Analytical Technologies in Biotechnology
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Module - 3
Chromatographic methods
Lecture - 2
Basic Concepts in Chromatography 2

In previous lecture, if you could recall we started the chromatography section and we were discussing about the basic concepts in chromatography. If you could recall we have talked about the very general principle of chromatography where we have discussed about distribution coefficient and effective distribution coefficient. We have also discussed about the various combinations of stationary and mobile phases. And then we have gone to discuss about a three modes of chromatography that is paper, thin layer and column chromatography.

We will continue our discussion on the terms and the performance parameters related to chromatography in this lecture. We were talking about the terms like chromatography, chromatograph, chromatogram, stationary phase, mobile phase and analyte. So, let us continue our discussion further. Now, there are certain other parameters which are very important to assist the performance of chromatography and we are going to discuss them today.

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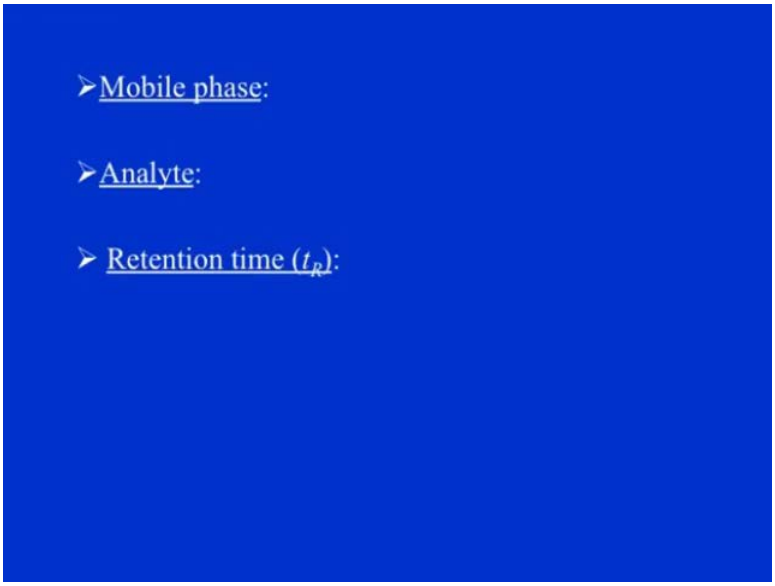

One is retention time. Now, retention time is a characteristic time taken for each analyte peak to emerge from the column. And as I said characteristic time, because under standard condition it will remain the same, that is for same column conditions temperatures, and other factors, the characteristic time for a particular analyte will remain the same.

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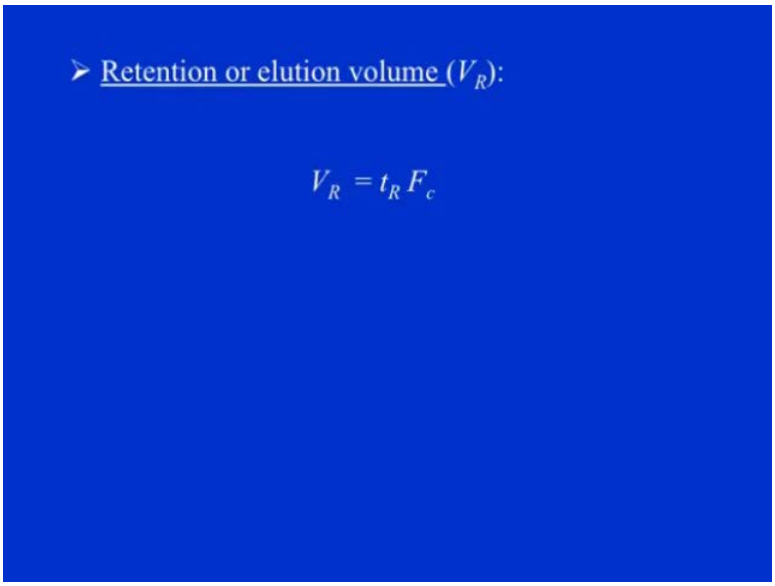

Then as the retention time is there, the another term called retention volume or elution volume. If we have to define this then retention or elution volume is the volume of mobile phase required to elute the analyte under standard conditions. The elution volume and retention time are related by the flow rate. So, again the retention volume or the elution volume is a characteristic feature under standard conditions. And both retention time and retention volume are related by the flow rate, that is at what flow rate you are moving the mobile phase through the column.

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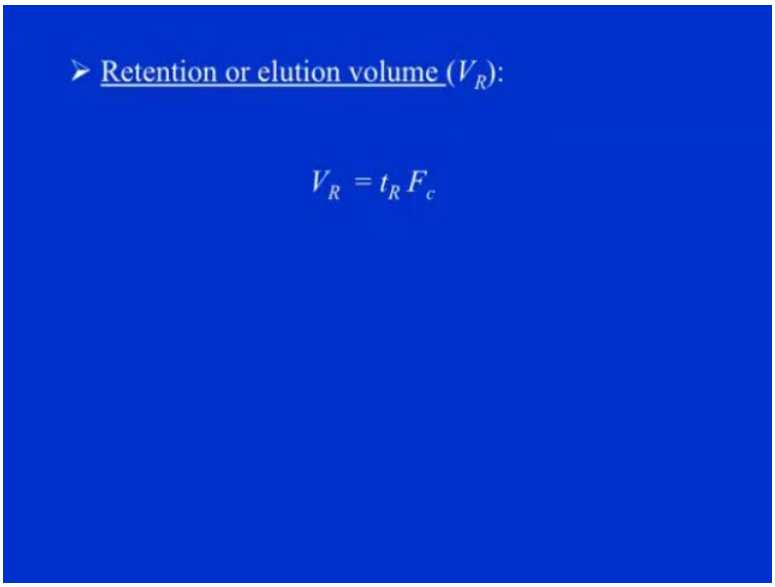

So, this relationship is given in here like the retention volume equals the retention time multiplied by the flow rate. So what you have is that, if you know how much flow rate is there you can calculate the retention volume. Now the flow rate could be influenced by a lot of different factors that could be physical dimensions of the column whether, column is narrow board or narrow diameter or it is a wide board column, what is the length of the column and all those characteristics.

Then it is also influenced by physical characteristics of the particles packed in column. That is, what is the size of the particle shape of the particle? What is the porosity of the particle? For example, if they are packed very well then certainly flow rate would be would be small, unless you pump or you make it so happen that you have a higher flow rate through a pumping system. Then viscosity of the mobile phase also will affect the flow rate. So, these are factors which will certainly influence the flow rate and then therefore, the retention time and the retention volume.

