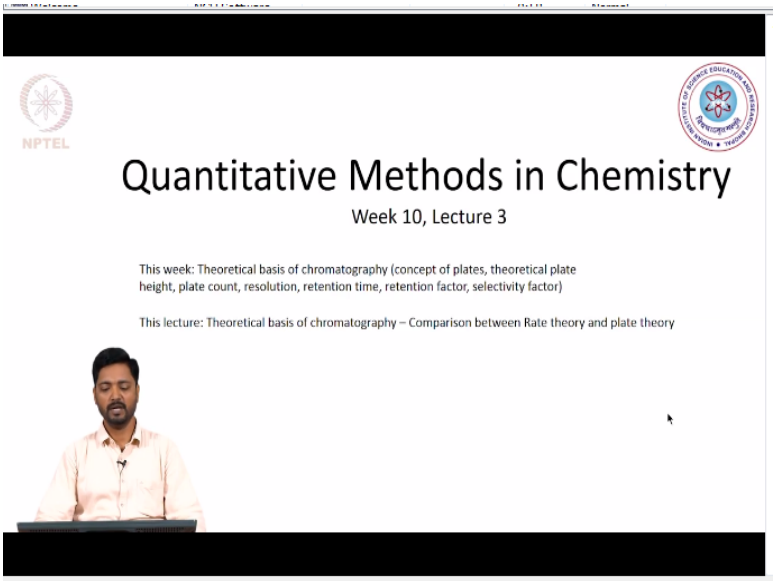
Quantitative Methods in Chemistry
Prof. Dr. Aasheesh Srivastava, Dr. Bharathwaj Sathyamoorthy
Department of Chemistry
Indian Institute of Science Education and Research-Bhopal

Lecture-42
Chromatography-Rate Theory

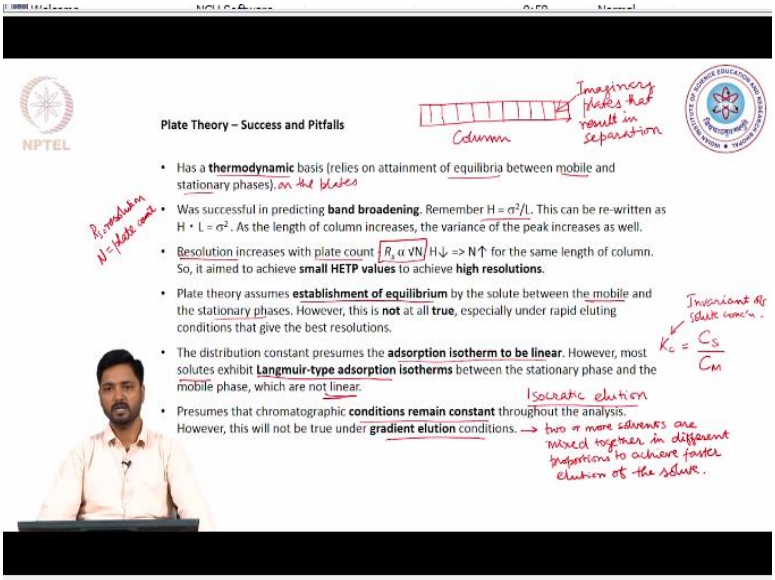
(Refer Slide Time: 00:29)



Hello, and welcome back to lecture 3 of week 10 for this course quantitative methods in chemistry. This week, our emphasis has been on establishing a theoretical basis for chromatographic separations. So we have already covered topics such as the play height, plate count, and concepts of resolution, retention time, selectivity factor and retention factor. All of these have helped us establish publish a certain understanding of how chromatographic separations proceed inside chromatographic column.

In the third lecture, we will continue exploring some of the theoretical concepts that are involved in the chromatographic separations and specifically, we will be focusing on having clear comparison between what is known as the rate theory and compare it with the older plate theory which was already covered in the second lecture of this week. So, let us get started with this week's lecture 3.

