Quantitative Methods in Chemistry

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Lecture-41
Chromatography-Concept of Theoretical Plates

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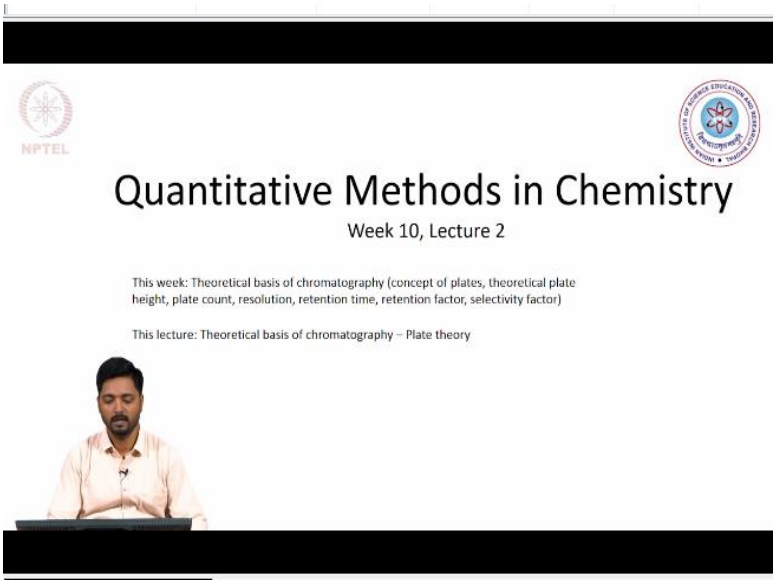

Hello and welcome back to lecture 2 of week 10 of this course quantitative methods in chemistry. This week we are focusing on understanding how chromatography works and we are exploring the theoretical basis of chromatography. We already got introduced to the concepts of resolution, retention time, retention factor and selectivity factor in our first lecture.

This lecture we will be focusing on the plate theory, which is the theoretical basis of understanding how chromatography work. So, we will get introduced to the concept of theoretical plates, we will know how we calculate what is known as the plate height of a column, the plate count and subsequently how we measure the resolutions based on these. We will also in this lecture get reintroduced to the original Gaussian error curve that we studied earlier in this course.

And we will see how this makes reentry in analyzing chromatographic profiles. So, let us begin this lecture 2.

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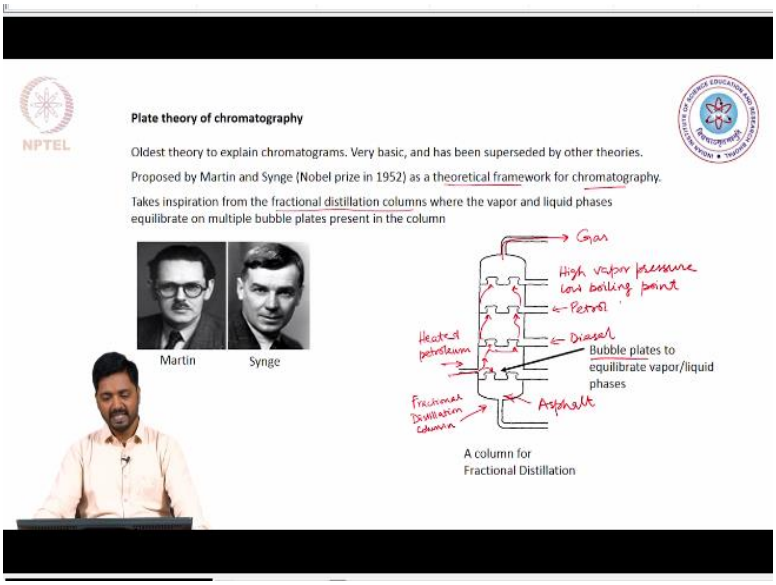

So, the concept of plate theory came early on and it is actually one of the oldest theories to explain chromatograms, it is in fact a very basic theory and has subsequently been superseded by other theories. However, because of its historical context, as well as its widespread usage, in the area of chromatography, we will benefit from getting introduced to this plate theory.

So, this theory was proposed by 2 scientists named Martin and Synge who were awarded Nobel Prize in 1952 for their work on chromatography, and they tried to develop a theoretical framework for explaining how chromatography work. Now, this theory got a significant input or inspiration from the fractional distillation columns that were prevalent at that time. And in fractional distillation column, if you might be aware, they are used in separating the petroleum fractions.

