, let us talk about the temperature programming in gas liquid chromatography. As we can expect that an isothermal elution that is a elution that is undertaken at a constant temperature of the column oven is analogous to what is considered as the isocratic elution in liquid chromatography. So, the vapor pressure of the carrier gas and then the analyte does not change in this kind of elution in the gas chromatography.

And sort of challenge or a drawback for isothermal elution in gas chromatography is that the late peaks the peaks that come out much later from the column exhibit poor resolution and of course, high boiling analytes may often not elute at all, if we have not chosen the right temperature for elution. So, for such challenges are programmed temperature gas chromatography, which is abbreviated as PTGC is utilized.

And this program temperature gas chromatography is actually analogous to the change in composition of the mobile phase during gradient liquid chromatography. So, in gradient liquid chromatography, we change the composition of our eluent or the mobile phase with time. Similarly, here, by changing the temperature, we are changing the vapor phase of or the vapor pressure of our analyte.

So, practically it is quite common to have temperature ramps from room temperature to 350 degrees Celsius. So, as you can see that we are talking about really high temperatures, and this needs to be kept in mind with regards to the stability of the analyte that is being passed through the gas chromatography column. And as already mentioned that the change in temperature alters the vapor pressure of the solute inside the column.

And allows it to elute out faster, it also influences the diffusion coefficient of the solute in the mobile phase. So, at higher temperatures, the diffusion coefficient increases that means, the analyte will be able to diffuse much faster into the mobile phase and again as a result elute out much faster. Finally, all of this results in the alteration in the movement of solute within the column.

So, this is in fact, schematically shown here, where we are passing our analyte through this column from the left and analyte with low vapor pressure such as the blue dots here will remain stuck closer to the walls of the capillary while the analyte red, which has high vapor pressure will be more diffusing into the mobile phase and hence will be moving much faster through the chromatographic column in gas chromatography.

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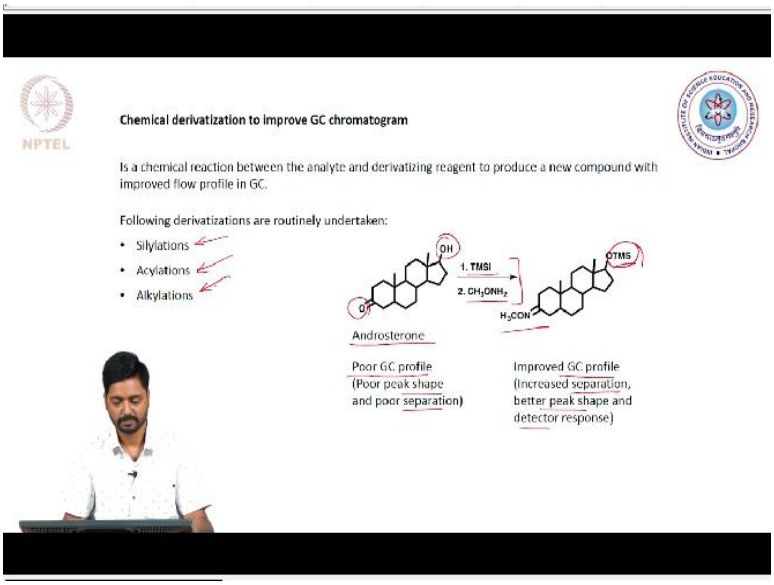

Now, let me give you a quick example of how chemical derivatization is utilized to improve the gas chromatography. So, here I have taken an example of androsterone which is a steroid present in our bodies. And this compound inherently has poor gas chromatographic profile and it does has poor peak shape and poor separation in gas chromatography but as you can see that this is a decently large molecule in terms of its molecular weight.

And as again as a result it has a low vapor pressure. So it is elution from the gas chromatography column is indeed difficult. And this can be overcome by undertaking chemical derivatization of this compound. And we use trimethylsilyl imidazole, which is abbreviated as TMSI and this methoxy amine to derivatize the hydroxyl residue and the ketone residue present in this molecule.

So, we as a result of this derivatization, we attach the trimethylsilyl residue on to the hydroxyl group and similarly, we make an oxime out of the ketone residue. And this new compound,

which is readily formed by using the reagents TMSI and methoxy amine will give much improved gas chromatographic profile that means, increase separation, better peak shape and improved detector response.

So, typically it has been observed that silylations for example, this trimethylsilyl group that we added here silylations and alkylations are very common derivatization protocols, that are utilized by chemists to improve the gas chromatography profile of the analyte of interest.