(Refer Slide Time: 01:50)



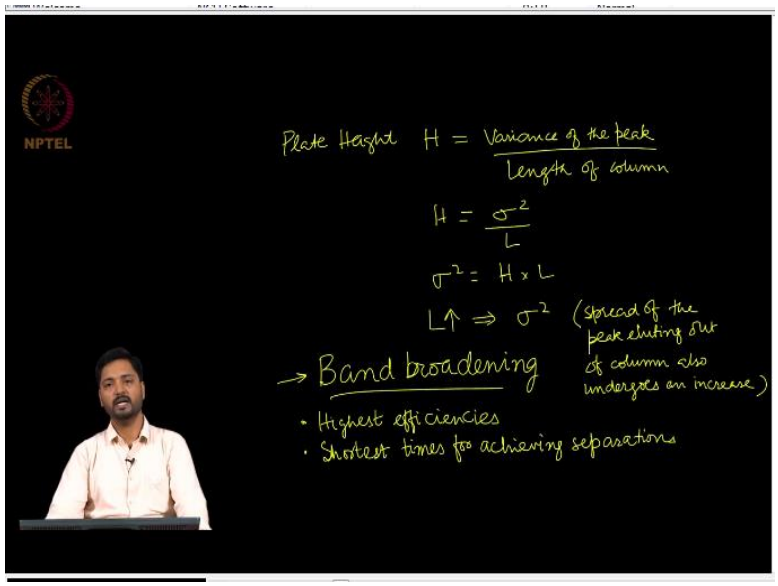
And first let us look at what was the plate theory. In nutshell what was success and where did it not succeed to the extent that it was required. So, as you might recollect, that plate theory was among the first theoretical descriptions for chromatographic separations, it was proposed by Martin and Synge and it presumed that a chromatographic column which is indicated by this rectangle here has multiple imaginary plates present in it.

And these plates result in the chromatographic separations. Now, of course, to increase the extent of separation, we need to increase the number of plates. So, we have a column and we have these imaginary plates that result in separation of analytes. And we need to increase the number of plates to achieve more efficient separation. So, we already saw in the last lecture that a more straightforward way to do this is to increase the length of the column.

However, it turns out to be more practical and more efficient, if we can reduce what is known as the plate height. So, plate theory was based on the thermodynamic concept, that is, it relied on attainment of equilibria between the mobile phase and the stationary phases on the theoretical plates. So, it presumed that on each plate there is an equilibration that happens between the mobile and the stationary phase.

Now, one of the first successes of this plate theory was in establishing that band broadening occurs as the solute passes through the chromatographic column. Now, let us try to understand how this happens and how this is explained in the plate theory. So, remember that we explained the plate height H in terms of the variance of the peak and divided by the length of the column.

(Refer Slide Time: 04:57)



So, let us go to the board and rewrite this. So, what we presumed is or what we established is that the plate height H is equal to variance of the peak that elutes out of the column divided by the length of the column which can be written as sigma square by L. And when we rearrange it, what we get is that the sigma square H into L. So, as L increases, the result is that the sigma square also increases or the spread of the peak eluting out of the column also undergoes and increase.

Now, this spread increase is termed as band broadening. And this is something which we would want to minimize while achieving chromatographic separations, because we want to achieve the most efficient separation within the shortest possible time that is sort of the main goal of chromatographic separations to have a minimal band broadening. So, that highest efficiencies can be achieved in separations and this should be done in shortest possible times.

So, let us quickly rewrite this. So, what we want is highest efficiencies and shortest times for achieving separations. And as we have already seen that band broadening results in compromising both of these aspects band broadening compromises both the efficiency and the time that it requires for elation. We saw through plate theory was that the resolution could be increased if we increase the plate count or the total number of plates present within the column.