So, what we do here is we take heated petroleum, which contains a mixture of fractions and pass it through this column, which is known as the fractional distillation column. So, this column is of very large height, but its construction is also unique in that it has these interesting bubble plates, which allow for equilibration of the vapor and liquid phases. So, as the heated petroleum enters this column, it starts migrating across these bubble plates.

And at every plate, there is an equilibration that keeps happening. And only the fractions that have high vapor pressure or low boiling point rise up this column. And this allows us to fractionate the petroleum products into the different useful fractions that we use in our daily lives. And of course, on the top of this column, comes out petroleum gas, which is then liquefied.

And we collect various fractions such as asphalt which is a very low boiling fraction is collected at the bottom while many other fractions such as diesel and petrol will be collected at intermediate levels. So, this fractional distillation column became a source for inspiration to Martin and Synge, when they were trying to explain the chromatographic profiles.

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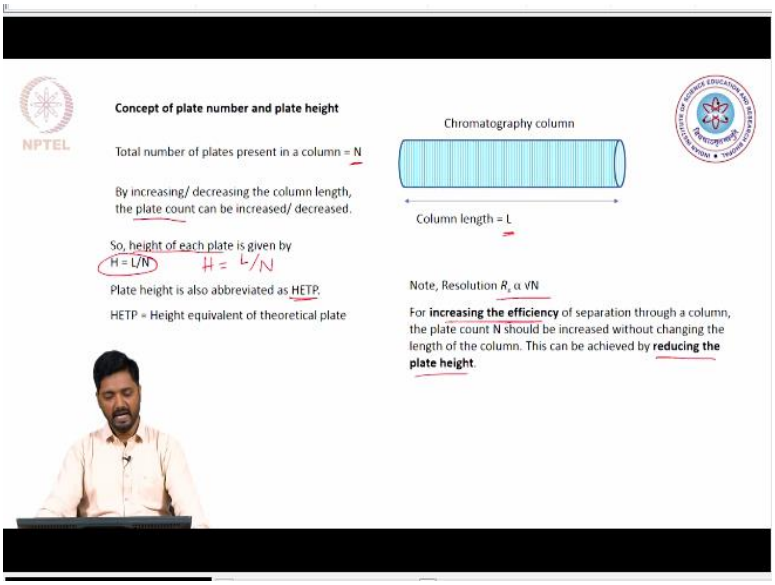

So, what they did here was they considered in their plate theory that the chromatographic column is composed of large number of plates, which are analogous to the bubble plates which are found in the fractional distillation column. Now, the solid moves down this column or across this column in this particular orientation by transfer of equipment rated mobile phase from one plate to the next.

So, what is happening is at each plate, there is an equilibration that is supposed to happen as the mobile phase moves and the equilibration of the solute also happens between the mobile phase and the stationary phase and that allows us to achieve separation of the different analytes within the column. So, it is very important to understand and note here that this concept of theoretical plates is really theoretical.

That means, it is an imaginary construct, which is used to explain how chromatographic separations happen inside a chromatographic column. There are no real players present inside chromatography column as has been described in the previous classes, we have stationary phase which is in a particulate form which is filled inside the chromatography column and it

is these particulates of the stationary phase that adsorb the moving analyte and give rise to the separation of the analytes.

Now, I have already explained to you that in the theoretical plates, the equilibration of the mobile phase happens as it passes through the chromatography column. So, what do we understand by this plate and what can we derive from it. So, this concept of plate number and plate height now comes into the picture. So, if we have a column of length L, we can describe that this column has say N total number of plates present in it.

So, again these are imaginary plates. So, we will have to find a way to calculate what this value of N is. And, more importantly for our purpose, it is clear that we can change the number of plates or what is also called as the plate count by increasing or decreasing the column length. So, when the column length is increased, the total number of plates present will also undergo an increase and vice versa when the column is decreased.

Now, we can also define another more important parameter which is known as the height of each plate. So, this height of the plate is given by this formula, which is nothing but the height is equal to the length of the column divided by total number of the plates present in the column. So, this is also abbreviated as HETP and various scientific articles. HETP is also representative of the plate height.

And is nothing but its expansion is height equivalent of the theoretical plate. So, what we have also known is that the resolution of the columns is proportional to the square root of the number of theoretical plates. This was the last point that we discussed in the previous lecture. So, if we want to increase the resolution or efficiency of separation of analytes through the column, we need to increase the effective plate count present within the column.

