Quantitative Methods in Chemistry
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Lecture-39
Basics Chromatography Part 01

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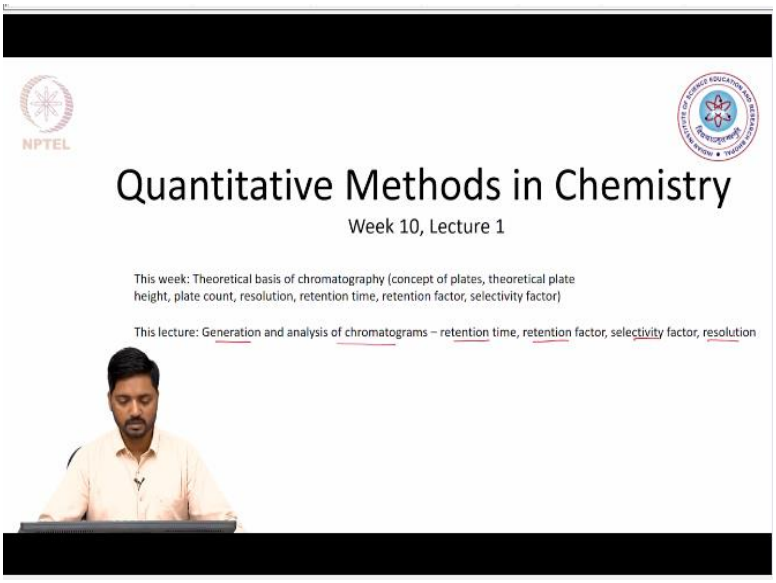

Hello and welcome back to this course quantitative methods in chemistry. We have reached week 10 of this course and this is lecture 1 for week 10. And this week our focus would be on establishing theoretical basis of chromatography. We will be understanding terms like the theoretical plates present in column and the plate height, the concept of plate count within a column, the resolution of the peaks, retention times, retention factor and selectivity factor.

So, this part of the lecture will focus on the generation and analysis of chromatograms. And we will get an understanding of what do we imply by retention time, retention factor, selectivity factor and resolution of the peaks. So, let us get started with this lecture.

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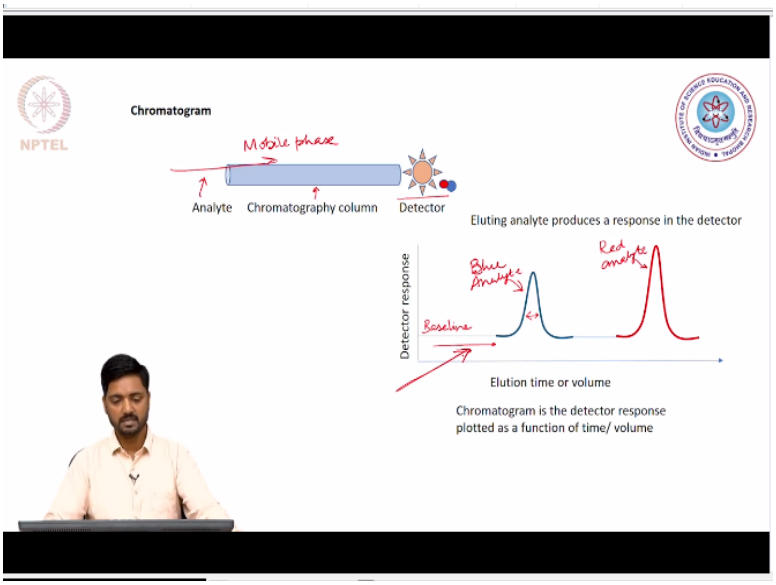

So, when we have a chromatography column which is shown here, and we pass the analytes So, here I have demonstrated 2 analytes, one with red, one with blue and we have a detector at the end of the chromatography column to detect the elution of the analyte. So, as we pump in the mobile phase, the analytes pass through this column. However, different analytes spend different time within the column and that generates a chromatographic profile.

So, for example, here we see that the blue analyte came out first and quite ahead of the red analyte, the red analyte on the contrary, spent much longer time within the column and hence eluted out later. So, if we are seeing the detector response with respect to the elution time, we will find a profile which is shown at the bottom, we will see that the detector response will be what is known as a baseline at the beginning.

Where only the mobile phases contributing to the minimal signal that may be generated, usually the baseline is set to 0. What we observe here is that as time progresses initially the blue analyte will come out and that will generate the signal which is indicated in the blue. So, this is coming from the blue analyte. And, as further time progresses, we see the signal being generated due to the elution out of the red analyte.