And this is discussed in the previous class and what we figured out is that the resolution R s is proportional to the square root N, where N is the plate count. So, R s is resolution and N is

the plate count or the number of plates present in the chromatographic columns. Now, of course, as has been discus repeatedly plate theory presumed that we establish equilibria on each plate between the mobile phase and the stationary phases.

So, the solute equilibrates between the mobile phase and the stationary phase on each plate and this was very clearly challenged and was indeed found not to be true in real life chromatographic separations, which are expected to happen at rapid pace. So, this establishment of equilibrium on each plate is important concept or presumption in plate theory, which was soon found to be not true.

Now, another point where plate theory had sort of a different presumption which finally did not hold true was that it presumed that the distribution constant, the adoption isotherm was presumed to be linear. So, if you recall, what we had written is that the distribution constant was the concentration of the solute on the stationary phase divided by its concentration in the mobile phase.

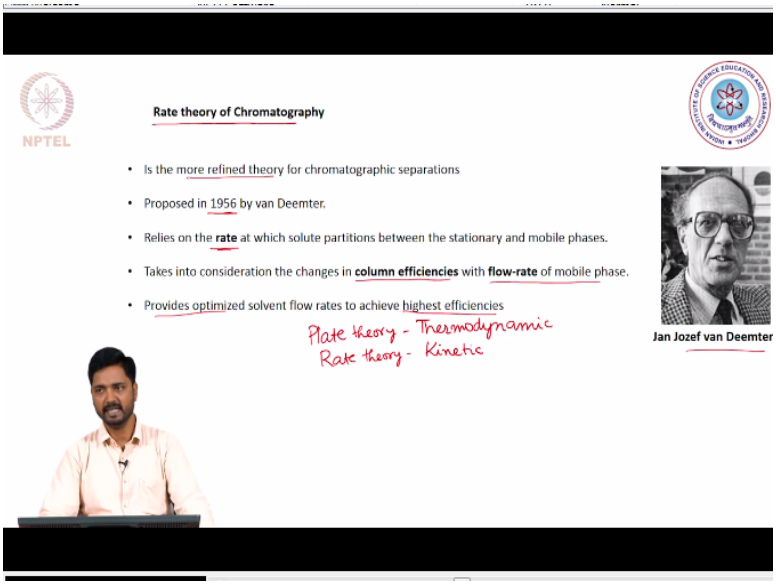
And this distribution constant was thought to be invariant of the solute concentration. Now, this of course, also does not hold true because the distribution constant and adsorption isotherms do change with the concentration. And indeed, what was found was that most of the solutes exhibit what is known as a Langmuir type adsorption isotherms between the stationary phase and the mobile phase.

And if you look out what these isotherms are, you will observe that they result in a nonlinear profile with the solute concentration. So, clearly the presumption that the adsorption isotherm for the solutes is linear in plate theory is found, again not to be true. Now, another point where the plate theory fell short was it is presumption that chromatographic conditions remain quite constant during the chromatographic separations.

This may hold true when we undertake what is known as an isocratic separation or isocratic elusion, but will not be true when we employ gradient elusion conditions where 2 or more solvents are mixed together in different proportions to achieve faster elusion of the solute and indeed gradient elution are very commonly implied in chromatographic separations and of course, are much more dominant way of achieving elusions.

Then, what is the isocratic elusion where only one solvent is passed over the entire course of the chromatographic separation. So, what we observed was that the plate theory, which was the initial theory had some success, but it had also its own share of pitfalls or drawbacks which required proposal have other theories that will be able to explain the chromatographic separations in a better way.