However, it is trivial to keep adding more columns to increase the plate count smarter way will be, if we can achieve the same through reducing the plate height, and we will see as we progress through the lectures, how this can be achieved in a practical scenario.

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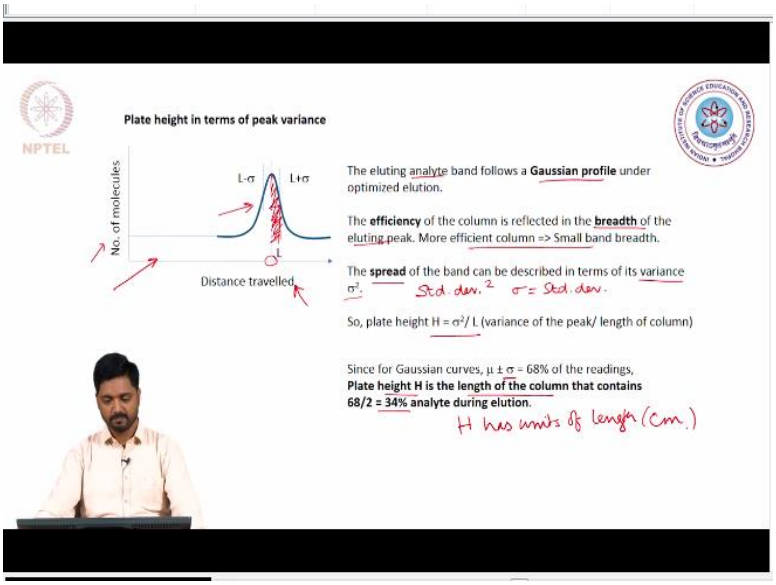

So, let us move ahead and understand how do we describe the plate height in terms of the peak variance. So, what you need to notice and here we come back to the concept of Gaussian error profile. We apply it to the chromatographic separation. And I reiterate that what you need to notice here is that the analyte that elutes out of the column gives a Gaussian profile under optimized deletion conditions.

So, this Gaussian profile arises for various reasons, which will be discussed subsequently. But, more what is more important is that more resolved or efficient separation would be the one in which the breadth of the eluting peak is small. So, a more efficient column will imply that the peak breadth that is coming out from the column of length L is small. So, here on the left hand side we have simply plotted the number of molecules that come out and the distance that they have travelled inside the column.

And what we have plotted is the migration of the solute or the analyte immediately outside the column of length L. So, this point is in terms of L, that is the distance L that that the solid needs to travel to come out of the column. And the spread of this band, which is shown here for the solute can be described in terms of it is variants. So we have studied in the previous classes, that for any Goshen profile, we can describe the spread of the curve in terms of the variance.

And that is nothing but the standard deviation squared, where sigma is the standard deviation. So, we can now define the plate height in terms of this variance of the peak and the plate height is now given as H equal to sigma square, which is the variance divided by L, which is

the length of the column. So, what we observe here is that the plate height can be reduced by either reducing sigma square or the standard deviation squared variants of the peak or the more trivial protocol would be to increase the length of the column.

Now, we also understood in our previous classes that moving plus minus one standard deviation from the mean value implies that 68% of the readings are covered in the Gaussian error profile. So, the plate height now is described by this distance which has been highlighted, which covers 34% of the analyte during elution, so, plate height is nothing but the length of the column that contains 34% and alight during the elution.

So, we can highlight this region to further bring out this point. This is the regime, which we are talking about, when we consider the plate height, it is given in terms of the length of the columns, it has a unit of centimeters. So, he has units of length, since the column length are typically given in terms of centimeters, the plate height is also denoted in terms of centimeters.

So, here I think it is very interesting to see how the Gaussian error profile has been employed to explain the resolutions within the column and in terms of the plate heights.

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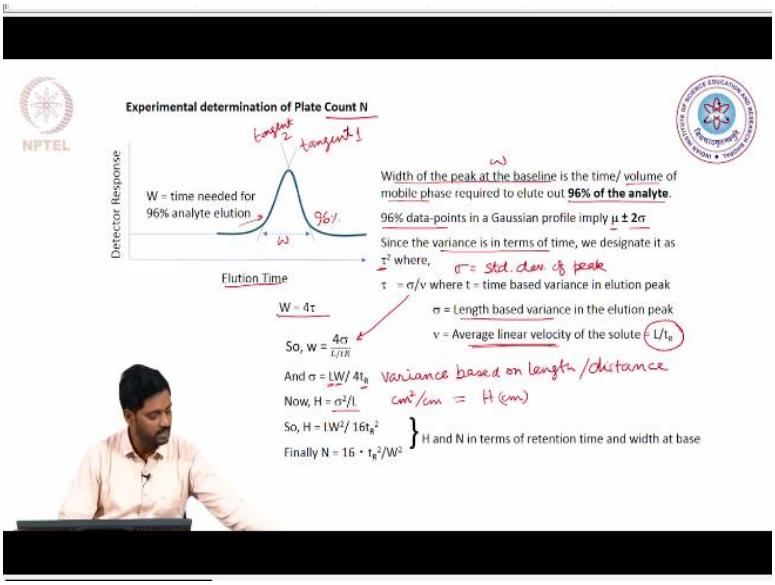