So for a liquid partition chromatographic column the elusion volume is related to volume of a stationary phase. Now it is not only stationary phase but it is related to stationary phase, distribution coefficient of the analyte between the stationary and mobile phase and the void volume that is the dead volume of the mobile phase around and within the stationary phase packed in the column. And this is related by the equation if you can see on your screen.

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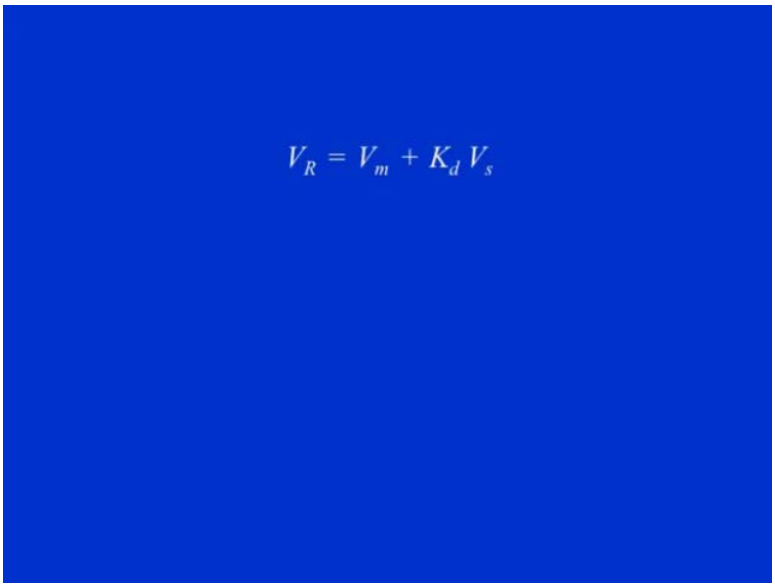

That is retention volume equals the dead or void volume plus the distribution coefficient and the volume of a stationary phase. So you can calculate again the retention volume if you know these parameters. For adjacent column chromatography, the stationary phase will be replaced by the surface area of the observant in place of a simple stationary phase. Here observant surface area will be taken. So, capus elusion volume or what we say retention volume is an important factor. To know, to asses that how analyte is being purified. Another fact another very important term and it is a performance parameter in chromatography is the capacity factor or the capacity ratio.

Now, the capacity ratio or the partition ratio or we call it capacity factor. This does not have any units and it is the major of the time it is spent by the analyte in the stationary phase relative to the time is spent in the mobile phase. And it is given by if you in terms of uh volumes here it is given Cm Vm upon Cm Vm where, all these are Cs is concentrations Cs and

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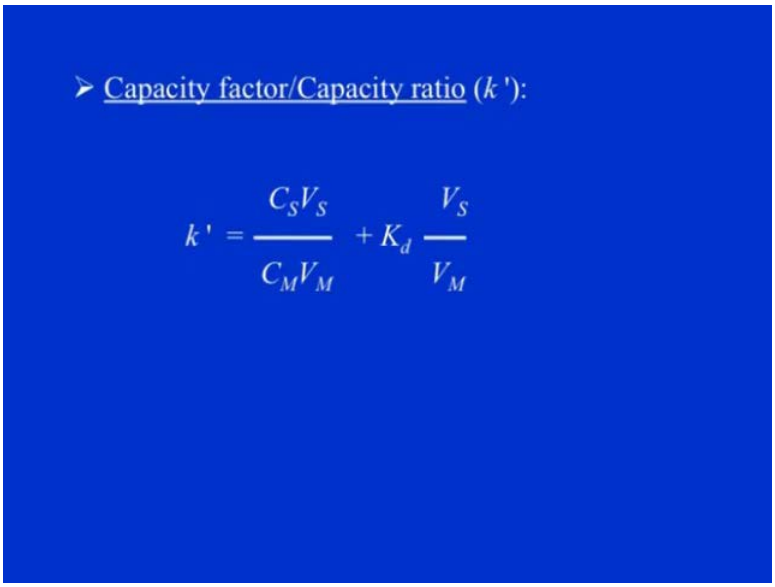

Cm is concentrations of the analyte in the stationary and mobile phase. This we have already discussed Vs is the volume of a stationary phase and Vm is the like it is the void volume. And then this is the ratio of which is also called volumetric ratio Vs upon Vm which is given here. So, capacity is an important factor now I said. It is the time spent in the two phases.

So, it is a measure of the additional time that the analyte takes to elute from the column relative to an unretained or excluded analytes. That does not interact with the stationary phase. So, if you a unretained or which does not really partition will just come out in the void volume. And will have a capacity ratio of zero. So, we can simply say in a very simple terms it could be that, capacity ratio is a measure of additional time that the analyte spends or takes to elute from the column.

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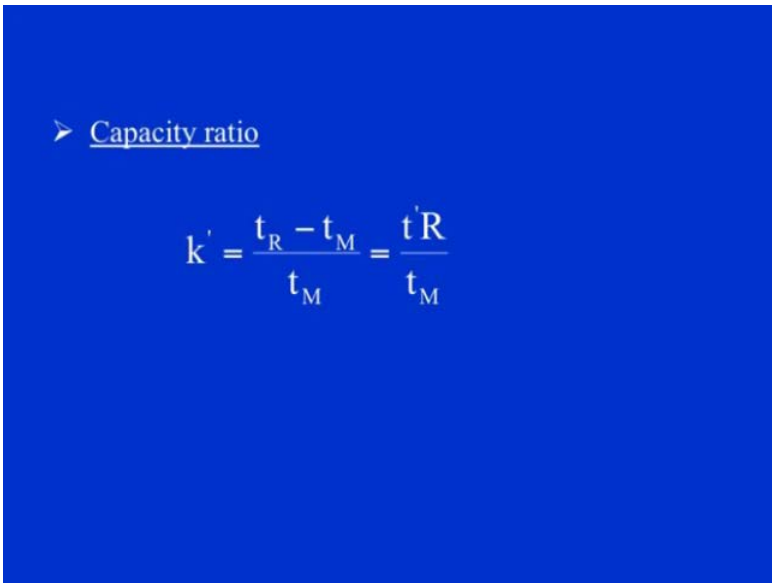

And this could be given by this equation where capacity ratio equals t R minus tm upon t M which is t R is retention time, tm is the dead time taken to pass through the column of the void volume and t prime R is the adjusted retention times that is t R minus t M. So, this you can calculate the capacity ratio on by this equation. Now in capacity ratio equation the tm or transit time of an unretained compound through the column is determined by the length of the column and the linear velocity of the mobile phase through the column. And it is given by this particular equation that is L upon tm.