(Refer Slide Time: 14:07)



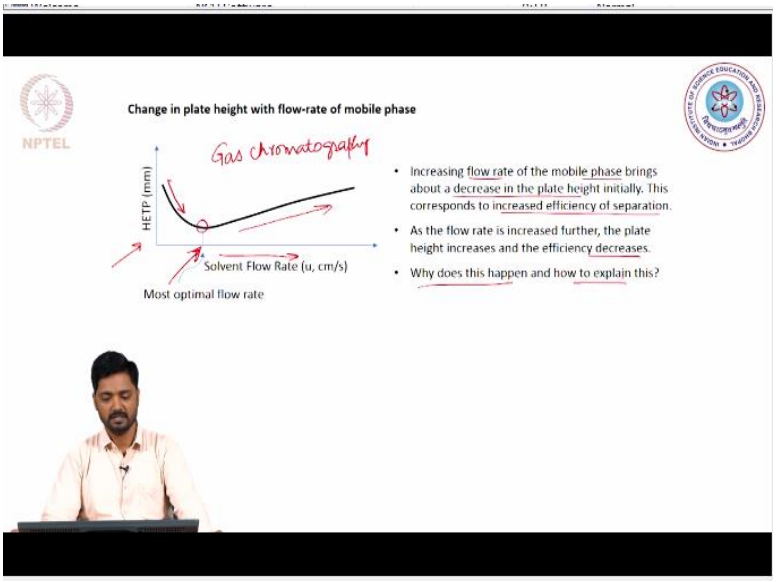
So, this brought us to the rate theory of chromatography. Now, this rate theory was proposed by many different people, but the theory that came to be most famous was proposed by this researcher, whose name was Van Deemter and he proposed this theory in 1956. And the rate theory of chromatography is thought to be more defined or more refined theory for explaining chromatographic separations.

Now, a major difference between the plate theory and the rate theory is that while the former is thermodynamic basis has a thermodynamic basis, the rate theory implies or utilizes the rate at which the solute partitions between the stationary and the mobile phases. So, while the plate theory has a thermodynamic basis, the rate theory on the contrary has a more kinetic basis.

Now, of course, an important consideration in this new theory, which is the rate theory is that it is able to explain and consider the changes in the column efficiencies or the changes in the plate height in our parlons with the flow rate of the mobile phase. Now, please recollect that in the original plate theory, there was no consideration of the mobile phases flow rate, while in the revised rate theory we do consider the flow rate.

And explain how it influences the column efficiencies during chromatographic separations. So, the rate theory also proposes or provides an optimized solvent flow to achieve highest efficiencies of separations in the chromatography. So, let us look at this point a little bit in detail.

(Refer Slide Time: 16:55)



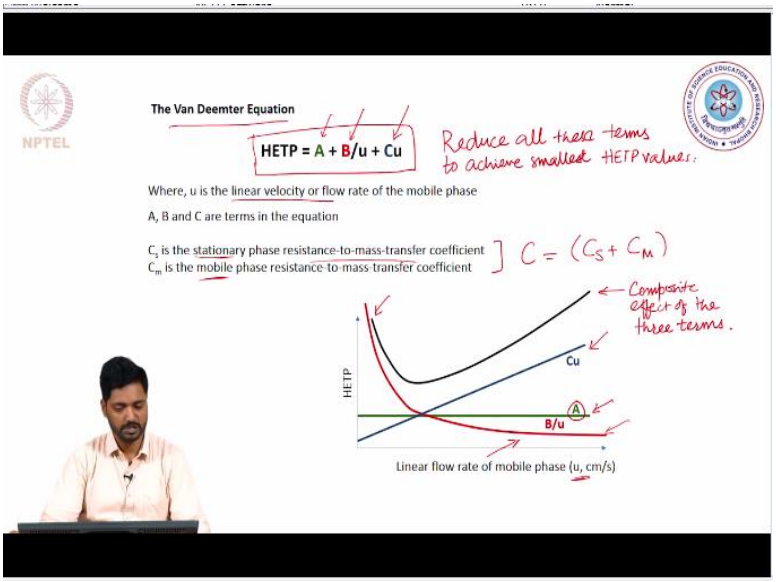
And what we have plotted here on the left is the height equivalent of the theoretical plate, how it changes as the solvent flow is increased. So, this was indeed found to be true for many cases, but is a quite true for seven separations which are undertaken in what is known as gas chromatography. And in the subsequent week, we will be discussing in detail what this gas chromatography is.