Also note that there is a certain width that is associated with this peak and we will consider these aspects as we move along. So, essentially a chromatogram which is shown in this profile here is a detector response, which is plotted as a function of time. Now, we will slowly progress on how this chromatogram can be analyzed.

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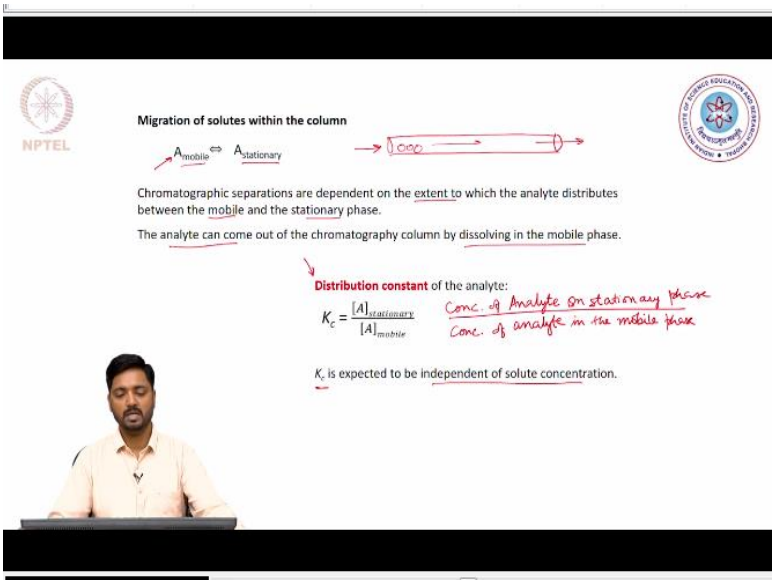

So, let us first understand the migration of the solutes within the column. So, if we consider ours solute or analyte A it will spend certain time in the mobile phase or will be equilibrating between the mobile phase and the stationary phase. So, the mobile phase will be moving the analyte out of the column, while the stationary phase will keep the analyte within the column. So, there is an equilibration that is considered to be happening here between the mobile phase and the stationary phase with regards to the analyte that is being eluted out.

So, the chromatographic separations in nutshell depend upon the extent to which the analyte distributes between the mobile phase and the stationary phase. So, for example, if an analyte is spending more time in the mobile phase, then it will elute out fast. However, if the analyte is spending more time on the stationary phase, so, it will stay longer within the column and will elute out much slower.

So, also it is important to understand that the analyte can come out of the chromatography column by dissolving in the mobile phase. So, the better it can dissolve the faster it can elute out. So, we can describe a distribution constant with regards to our analyte and this distribution constant is denoted by capital K and C and that is nothing but the concentration of the analyte on the stationary phase divided by the concentration of the analyte in the mobile phase.

So, this essentially tells us how the analyte gets distributed between the 2 phases present in a chromatographic column. And what is also expected is that this K c value of distribution constant is independent of the solute concentration. So, essentially what we are trying to say

here is that, irrespective of how much solute is present within the column, the distribution constant remains in variable and is not changing with the solid concentration.

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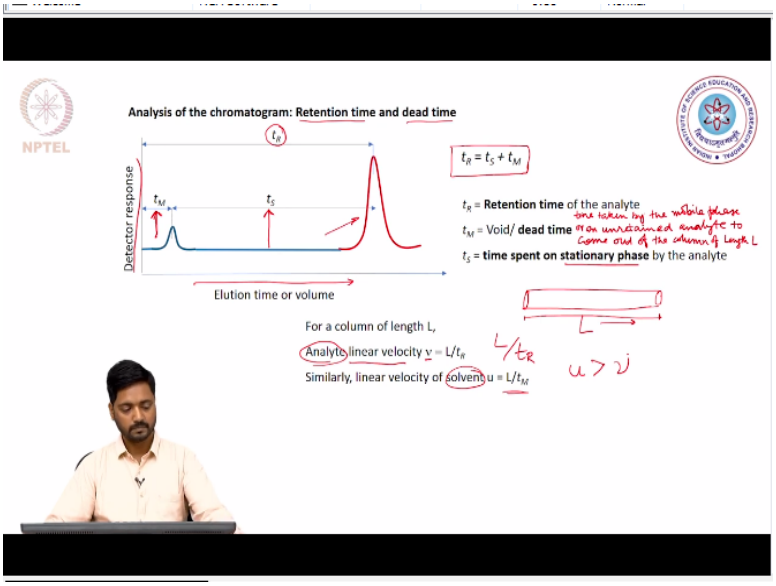

Now, let us see how chromatographic profiles are analyzed. So, here we will be talking about 2 terms which are known as the retention time and the dead time or the void time. Now, both of these terms are very relevant in terms of chromatography. And they provide us important information with regards to the system that we are dealing with. So here, for example, we have again plotted the detector response with regards to the elution time or the volume of the mobile phase that elutes out of the column.