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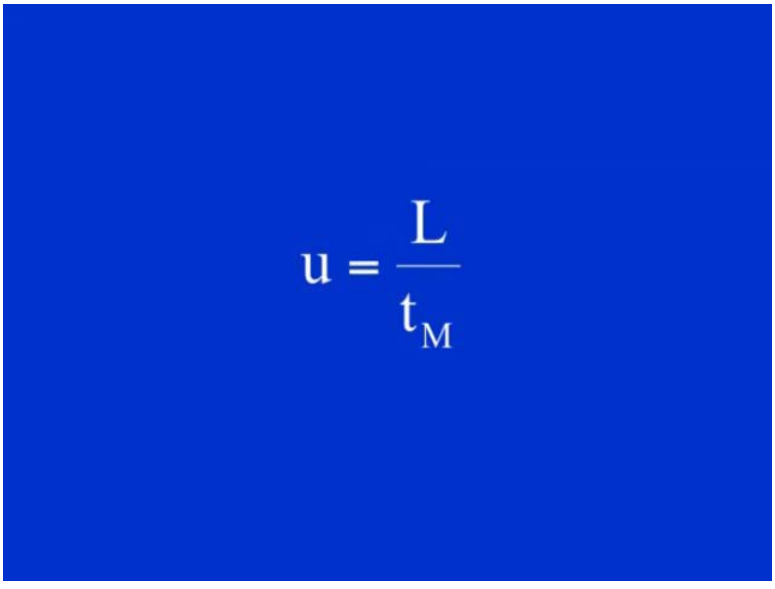

Where L is the length of the column and tm is the dead time as we were discussing. So this way you can determine this particular like through the linear velocity you can determine the transit time or the time taken for the void volume to come out. Now, it can be derived from the capacity ratio equation that if an analyte spends an equal time in both phases that is the stationary phase and the mobile phase. Then it is t R equals 2 tm that is tm is the transit time or time taken to come out by unretained analyte. So, the capacity ratios will be 1.

If the analyte spends 4 times as long in the stationary phase as compared to the mobile phase, then the retention time for the analyte will be equal to 5 times that of the transit time or time spent by unretained analyte. And hence the capacity ratio will be equal 4. So, that means there will be 4 times the amount of analyte in the stationary phase than in the mobile phase at any point in the column at any time, if that is if the capacity ratio is 4. Therefore, capacity ratio is related to distribution coefficient. Since, amount and concentrations are related by volume.

Therefore, the capacity factor for an analyte will increase with both distribution coefficient and volume of a stationary phase. Now capacity ratio values may normally range from 1 to 10. So, this is just to understand in terms of that how much time an analyte spends. More time it spends more will be the capacity ratio of the column, less time it spends less will be the capacity ratio of the column.

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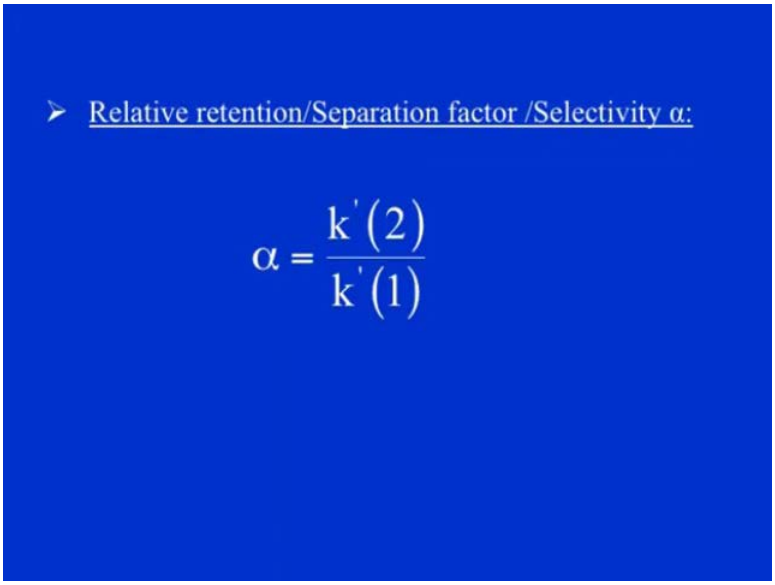

Another important factor is the separation factor or selectivity factor alpha. Now it is a relative retention or you can say separation factor or selectivity factor. And this is the measure of inherent ability of a system to discriminate between two analytes. That is capacity ratios are reflections of the selectivity of the system. Say, if it is it has more capacity ratio it will be having high higher selectivity. And this is expressed by the selectivity factor which is given in here that is it is a relative retention ratio for 2 analytes.

If you see it is like one it is relative retention ratio for analyte 2 in comparison to analyte 1. So the selectivity factor is also important in assessing the functioning or performance of a particular column time. Now, the selectivity factor is affected by the chemical nature of the two phases some systems like affinity chromatography are highly selective. So separation of two components is only possible, if alpha that is selectivity factor is greater than one. So, unless they could be separate out as we have discussing different performance parameters.

These are important to really purify or to separate two analytes. We have discussed about capacity ratio, we have discussed about selectivity factor and we will also discussed about retention volume retention time. Now, another important factor in column chromatography or any chromatography is the resolution actually. Now, what is resolution here? Now, resolution in terms of chromatography is the ability of a chromatographic system a particular chromatographic system to resolve or to separate one analyte from the mixture of similar compounds or we can say to separate two analytes or to resolve two analytes from one another.

And this is the measure of success of a chromatographic separation. So, unless you can completely separate two analytes in a column whole purpose of chromatographic separation is not served. Now, resolution which is given by Rs is defined as the ratio of the difference in the retention time between the two peaks of the mean of the base width. So, let me say it again so resolution is defined as the ratio of the difference in the retention time between the two peaks to the mean of their base widths. What does that mean.

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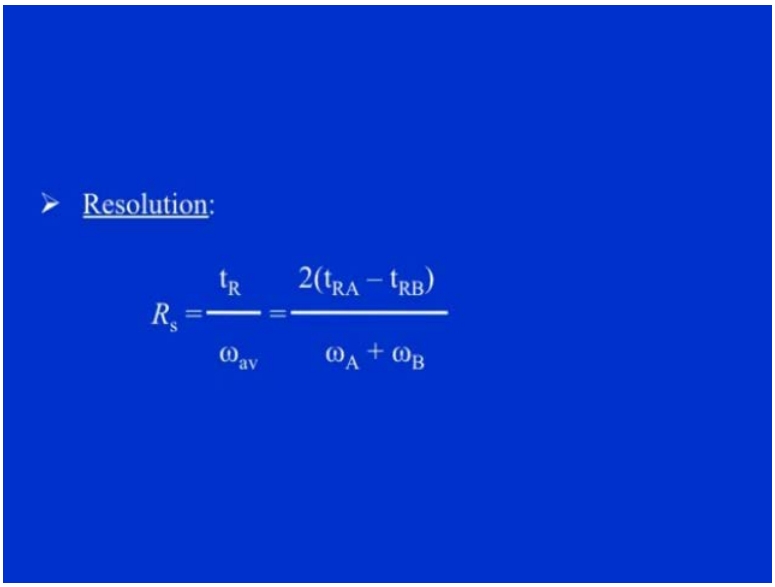

Let us see this equation here, so resolution equals this is t R divided by the w or which is called the base width or the peak I will just show you. So, if it is a two peaks here just say peak A and peak B and these are the retention times for the two peaks. Then it is the ratio of the difference in the retention time to the mean of their base width that is the base width of one peak to the width of another peak.