So, but what for our purpose what we observe here is that, increasing the flow rate of the mobile phase initially brings about a decrease in the plate height. So, we are talking about this regime where the plate height reduces or the efficiency of the column increases and this is already mentioned in the text here. So, a reduction in the plate height implies that the efficiency of separation is improved.

However, as we increase the flow rate further, which is this regime, the plate height starts increasing again and the efficiency undergoes a reduction. So, this was a common observation and based on that and optimized flow regime was to be used for achieving the most efficient separation. So, people figured out that there is a most optimal flow rate at which our plate height will be the smallest.

However, we would want to understand that why this happens and how do we explain this unique change in the plate height with the solvent flow rate, what are the factors that govern these changes and how do we explain these is what we will be dealing with in the subsequent slides.

(Refer Slide Time: 19:23)



So, Van Deemter equation was able to explain this in a very efficient manner, and the equation that Van Deemter proposed in 1956 was this, that he considered the plate height to be composed of 3 components or 3 terms, the A term, the B term and the C term. Now, u is the linear velocity or the flow rate of the mobile phase and A, B, C are the 3 terms present in these equations.

In the subsequent slides, we will be understanding what these in terms individually are. At this moment, it is important to also remember that the C term the third term in this Van Demeter equation is actually composed of 2 terms the C s and the C m, where C s is the stationary phase resistance to mass transfer coefficient and C m is the mass transfer resistance for the mobile phase.

So, when we start plotting the change in the height equivalent of the theoretical plate based on the linear flow rate of the mobile phase, which we say as you, and see the influence of these 3 terms what we observe initially is that of course, the A term is invariant to the change in the rate of the mobile phase flow. So, A term remains constant at all flow rates of the mobile phase.

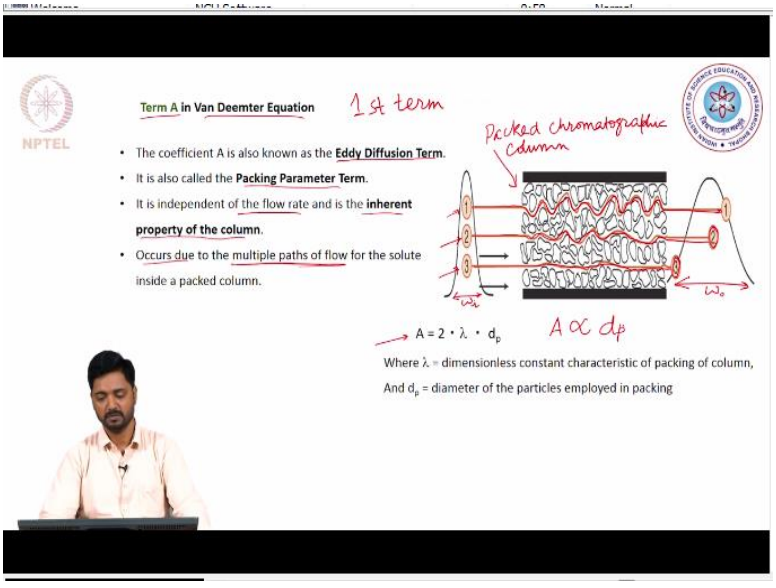
When we consider the second term B, this follows our unique profile, which is shown here, that is at small flow rates, this value of this term is having a very high value and as the flow rate increases, this value undergoes significant reduction and is negligible at very high flow rates. Now, finally, the C u term the third term of the Van Deemter equation will give a linear profile with the change in the flow rate of the mobile phase.