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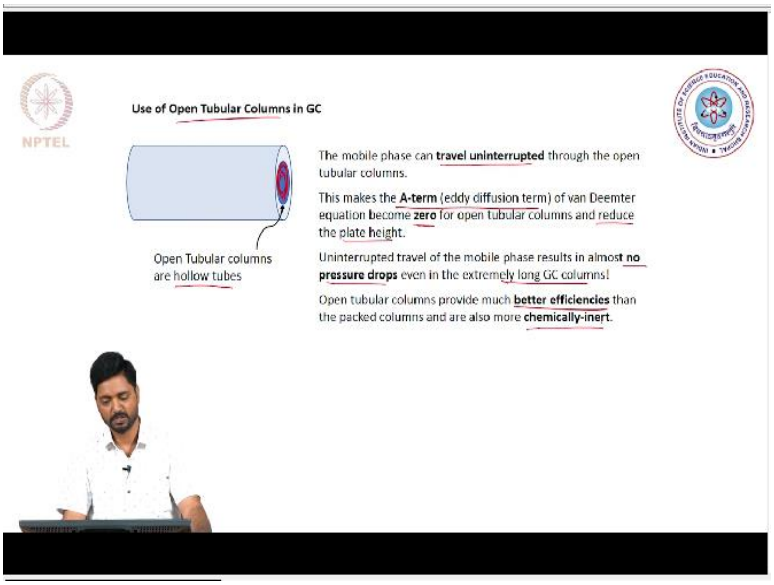

Now, let us talk about an important aspect which is the use of open tubular columns in gas chromatography. Now, this again is in clear contrast with the liquid chromatography systems in that in liquid chromatography systems, we have a solid stationary phase or a gel type stationary phase in case of gel filtration chromatography or size exclusion chromatography. And the stationary phase is very tightly packed into the column.

While in the gas chromatography, we have columns that are often hollow tubes. So, we have a hollow structure inside this column through which the carrier gas can travel uninterrupted. So in these open tubular columns the mobile phase travels uninterrupted. And that is very advantages because it makes the A-term of the, which is also known as the eddy diffusion term of the van Deemter equation to become zero.

And this results in significant reduction of the plate height. Moreover, because of this hollow nature of these columns, there is almost no pressure drops, even when we use extremely long gas chromatography columns. And this indeed allows us to use really, really long columns such as a 100 meter column, which can never be realized in liquid chromatography systems. Now, of course, the result of having smaller plate height to do to almost zero A-term is that we achieve much better efficiencies.

And we also improve the chemical inertness or reduce the reactivity of the stationary phase with the analyte that is moving through the column. So, this has indeed resulted in open tubular columns becoming the most useful or the most widely used type of columns in gas chromatography systems.

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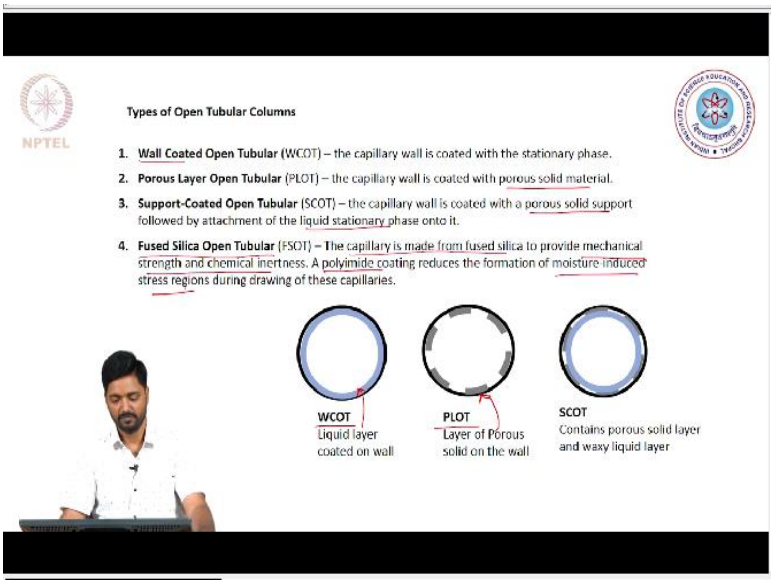

Now, people have also designed a wide variety of open tubular columns. For example, one can have a wall coated open tubular which is abbreviated as WCOT W part and this has a liquid stationary phase, which is indicated in this blue circle. And this stationary phase is coated on the inner wall of the capillary. The contrasting system is what is known as the porous layer open tubular or PLOT columns, which has a porous solid material which is indicated by these gray structures coating the inner walls of the open tubular column or the capillary that is being used as a chromatography column.