So that is the average way of taken here now here when r resolution equals one then the separation of two peaks almost 97.7 percent. And the overlap is quite low 2.3 percent. And when Rs is 1.5 separations is 99.7 percent almost complete purification and overlap is very low that is a 0.2 percent. Now, let us see this how this whole thing is happening if you see this figure here.

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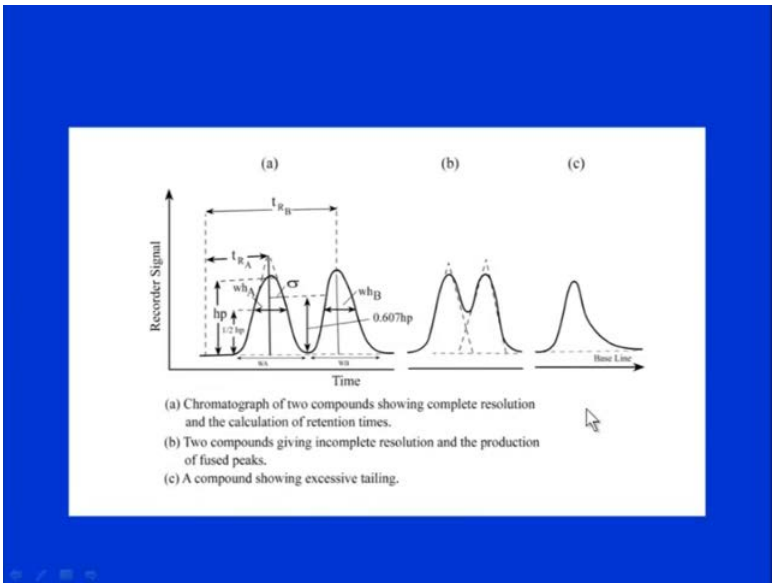

Then what you can see here that we can see certain peaks in here. Now if you see here this peak here they are two completely resolved peaks here. And here you have a retention time of peak A, this one here which is from origin we are taking and there is another peak with retention time t R B of second peak with increased retention time that is this peak comes later on. But, you can see these two peaks are almost separate or we can say these are completely resolved.

Now base width here, this is the base width of the two peaks. And at this point will be you can say half the width of the peak at the infraction point. And this I calculated in terms of sigma, so base width is calculated to be 4 sigma and then at infraction it is 2 sigma. Now if you see here these 2 peaks like we say resolution of 2 peaks or it can separate completely like if it is a 1.5 resolution then it is kind of purifying or separating by 99.7 percent.

If you see these two peaks then these are overlapping. If you draw these lines here these are overlapping peaks what does that means. These two peaks are not completely separated or they are not completely resolved in chromatography system or particular chromatography system. So, we say that it is incomplete resolution here. Then you are doing the purification or you are purifying an analyte by a particular chromatographic system depending on the efficiency of system, you might get these different types of peaks. Like for say example, hp se will give you a very high resolution separation and

very good peaks that is completely separate peaks as compared to a simple gravity run chromatography system.

Here could also be other problems like if you see here there is a peak tailing actually so. And there could be fronting also where peaks are not very sharp and they kind of drag here. And what you get is you do not get a very good purification. So there could be lot of different conditions and you can see the resolution in terms of peak shape and peak heights, how they are resolved are they separate from each other and all those things could be looked from this figure.

So, this was like resolution is very important factor and it is a mean determinant of the success of a chromatography system. There is another term which determines the resolution which is called theoretical plates. Now the chromatography system columns are considered to consist of a number of adjacent zones in each of which, there is sufficient space for an analyte to completely equilibrate between two phases. Now each zone is called theoretical plate.

And plate height is the length of the column for one theoretical plate. And it is units will be length. So, there could be n number of plates in a column depending on what is the height of the plate theoretical plate. If we have more number of say plates or you have a smaller plate height of the plate, then you will have more number of plates that is n will be higher. And if you have less plate height is higher then number of theoretical plates will be lower.

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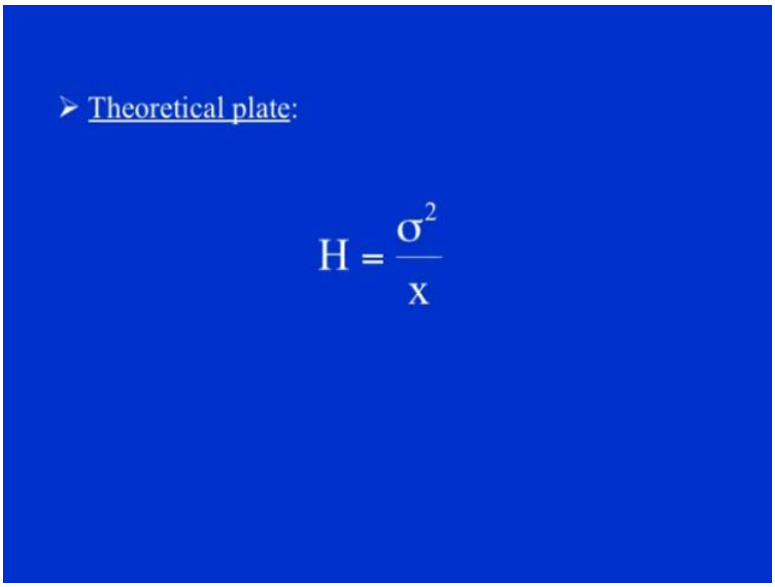

So, here an equation is given, where H is given by sigma square upon X. So, H is the plate height that is the length of column containing one theoretical plate. Sigma is standard deviation of the Gaussian peak. And X is the distance travelled by analyte within the column. So this could give you the plate height. Now, for symmetrical Gaussian peak, the base width is equal to 4 sigma as I had told you. And the peak width at the point of infraction is equal to 2 sigma. So, hence the value of H can be calculated from the chromatography by measuring the peak width. Now number of theoretical plates in the whole of the column is given by this particular equation.