Now, let us see how on a practical basis do we determine the plate count and from there, it would be straightforward to calculate the plate height. For that, we simply take the Gaussian error profile of the elution of the analyte. And we draw 2 tangents, which are already drawn

here, tangent 1 and tangent 2. And these 2 tangents essentially provide the width at the baseline that this Gaussian profile makes.

And this width is covering 96% of the peak area and we have seen again from the our previous investigations and studies that 96% of the data points in Goshen profile are described in terms of the mean value plus minus 2 standard deviations. So, I repeat that the width of the peak at the baseline which is given as w can be described either in terms of elution time or in terms of the volume of the mobile phase that comes out.

And this width of the peak at the baseline describes the time or the volume that requires to elute out 96% of the analyte from the column. So, we can write the variance in terms of the time. So, we designate it as tau square and tau is given as sigma by V, where sigma of course is the standard deviation of this peak. And nu is as we had discussed earlier is the average linear velocity of the solute.

And the average linear velocity of the solute we have already seen can be described as the length of the column divided by the retention time of the particular solute. So, we have now described the variance of this peak in terms of the length based variance which is the sigma and the average linear velocity, which is L by t R. And we also understand that the width at the base is equal to 4 times the standard deviation value.

So, which is w is equal to 4 tau. Now, since w is equal to 4 tau, we insert the terms related to tau here and we get a new equation, where w is described in terms of sigma length of the column and the retention time t R. We also know that now we can describe the variants based on length or distance, in terms of the length of the column, the width at the baseline and the retention time.

All of these three parameters are very straightforwardly available from a chromatogram. So, we can figure out what is the variance based on the length from these inputs. And finally, we can calculate the H value, which is the height equivalent of theoretical plate, or the plate height, in terms of the variance in terms of length divided by the length of the column. So, again please understand that the variants based on the length will have a unit of centimeters square, while the length has the unit of centimeters.

So, the H value will be given in terms of centimeters. So, when we insert the sigma value here, what we observe is let us go to the board.

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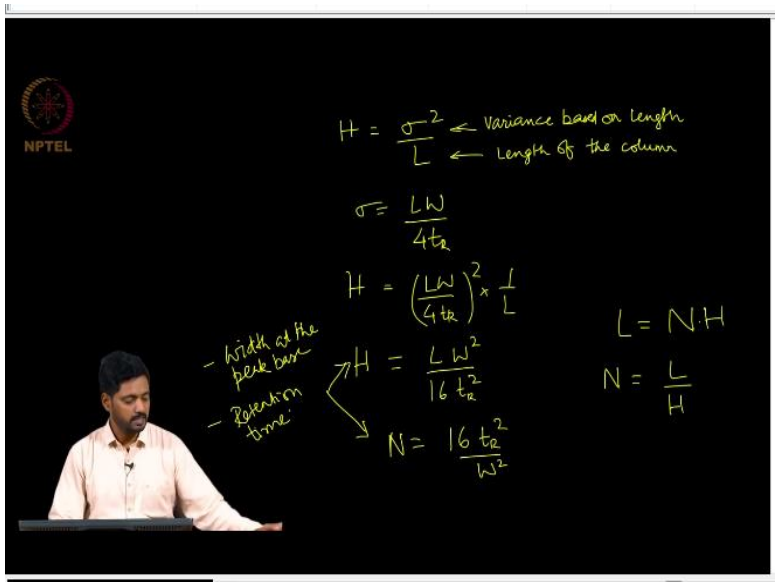

That H is equal to sigma square by L is what we described our height of the plate as. So, sigma square was the variance based on length and L is the length of the column that was used to elute out the analyte of interest. Now, we already saw that the sigma square value was Lw by 4 t R, when we insert this into this equation, we have H is equal to Lw by 4 t R squared into 1 by L.