So, we can have 2 scenarios here. One is where we have an analyte or solute, which is not at all retained in the column and it moves with the mobile phase. So, for a column of length L, there would be a finite time that would be taken for the mobile phase to move out of this column or to travel the length of this column. So, this time is denoted by t mobile or t M, and is the time that the mobile phase takes to come out of the chromatography column.

Now, if we have an analyte, which is retained within the column, then this analyte obviously spends more time within the column and is eluting out much later and that is shown here through the red peak. And we can now define a retention time, which is the time that this solute takes to traverse the length of the chromatographic column and reach the detector. So, essentially, this retention time t R is composed of 2 components.

One, of course, is the time that the mobile phase takes to come out of the column, which is the t M. And there is an additional time that our solute is spending inside the column, which is known as the T s or the time that the analyte is spending on the stationary phase. So, we define the retention time as the sum of the time the analyte spent on the stationary phase plus the time that is denoted as the dead time or the void time.

Now, we already described the dead time **or** or the void time by explaining that it is the time that an analyte which is not retained is the time taken by the mobile phase or an unreturned analyte to come out of the column of length L. So, obviously, the longer the column, the more time the analyte will take to come out, or the more would be it is dead time and we can also define what are known as the linear velocities of our analyte.

And of the solvent and they are described by the term nu for the analyte and u for the solvent. So we have the linear velocity for the analyte being described as the length of the column divided by the retention time of the analyte. So this describes the average linear velocity of the analyte. Similarly, for the solvent, we have the linear velocity being described by the length of the column divided by the time taken by the mobile phase to come out of the column or the dead time or the void time.

So, of course, the u value is much greater than the nu value. And that essentially tells us that the solute or the analyte that is retained within the column is moving with a much slower velocity.

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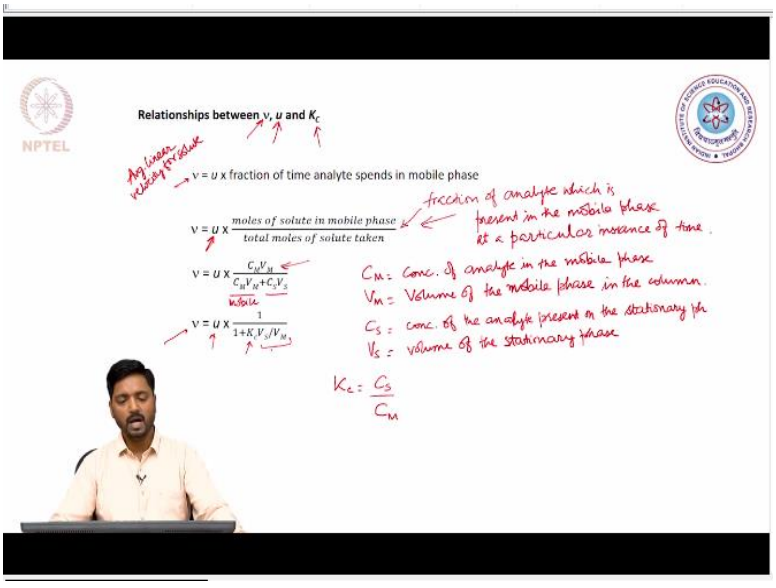

So, let us understand now in this slide, what are the relationships between the linear velocity for the solute or the analyte, the linear velocity for the mobile phase and the distribution constant, K c. So, what we can think of is that the average linear velocity for the solute which is described by nu is equal to the linear velocity for the solvent multiplied by the fraction of time the analyte spends on the mobile phase.

So, if the analyte is spending more time on the mobile phase, then it will elute out much faster and will have much higher average linear velocity. On the contrary, if our analyte is spending much longer time on the stationary phase, or is getting stuck within the column, then the time fraction that this analyte is spending on the mobile phase is much shorter, or it is average lenient velocity will be much lower.

So, this is described in the next equation shown here, where we have said that the average velocity for the solute is nothing but the velocity of the solvent, which is given by u multiplied by the moles of the solute in the mobile phase divided by total moles of solute that was present in the column. This term on the right is essentially a fraction of analyte which is present in the mobile phase at a particular instance of time.

So this can now be described in terms of the concentration of the analyte in the mobile phase multiplied by the volume of the mobile phase in the column and this value, which is the numerator here is divided by the total amount of the solute present in the column which is nothing but the amount of solute present in the mobile phase, which is denoted by C m into V m. And to that we add the amount of the solute, which is present in the stationary phase.