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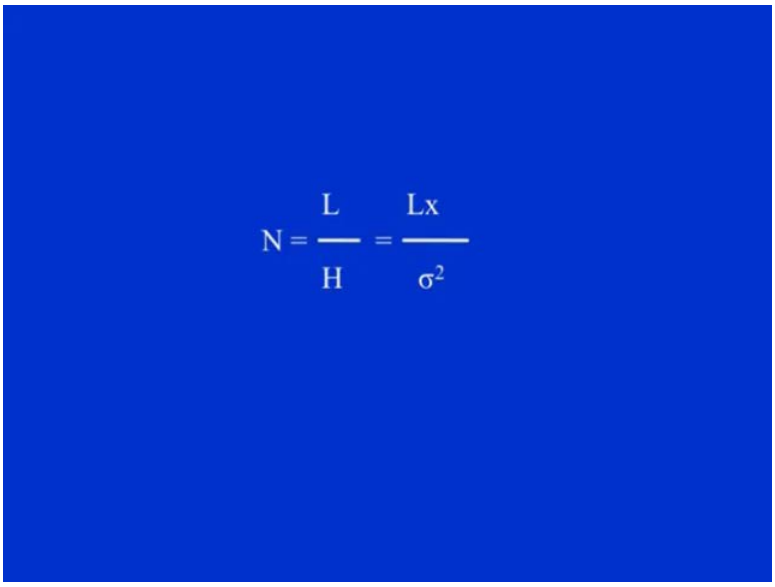

That is L upon H. Now L is the length of the column, H is the height of the plate as we have discussed earlier. If you have a condition like previous equation if you could remember it was sigma square upon x that could be substituted here in place of H and what you get is L x upon sigma square. Now this could be converted to if L equals x that could be L square. Now if you consider the position for a peak m emerging from the column so that X equals L and from the knowledge of the fact that the width of the peak at its base obtained from tangents drawn from the two steepest part of the peak is equals 4 sigma.

Hence sigma equals one-fourth of the base width. And so you can convert the previous equation here, which is that number of theoretical plates equals 16 L square upon the base width square. So, both these things could be measured in units of time also rather than length. Here we were measuring in terms of length, but you can measure that in terms of time. Then the equation will be something like this

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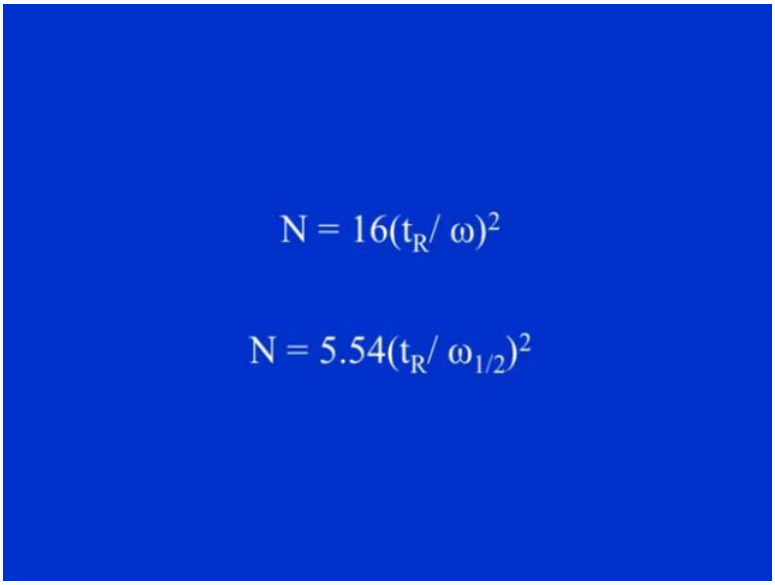

Where number of theoretical plates equals sigma 16 tr upon omega square. And can also be measured in terms of peak width at half the height. And this will be represented by this particular equation. So, there could be various methods particularly to measure this number of theoretical plates in a particular column. Now, the value of n represents the efficiency of the column. And can be very high it could be say 50,000 plates per meter or 100,000 plates per meter could be a possibility for a very, very efficient column. And

corresponding value of H can be very small in a few micrometers. So, remember as the as the height of the plate or the in terms of length reduces, number of theoretical plates increases. So, as we said that theoretical plates it is the analyte which separates it is like different zones present so more is number of zones more will be number of theoretical plates. Now if we consider like just showing, how a peak will look like, if we have to say that on the basis of number of theoretical plates.

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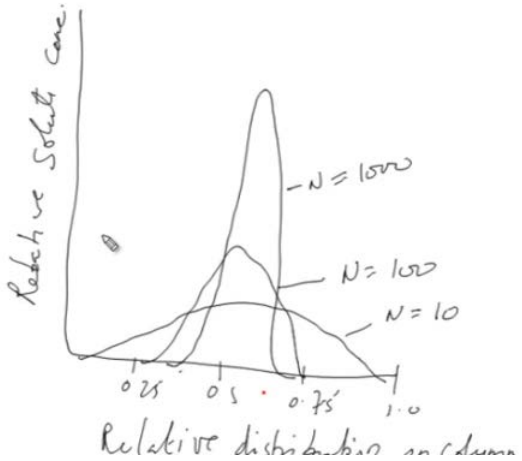

So, let us if I just want to show you a depict something in terms of theoretical plates. And so if on this axis it is a relative distribution on a column. And on the y axis is the relative solute concentration. So what we are trying to show is that how number of theoretical plates will affect the peak shape or type of a peak of a particular analyte. Now supposing the theoretical plates are very low number, say there are 10 theoretical plates in a column.

Then peak will be spread out sort of in a sense that peak will not be very sharp peak it will be something sort of spread out peak where you have N equals 10. So, less the number of theoretical plates the quality of the separation will be also low. Now supposing let us increase the number of theoretical plates. Now, let us say if we say that 100 numbers of theoretical plates then there will be certain improvement in the peak certainly.

And you will see that the peak is better than the previous one where theoretical plates were 10. So, you can see that as number of theoretical plates is increasing, the base width is also decreasing and the peak is become sharper. Now, let us say if there are 1000 theoretical plates, the peak if I say these are 1000 theoretical plates then peak has become much more sharper the base width decreases. And you can see that the amount of solute will remain though peak height goes up, but amount of solute or the area under the peak might remain the same. But certainly it is coming in a very small fraction here.

It is like it is distribution in the column or in the column it is distributed in a small area rather than in a bigger area where theoretical plates were less number. So if you have no theoretical like if the theoretical plate is 1, then the sample just comes out in void volume. But as number of theoretical plate increases, the analyte also distributes itself into the stationary phase and mobile phase in terms of that adjacent zones are very small and it distributes more or spends more time in there. And therefore, you get a peak which is where it is present in a smaller area as compared to where it is present in a larger area. So, this was just to show you the importance of the importance of the theoretical plate in here.

So, this is like both theoretical plates, the theoretical plate will determine the resolution as well actually. More is the number of theoretical plates more will be the resolution. But remember there is a limit to the increasing plate number by increasing the column length. Like say if you increase the column length supposing in a particular column length there are certain number of plates. And if you increase the column length certainly number of theoretical plates will also increase. Now, to a certain extent it will help to improve the resolution.