And this solves out to Lw square by 16 t R square, that is the height of the theoretical plate. Now we also know that the length of the column is described in terms of the total count of the plates or the plate count into the height of each plate. So, when we have to figure out the value of N, we simply do L by H. So, the length divided by length of the column divided by the height equivalent of the theoretical plate will give us the total plate count.

When we insert this here, what we get is the value of N here, which is nothing but 16 t R square by w square. You can undertake this calculation or this transformation yourself and see that this is indeed correct. Now, what we have described here are the values of H and N, which are 2 important parameters in the plate theory in terms of the width at the base, width at the peak base and the retention time.

So, these numbers can indeed be very clearly obtained from the chromatograms that we generate during our analyte separations.

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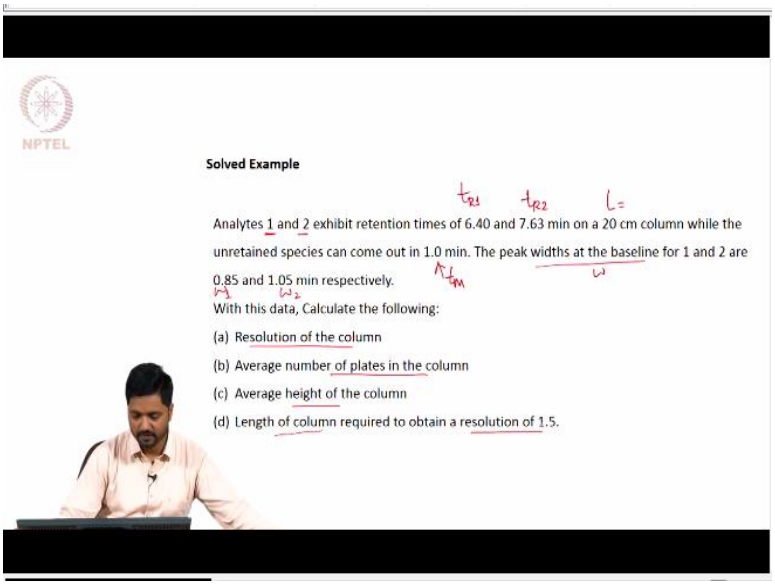

Now, in the final part of this lecture, I have for you a solved example, where we will be calculating the H and N values as well as the resolution of the column based on the chromatographic data that is provided. So, here for example, we are talking about separation of 2 analytes analyte 1 and analyte 2, that has retention times we can describe this as t R 1 as 6.40 and t R 2 as 7.63 minutes.

And the column length L is equal to 20 centimeters and we are also told that there is an unreturned species that comes out of this column within 1 minute. So, this is nothing but our t M value in terms of what we have discussed in the previous class. And we are also given the peak width at baseline, which is our w values for analyte 1, we can describe it as w 1 as 0.85 minutes and w 2 as 1.05 minutes respectively.

So, with this amount of information available, it should be possible for us to answer the queries that are given here, and we are asked to calculate the resolution of the column, the average number of plates in the column and of course, based on these 2, the average height of the column and finally, we are asked to also tell what is the length of the column that will be required to achieve a resolution of 1.5. So, first of all, let us go to the board and start writing these values.

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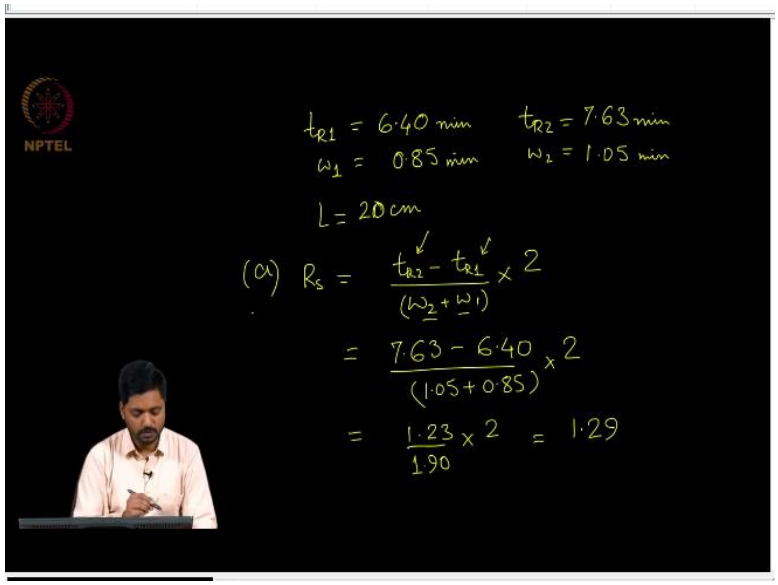

So, we have a retention time 1 is given as 6.40 minutes. The retention time 2 is given as 7.63 minutes. The width at the base for peak 1 is given as 0.85 minutes. And finally, the width at baseline for the analyte 2 is given as 1.05 minutes, we are also provided the length of the column as equal to 20 centimeters. So based on this information, it should be possible for us to calculate the resolution R s which is nothing but t R 2 minus t R 1 divided by w 2 plus w 1.