So, we observe that these 3 terms have very different sort of changes that happened to them as the solvent flow rate is increased and a composite effect of these is the actual change in the HETP value or the plate height with the solvent flow rate, which is shown in the black profile. So, the black profile is the composite effect of the 3 terms. So, what we see is that indeed, it mimics the original change in the play tight with the solvent flow in a very decent manner.

So now our aim would be to understand and make a comparison between what these 3 terms are of the Van Deemter equation and how do they influence the chromatographic separations. Please note that our aim is to reduce all of these terms smallest H values or HETP values. So, we want all the 3 terms A B by u and C u to be smallest so that we achieve the smallest plate height values.

So, let us see what these individual terms are and we will also understand how in chromatographic separations we can minimize these terms to achieve the highest efficiencies.

(Refer Slide Time: 24:46)



Now, coming to the A term of the Van Deemter equation, which is the first term, this terms are this coefficient is also known as the Eddy diffusion term and it is also called the packing parameter term and we will understand why this is called first of all let us try to understand what is the origin of this term. Now, consider 3 molecules of our solute, which are injected into the chromatographic column, which is what is known as a tagged column.

So, this is our bagged chromatographic column and the solute molecules are passing through this column under the influence of the mobile phase. So, the solute is partitioning between the stationary phase and the mobile phase and while passing through this column, but what we observe here is that these solute molecules will follow very different parts as they traverse through the column.

For example, the molecule 1 takes a longer path within the column and elutes out pretty late. Similarly, the molecule 2 takes little shorter path inside the column and hence elute out somewhat earlier from the column. Finally, the molecule 3 of the same solute may indeed take a really shorter path out of the column and elute out much, much faster. So, these differences in the path that our solute molecules take inside the chromatographic column will dictate how spread out this peak is.

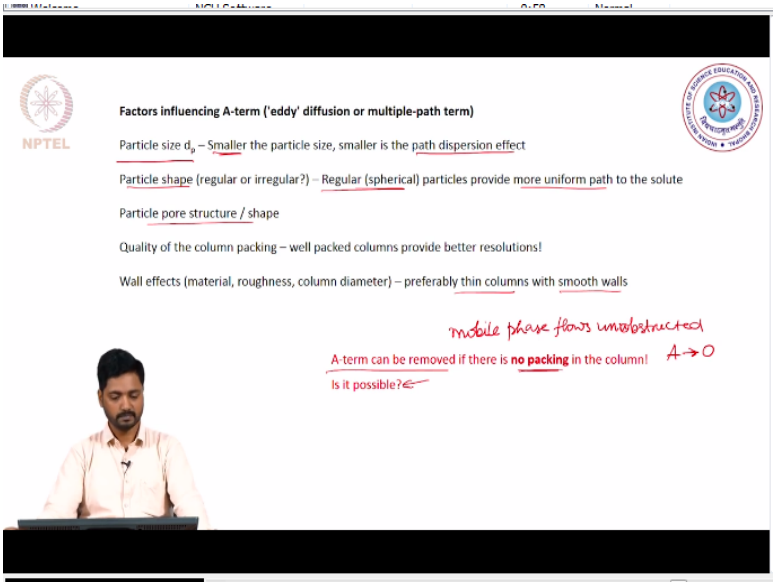
And we have already explained that the peak spread can also be explained in terms of the width at the baseline. So, what we observe is that, as the solute enters the column, it has much sharper profile or a smaller w value at the input stage while at the output stage, the w are the width at the base is significantly increased and this increase is coming mainly because of the different paths that our solute molecules are taking inside the chromatography column.

Now, what people realized is that this A term or A coefficient is independent of the flow rate and the flow rate has no influence on it, we have already seen it in the slide, which are shown previously. And this was considered to be an inherent property of the column based on what material is used and how well the column is packed. So, in nutshell, the term A in the Van Deemter equation comes due to the multiple paths of flow for the solute inside the packed column.