But there is a limit to that and as you increase plate number by increasing the common length, the retention time and peak with will increase proportionally with L. Whereas, the peak height decreases as the square root of N. So, the quality of the purification will go down if you keep on increasing after a certain limit. Now, the maximum number of peaks which can be separated by a chromatographic system is known as peak capacity. And it is related to the retention volumes of the first and last peak. So, remember unless the first and last peaks how many like in between peaks could be taken. And if the retention volumes are overlapping then certainly you cannot purify them, but if the

retention volumes of the first and last peak has to be taken to a certain how many number of peaks could be separated.

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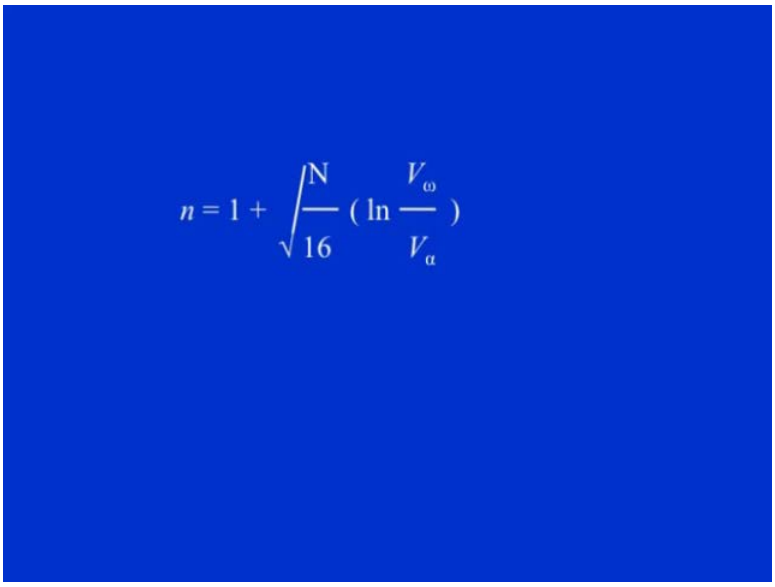

Now, this is given by this particular equation here, which could be utilized to calculate the peak capacity of a particular column. So, we have gone through lot of different factors that is performance factors in chromatography. Now, the success of a particular chromatographic system is just by its ability to achieve good resolution. And is determined by the following some like 3 functions are very important in here. And these are selectivity, capacity and efficiency.

So, let us discuss them one by one. The first is selectivity, if you could remember, we talking about selectivity factor also. So, selectivity is a measure of inherent ability of the system to discriminate between structurally related compounds. Now, this is reflected in their distribution coefficient and capacity ratio values, such that the ratio of the partition coefficient for the two compounds gives their relative retention ratio or selectivity factor. So, it is influenced by the chemical nature of the mobile and stationary phases.

Now, one example of highly selective chromatographic system is affinity chromatography. Affinity chromatography is highly selective. And it can like I said it is an inherent ability of the system to discriminate between structural related compounds that could be done, by say one example is affinity chromatography. So, selectivity is one important parameter to achieve good resolution. Another parameter is efficiency. Now,

efficiency is a measure of the diffusion effects that occur in the column to cause peak broadening and overlap. So, it is influenced by physical parameters of the mobile and stationary phase and by the quality of the packing of the column.

Remember, we were talking about trailing of the peak or fronting of the peak that could come as a problem and your efficiency goes down. So, diffusion effects might occur in the column and they might cause peak broadening or trailing and other problems. And therefore, peak overlap. And so what will happen again resolution is affected. So, this is also the capacity or its ability of a column to avoid these things and lot of physical parameters will also be affecting the quality of the packing quality of the run in these particular columns. And third factor is capacity for a successful chromatographic separation.

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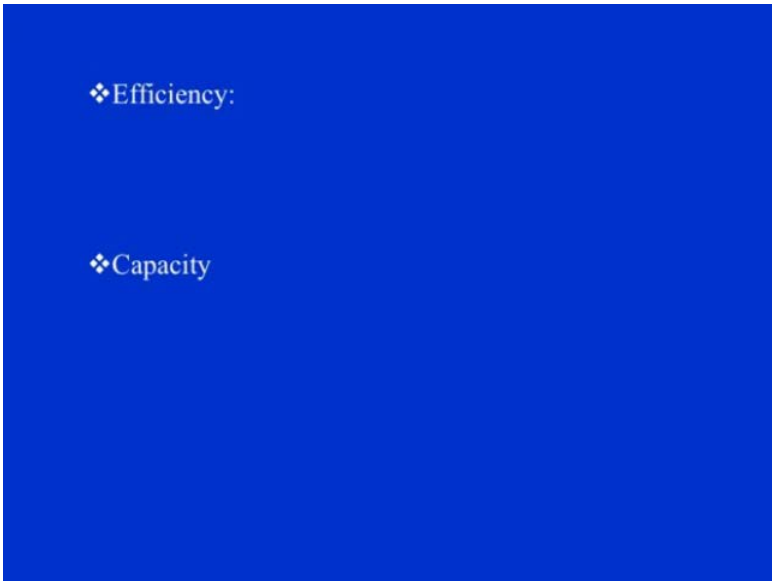

Now, capacity is the measure of the amount of material that can be resolved without causing peaks to overlap. Now, this is irrespective of such extent as gradient elusion and temperature programming. So, capacity is how much amount of the material which could be purified without causing two peaks. Like, if you can load if you are loading too much of the sample then certainly peak will broaden. Now, if you are loading in such a way that the two analytes though the peaks are broadening, but the two analytes which needs to be resolved do not overlap.

The peaks of those two analytes do not overlap. So, there is a limit to a capacity of that column, if you load too much then certainly if these two peaks could overlap and then resolution goes down. One example is an exchange chromatography which has high capacity. And the other columns like chromate focusing which also has a high capacity because these are the commonly used techniques to concentrate impure materials. So, the three things which are important like we said selectivity, efficiency and capacity are very important. And resolution is related to capacity ratio, relative retention ratio and plate number by this particular equation.