So, the resolution R s is given as the retention time for analyte 2 minus the retention time for analyte 1 divided by the width at the base for analyte 2 plus the width at the base for analyte 1 and this whole multiplied by 2. So, please note here that we will not require the t M w value even if it was given for calculating the resolution for the 2 peaks. So, let us go ahead and plug in the values in this equation.

This comes out to be 7.63 minutes minus 6.40 minutes divided by the width at the baseline is 1.05 w 1 is 0.85 and this whole is multiplied by 2, when we solve this out, this turns out to be 1.23 divided by 1.90 into 2 and this finally solved out to be resolution of 1.29. So, this is the resolution between the 2 peaks and what we have seen in the previous class is that resolution of 1.0 implies that there is 4% contaminant present.

And a resolution of 1.5 will imply that there is only 0.3% contaminant present. So, here we are talking about just about this N separation, where we will still have a little bit of the 2 analytes being present as contaminants in the peaks that come out during the elution of these analytes from the column. Now, the next part requires us to calculate.

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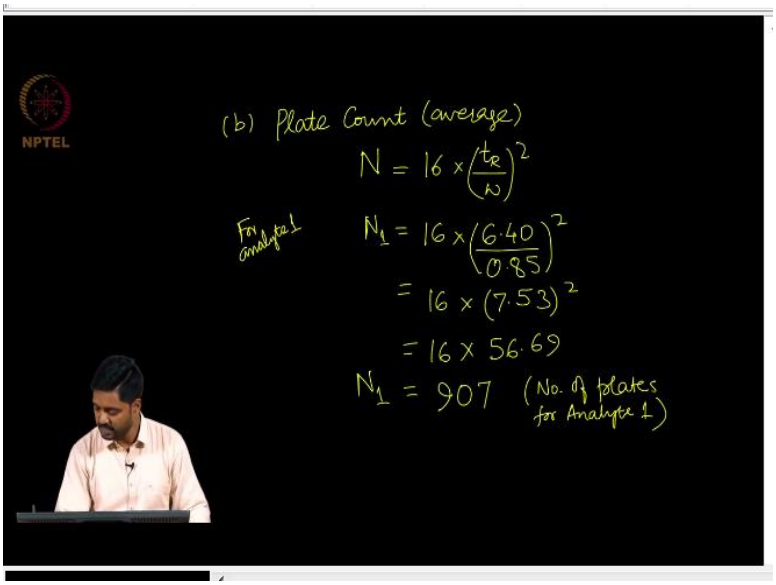

In the part B, we are required to calculate the plate count every average for this column. So, what we will do here is, we will calculate the plate counts for analyte 1 and analyze 2 and average it out to figure out the plate count average for this column. So, let us do that by plugging in the values. Now, plate count or the total number of plates present in this column will be given by 16 into the retention time divided by the width at the base squared.

This we already saw just a few minutes back in this class. So let us do again the calculations here, that would be 16 into the retention time of 6.40 for analyte 1, we call it N 1 and the width at the base for this was 0.85 and when we solve this out, we get an N 1 value by the way this whole has to be squared, because that is what the formula requires us to do. So, this solves out to 16 into 7.53 squared.

And finally, this is solving out to 56.69 into 16. And the N 1 value comes out to be 907 rounded off. Let us also calculate the N 2 value, which is the number of plates for analyte 2 So, N 1 is the number of plates for analyte 1.