And interesting interplay of these 2 is what is known as the support coated open tubular, here we utilize both a porous solid support and a liquid station phase which is attached on to this porous solid support to create interesting chromatography column which now is called the support coated open tubular, because the liquid stationary phase is coated on the porous solid support and revised version of open tubular column is what is known as the fused silica open tubular or FSOT.

This is nothing but if the capillary that is used for the gas chromatography column is made from few silica which provides better mechanical strength and chemical inertness compared to the metal columns that can also be made and important technical requirement for creating really long and flexible silica tubes or capillaries is that when the capillary is drawn, it is immediately coated with a polyimide coating, which reduces the formation of moisture induced stress regions in the capillary and prolongs its shelf life.

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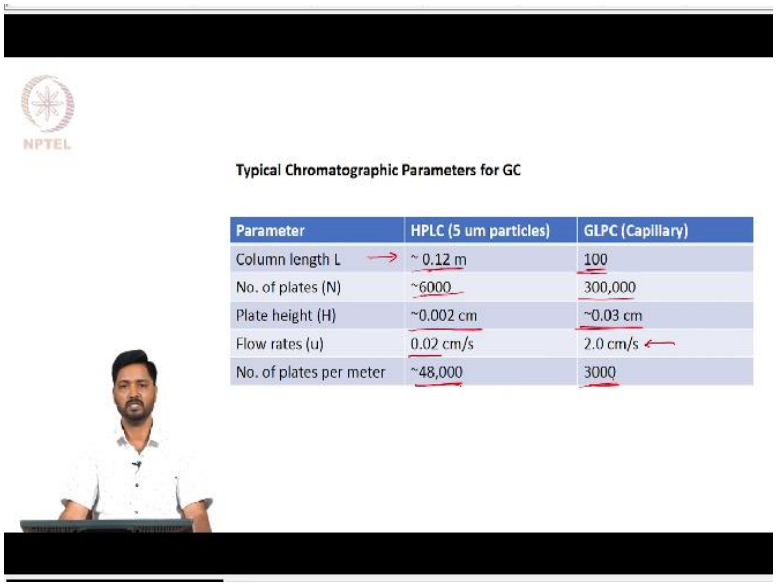

Let us look at what are the typical parameters in gas chromatography and sort of contrast it with the high performance liquid chromatography which we discussed in the previous lecture. So, the column lengths in these 2 techniques are widely different. In high performance liquid chromatography we use much shorter columns compared to gas liquid partition chromatography, organic gas chromatography where the column lengths of 200 meters are available commercially.

Now, this results in very different number of plates that can be present in these 2 columns. In HPLC, we can expect number of plates in the range of a few thousands, say up to 6000. But in gas chromatography this number can reach up to few lakhs. For example, in 100 meter column, many of the analytes will have a theoretical number of plates equal to 300,000 or a number similar in a similar range.

Now, the plate height which indicates the efficiency of separation is of course, much shorter in the liquid chromatography system, because the mobile phase is a dense phase. So, the longitudinal diffusion is avoided in liquid chromatography and the shorter column also reduces the plate height. In comparison, in gas chromatography, the plate height is somewhat larger, but that is well compensated by using columns that are extremely long.

The flow rates are also pretty different between these 2 in liquid chromatography because of the high density of the mobile phase. Our flow rates are restricted to about 0.2 centimeters per second, while in gas liquid partition chromatography, flow rates of up to 2 to 5 centimeters per second are often used. Now, we can derive an interesting parameter which we call as number of plates per meter of the column.

Although that is sort of a number that we just scratch out, it has no real meaning, because the column lengths are very different. However, when we do this, what we find out that the liquid chromatography systems have much larger number of plates per meter compared to the gas chromatography systems. Nonetheless, as mentioned earlier, the column length in the liquid chromatography systems are restricted.

And this is where the gas chromatography systems sort of take advantage of utilizing long columns for achieving efficient separations. So, with this, we come to an end of this lecture. And we have seen in this lecture, how gas chromatography contrasts with the liquid chromatography, which is more often use the gas chromatography systems are mainly limited to volatile compounds.

However, there are a large number of small organic molecules and biological metabolites for which gas chromatography can be readily used. In the next lecture, we will focus on supercritical fluids for their use in chromatographic separations. Thank you.