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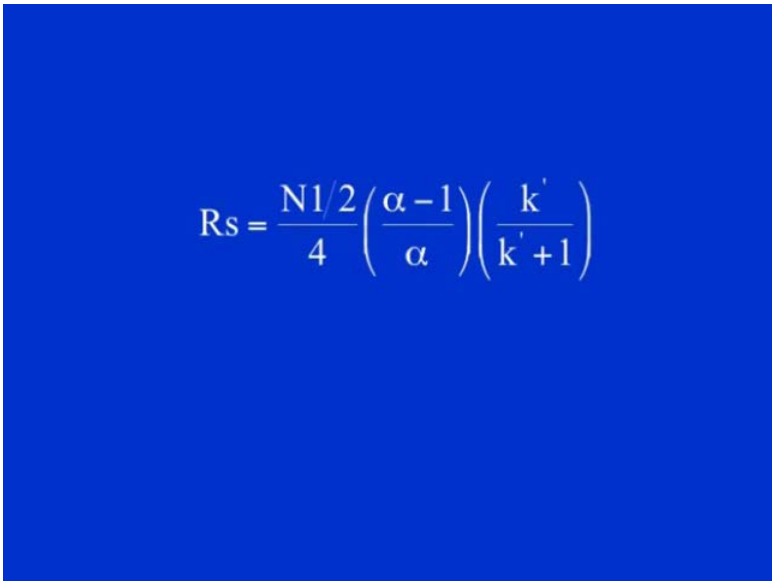

This is N half, and this is selectivity factor, and this is the capacity factor taken into account to calculate how the resolution is related to these factors. So, if you could see we have discussed certain number of parameters here, to understand or to assess the performance of a particular chromatographic system. Now, chromatography could be qualitative chromatography and it could be quantitative chromatography.

In qualitative chromatography you are simply analysing a particular analyte for a particular application, but in quantitative chromatography what you are doing is, you are also kind of purifying compound for a certain application or you want to do something else. Like say for example, you want to characterise that particular analyte say you want to characterise protein or other say you want to use nucleic acid for further applications.

Now, when we have a quantitative separation or you want to quantify certain compound in the system then you can apply or you can utilize. For example, you want to quantify a particular analyte to the chromatogram, then the area under the peak could be calculated for quantification and there are methods to do that. Like you know the base width, you know the area it could be calculated underneath the peak and you can quantify the substance. There could be internal and external standards could be utilized for this particular purpose. Another important part in column chromatography is that how the kind of chromatographic systems, where it could be simple displacement or simple aristocratic one also, that we are going to discuss later on.

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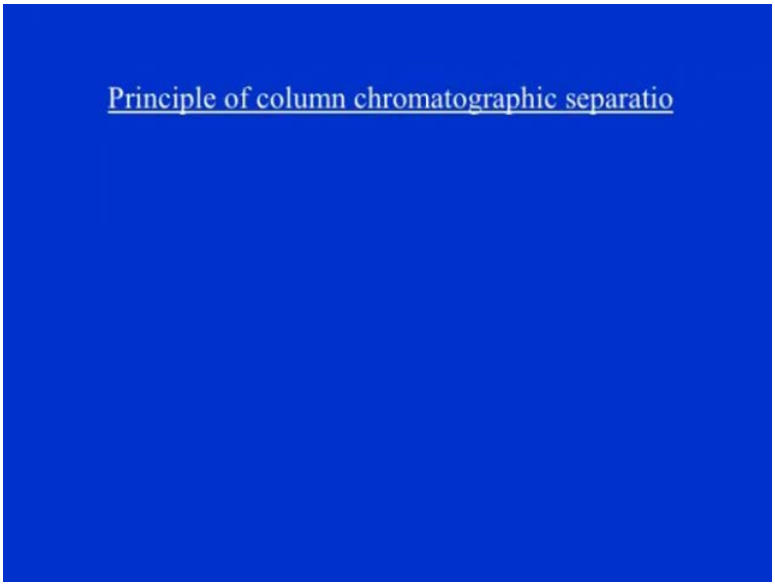

Now, let us see the principle of column chromatography separation in very simple terms here. Now, if I say there is a column and that column as we were talking about that column is divided into separate zones actually. So, in terms of how separation of the analyte will take place. Let me show you then we will go through the discussion.

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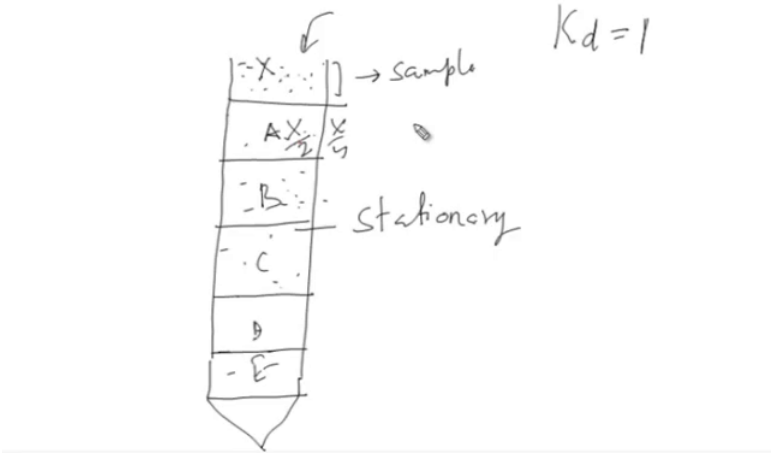

So, if you can see on your screen. As we have showed you there is a simple column, it could be a glass column which is enclosed from one side. And you have filled in a matrix here. There will be a mobile phase which will be entering now. This could be called sample or your mixture of analytes, which is in a mobile phase; this could be a stationary phase. Now, if I could divide this column into different separate zones here. Just to detect like this is just to understand the whole thing. Now, see a sample is present in X quantity here.

And just for assumption the distribution coefficient that is your Kd value is one, that is it separates equally in mobile and stationary phase. So, what will be the consequences, as this whole thing enters like say separate this area into different zones here. Now, what will happen if X amount of substance in the mobile phase enters this area it will just like you have taken equal amount here equal area. As it reaches here what will happen the X will be distributed here between the two phases equally. Let us phase the area A and between the mobile phase.

So, what will happen here it will be X by 2 that is half the amount will be in the stationary phase and half the amount will be in the mobile phase. Now, supposing more so once this is gone then mobile phase will be put in here. And extra mobile phase as it goes along, it will redistribute the whole analyte or sample mixture. Now, in that way

what will happen that the X half like more analyte comes in, then X half which is in mobile phase will move on and will interact here.

So, X by 4 and by four will come out and then more mobile as you put in then this will also be distributed which is present in a stationary phase. So, this will then become X by 4 and X by 4 will go to the mobile phase. So, there will be a bending pattern will be created here, which will move as a central thick band and adjacent thinner or you can say less concentration bands. Now, let us let us go back to discussion and understand this whole thing that is the whole concept of how the separation is being or how this whole thing is happening in the column chromatography.

So, what you have is when you are doing this separation it could be depicted by considering a column packed with a solid granular stationary phase as I have shown you. And it has a particular height which could be anything. Now, this one is surrounded by the mobile liquid phase. You will apply the mobile liquid phase from the top. And of which there is 1 centimetre cube per centimetre of column as we will be showing. I have shown you, but I will show you another figure also.