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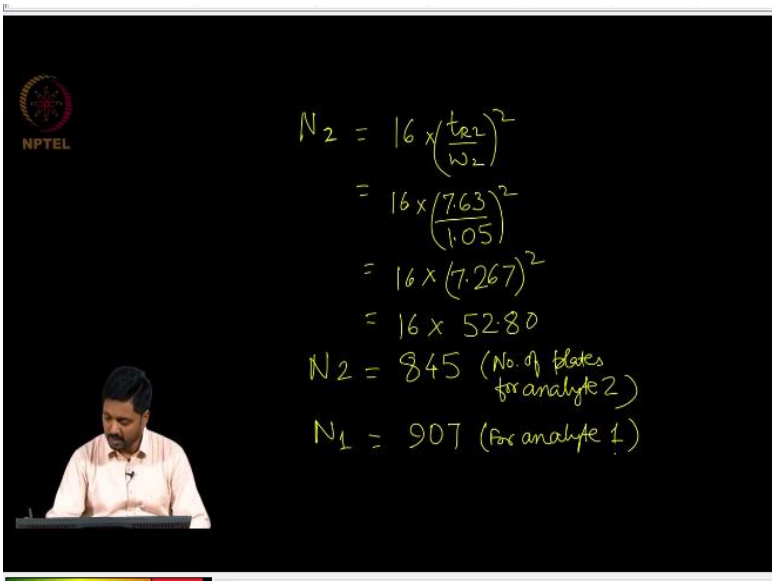

And in the next page we will calculate the N 2 value which will be 16 into t R 2 by w 2 whole squared. And these numbers are plugged in straightforwardly as 7.63 divided by 1.05 whole square. And finally this solves out to be 16 into 7.267 whole squared. And when we solve this further out, it comes out to be 16 into 52.80 rounded off and this now can be solved to about 845 is the number of plates for analyte 2.

So, let us pause here for a minute and take note of an important observation and that is that N 2 value is 845 while N 1 was coming at as 907. So, I want you to carefully note this point that for the same column we can have different plate height for different analytes. And this is an important point to be kept in mind when you are doing your own calculations. But this question currently asks us to calculate the average plate height for this column.

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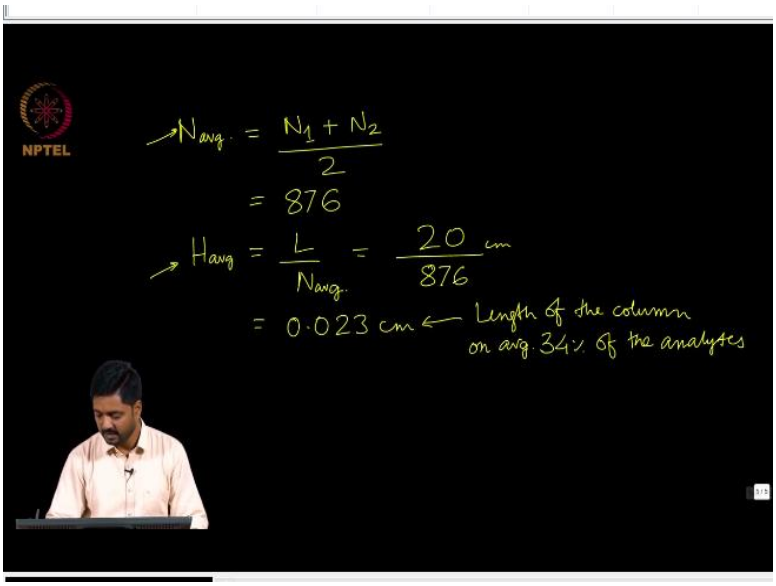

So the average would be nothing but the number of average is nothing but the number of plates for analyte 1 plus number of plates for analyte 2 divided by 2, and the average plate count for this column as a result comes out to be 876. Now, with the average number of the plate count available to us, it would be straightforward to calculate the average height of the plate.

And that would be nothing but the length of the column divided by the number average of the plate count. And we insert in the numbers 20 centimeters was the length of the column that we used here and an average is coming as 876. So this gives us a average height of the theoretical plate as 0.023 centimeters. So in other words, this is the length of the column that contains on average 34% of the analyte okay.

So we have already calculated the total number of plates present in this column for analyte 1 and analyte 2 and these 2 numbers were different from this we calculated the N average. And finally, we also calculated the average plate height for this column.