So, C s is the concentration of the analyte present on the stationary phase and V s now becomes of the stationary phase present in the chromatography column. Now, we have defined the distribution constant K c as equal to the concentration of the analyte in the stationary phase divided by the concentration of analyte in the mobile phase. So, we can write the average linear velocity for the solute in terms of the average linear velocity of the solvent multiplied by the distribution constant, K c. And the volumes of the stationary and mobile phases present in the column.

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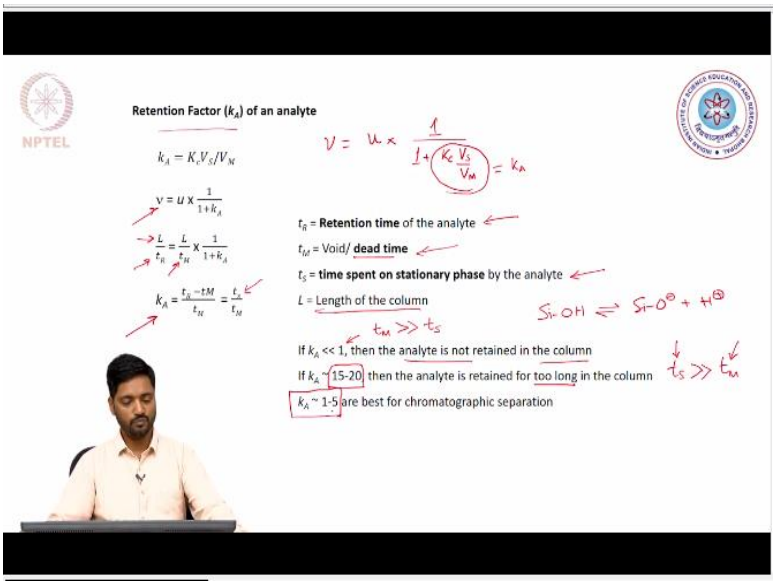

Now, let us move ahead and try to understand what do we imply by the term retention factor. Now, in the previous example, or the previous equation, where we had the average linear velocity for the solute being equal to the linear velocity of the solvent or the mobile phase into 1 by 1 plus K c which is the distribution constant into the volume of the stationary phase divided by volume of the mobile phase.

So, this term which is now encircled in the equation can be equated as small k a, which is being described as the retention factor. Now, there is a small k a can replace the term K c into V s by V m and we get a different equation for the average velocity of the solute in the chromatography column. Now this everything can be written in terms of the length of the column L, and the retention time taken by the analyte as well as the void time, which is the time taken by the mobile phase to elute out.

So the equation boils down to L by t R, which is the velocity of analyte is equal to L by t M, which is now the velocity of the mobile phase multiplied by 1 by 1 plus k A where k A now is our retention factor. When we rearrange all of this, we get the equation which is shown here, which now allows us to calculate or measure the retention factor based on the chromatogram that we have generated.

Because the information related to t R, which is the retention time of the analyte t M, which is the void time or the dead time for the column as well as the T s, which is the time spent by the analyte on the stationary phase. can all be figured out from chromatogram and the length of the column is usually known when we are undertaking chromatographic separation. So, the

retention factor essentially boils down to the fraction of time the analyte spends on the stationary phase divided by the time spent by the analyte on the mobile phase.

So, this fraction if it is much less than 1, then we are implying that the analyte is spending much more time on the mobile phase or is eluting out with the mobile phase or in other words k A value or a retention factor, which is much less than 1 indicates that our analyte is not being retained in the column. So, it is coming out almost along with the mobile phase and the time that it is spending inside the column is very close to the void time or the dead time.

So, on the contrary, if the K value or the retention factor is in the range of 15 to 20, then what we are implying is that the time spent by the analyte on the stationary phase, which is denoted by T s is much larger than the dead time or the void time of the column. And the analyte is staying too long within the column. So, the best scenario for us is when the retention factor is in the range of 1 to 5, and this is where the best chromatographic separation can be achieved.

Now, it is important also to note that a large K value of say 15 to 20 indicates that the analyte is a spending long times inside the column. This is detrimental not only to the chromatographic separation that we may want to achieve. But can also result in the degradation of the analyte because the analyte can start reacting within the column the silica that is typically used in our chromatographic columns is considered to be slightly acidic.

We saw yesterday that it has this silanol residues that means silicon OH bonds, which can be a source for protons at the appropriate pH and these protons can now catalyze certain reactions, which may result in degradation of our compound. So, indeed, it is in the best of our interest to have the k values which are intermediate that is in the range of 1 to 5.