Now, if 48 supposing like X concentration what I was telling you its 48 Microgram of the compound is added to the column to occupy the position A. And what will happen as you put one centimetre cube of mobile phase then it will leave same amount will leave from the base of the column. And if the compound added has an effective distribution coefficient of one, as were talking about, then it will distribute equally itself between the solid and liquid phase.

Now, remember here we are keeping the amounts also equal in terms of one centimetre cube, but otherwise we are talking about here is distribution coefficient and effective distribution coefficient is different. Now, as you go along you add more same amount of mobile phase is introduced on to the column from the top. The mobile phase in the section B will move down to section B. Now, what will happen the mobile phase and the amount of the material that is 48 microgram will be distributed to 24 microgram of the compound and 24 microgram will be moving down. So, at both B and B a redistribution of the compound will occur, so there is a 12 microgram in the mobile phase and 12 microgram in the solid phase.

So, remember again understand this, first time when the substance or the analyte enters the 48 Microgram is distributed to 24-24. 24 in mobile phase and 24 microgram in stationary phase. As you introduced more mobile phase then the 24 microgram which was present in the stationary phase in the section A will be again redistributed into 12 and 12.

And as you introduce more of the mobile phase it will keep on distributing, so 12 becomes 6-6 will become 3 and likewise. So, addition of further equal amount of mobile phase to the column will displace the mobile phase in sections A, B and so on and it will move to C. And the distribution will occur as I will show you in a little while. So, as you keep on moving the band also keeps on moving here alright.

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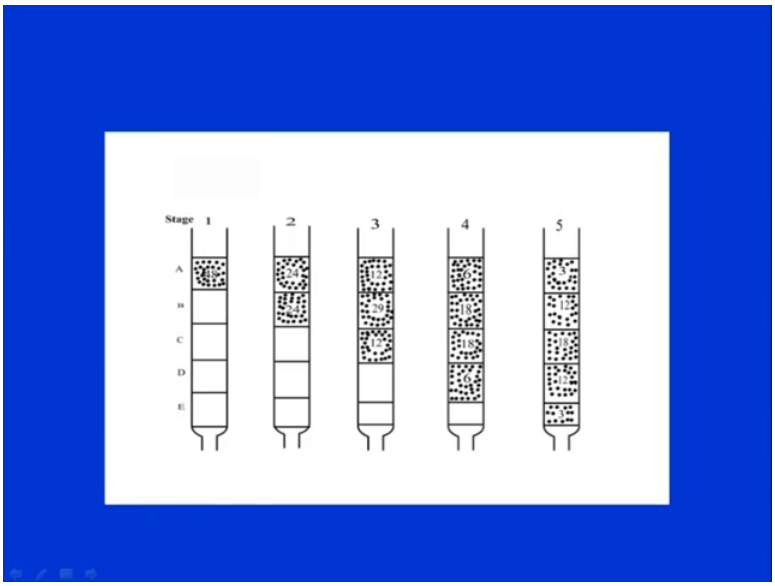

Let us have a little bit try to understand through this figure. You can see this figure there are lot of zones here which are A B C D E. And here you are adding a particular amount of material is being added in here. So, like I do not know whether you can see it here there is a 48 microgram in here and it distributes into 24, 24. Now, 24 in terms here 24 is in the stationary phase and mobile phase and 24 with the mobile phase as you introduce more mobile phase goes down.

And as you move on it will be distributed into 12 24 12 because as you are introducing more mobile phase it will also go down again 50, 50 distribution will take place as distribution coefficient is one. And again as you introduce more mobile phase it will be 6

18 18 6, that is how it will work out. And as you move again you will introduce it will be 3 12 18 12 3. So, you have a central band which is thicker or more concentrated and then you have adjacent bands as they go along they are less concentrated.

So, your analyte moves as a thick or concentrated central band surrounded by less concentrated bands. And this is how the separation takes place. And these were talking about when distribution coefficient is one. Now, this could be a different distribution if the distribution if the distribution coefficient is somewhat different actually. So, what is happening that it is quite apparent that after a relatively small number of equilibrations the compound distributes itself symmetrically within a band.

Like I said it is a central band and there are which is more concentrated and there are other bands adjacent both on top and bottom of the central band, which are less concentrated. So, it should be equally like so what you see is a mixture of 2 compounds one having distribution coefficient of one and the other distribution coefficient of say 100 will be able when they are added to the column. They will be able to separate rapidly into the distinct bands because one will be retained more and another will be retained less.

So, all these things which we have studied so far in terms of capacity ratios, in terms of retention, in terms of say selectivity they will define how a particular analyte is retained, so more is the distribution coefficient or higher distribution coefficient. Then it is retained more and subsequently depending on different analytes having different distribution coefficient will be separated will distribute themselves in the column into different bands and they will be separated.

Now, if the distribution coefficient is very close then it will be hard to separate those two analytes, but if the distribution is quite apart then the separation will be a very easy proposition. So, this is how the whole thing will happen. So, hope you have been able to understand how on a column chromatography separation will take place and the distribution of analytes will occur depending on the distribution coefficients. So, in this lecture what we have gone through is, we have discussed about different parameters which determine performance of a chromatographic system.

And which will determine whether two analytes could be resolved or not like high efficiency columns will have high resolution, and low efficiency columns will have low

resolution. Now, when we say resolution is high also high efficiency columns will have larger number of theoretical plates as compared to the their counter parts that is low efficiency columns. So, if you have like a particular number of theoretical plates then height of the plate will be smaller and number of theoretical plates will be larger.

Likewise, capacity ratio or selectivity will determine the efficiency of a particular column. As we have discussed 3 main factors, which are important and these are selectivity, efficiency and capacity. Capacity is important, because if you cannot purify a particular amount of sample then you have to do it many runs of that. And so it is important that maximum amount of sample could be separated. So, but not all columns are highly having high capacity like say iron exchanges have higher capacity.

Likewise, selectivity is another important term where unless a column could differentiate between very closely related substances, it will very hard to purify them like affinity chromatography has very high selectivity. And likewise efficiency where diffusion and other problems might occur and you know broaden the peak or may result into overlapping of peaks those things has to be taken into account. So, this kind of completes our basics in chromatography.

In the next lecture we will start with we will be explaining about low pressure liquid chromatography and high pressure or high performance liquid chromatography systems also the basic chromatograph that is the instrument part. We will be discussing what the main basic constituents of a chromatograph are and we will be comparing low pressure and high pressure or high performance liquid chromatography systems. And then in the subsequent lectures were going to discuss about particular column chromatographic techniques. And these are affinity chromatography, unexchange chromatography, exclusion chromatography and guest liquid chromatography systems.

Thank you.