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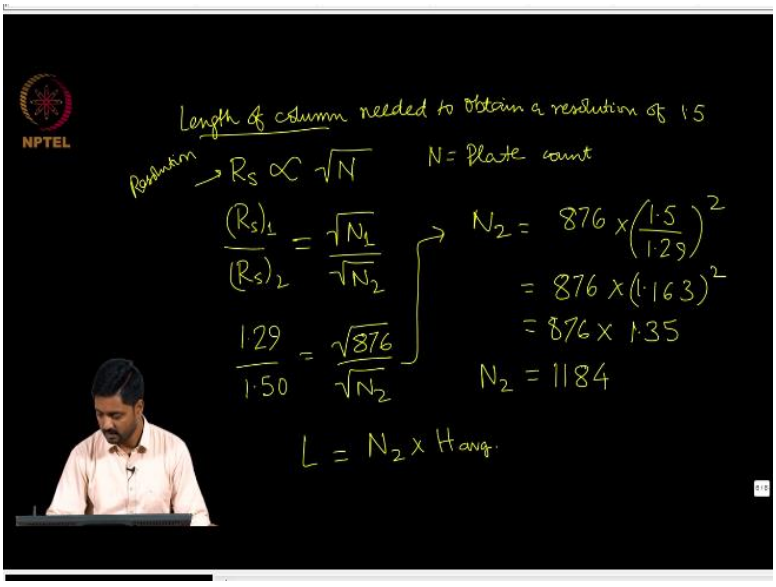

Now, the last part of this question asked us that what is the length of the column needed to obtain a resolution of 1.5 because as we have seen previously that at this resolution only 0.4% of the contaminant will be present in the 2 peaks. Now, let us understand that the resolution of the column is proportional to square root of the number of plates present in the column or the plate count.

So, N is the plate count and R s is the resolution. So, we can rewrite this as the resolution 1 by resolution level 2 is equal to square root of N 1 by square root of N 2, where R s 1 is the resolution that we currently have, which is 1.29. And R s 2 is the resolution that we are seeking which is 1.50. We know the N 1 value here, which is the average value as 876. And we do not know what is the N 2 value.

So, we plug these things into the equation. And when we solve this, we get n 2 is equal to 876 into 1.5 by 1.29 squared and this solves out to 876 into 1.163 and finally 876 into 1.35 and all of this gives us the N 2 value as 1184. So, we will require the plate count of 1184 to achieve a resolution of 1.5. So, what is the length of the column that will be needed, length will be nothing but N 2 into the average height of the theoretical plate. So we calculate that.

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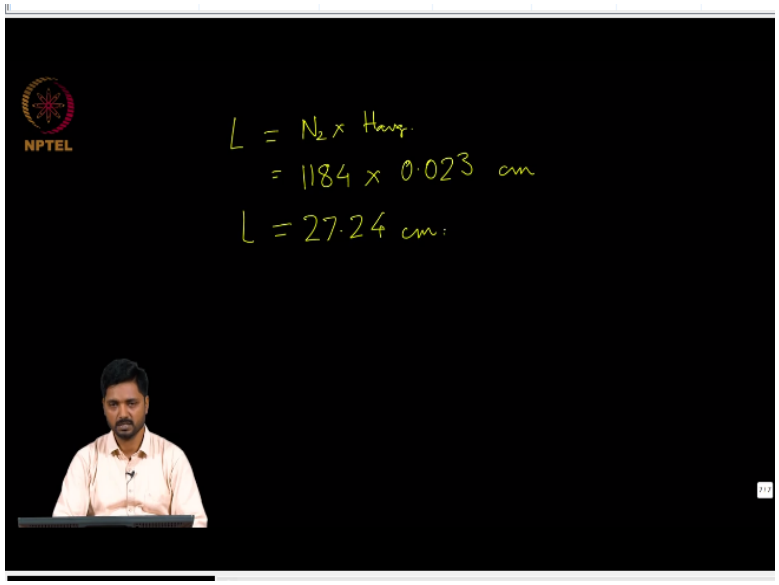

So, length of the column is n 2 into the H average. We know that N 2 is equal to 1184 and H average, we calculated previously as 0.023 centimeters. So this solves out for the length of the column to be 27.24 centimeters. So in other words, to achieve a resolution of 1.5, we will have to increase the column length to 27.24 from our original length of 20 centimeters. So, I hope that this lecture has clearly told you how we utilize the simple plate theory to estimate the various parameters associated with chromatographic separation.

And these parameters include the plate count, the plate height, the resolution, and all of these can be easily figured out, if we know the width at the base and retention time for the solute that is coming out along with the length of the column. So, with these parameters, it would be

straightforward to provide a quantitative analysis of the chromatographic separations that we are undertaking.

Now in the final lecture of this week, we will take the basics of another very interesting theory that has been developed for explaining the chromatographic separations and that theory is called as the rate theory of separation. And we will be looking and contrasting it with the current plate theory that we discussed in this lecture.