So, the term A can also be expressed in terms of the size of the packing particle that is used. So, the term A is found to be proportional to the size of the particles that are used for packing

the chromatographic column, which is denoted by dp. So, what we would want to do is to use smaller particles, so, that the path differences are minimized and the A term is minimized to achieve the lowest H values or the plate height values.

(Refer Slide Time: 29:43)



What are the factors that influence our Eddy diffusion term or the multiple path term. Of course, we discussed that the particle size has a very clear and major influence on the Eddy diffusion term, and we would want to utilize smaller particles, so that the path dispersion effect is minimized. Now of another point which is to be considered is the shape of the particle, we would want to be dealing with regular spherical particles of very uniform size, which are mono disperse in our terminology.

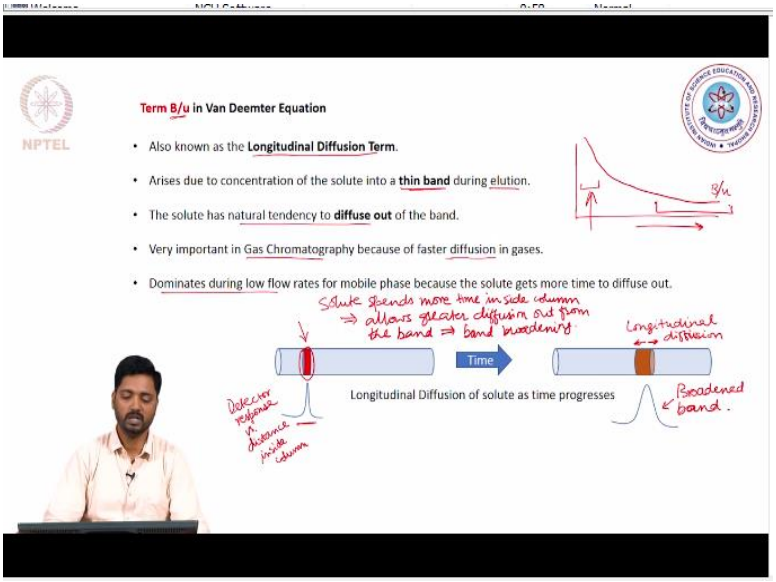
And because they provide a uniform path to the solute, so, the path differences are minimized if our particles are mono dispersed and uniform. Now, other terms that may influences the pore structure and shape of the stationary phase. So, particles present in the stationary phase now, here what we have not considered so far but which becomes increasingly important is that the way these chromatographic column materials are made.

They result in particles which have good number of pores present inside them, and these pores will now result in increasing the path differences. And we would want to therefore, use very uniform particles with a smooth surface to achieve smallest A term values. Finally, the quality of the column packing is also very important indeed in achieving the highest efficiencies, we require columns that are very properly packed.

And there are specialized depredators that are available to achieve the best packing of chromatographic columns. There can also be wall effects which are somewhat minimal, but are still important to be remembered and what we need our columns that are preferably thin and which have smooth walls , the path differences happening due to these is minimized. Now, what we can think here is that the A term can be indeed completely removed from the plate height calculations if we are able to use no packing material in the column or if the mobile phase flow is unobstructed.

So the mobile phase flows unobstructed will result in A term going to 0 which is what will be an ideal case scenario. For today's class, I will leave this question to you is it possible in the subsequent classes, we will see how this is made possible.

(Refer Slide Time: 33:30)



Now, let us look at the second term in the Van Deemter equation, it is also known as the longitudinal diffusion term and it arises due to the concentration of the solute into a thin band during elution. So, please remember that we would want our solute to be present as a very fine band inside the chromatographic column, so if I am plotting the detector response versus distance of column distance inside column.

I will observe a very sharp profile for the solute, which is eluting out of the column initially. Now, as time goes by, we observe that the solute because it is concentrated within this regime would want to expand out of this concentrated region through diffusion, and this will result in a broadened band of the solute. And we already understand that such band broadening is detrimental to the efficiency of separation.

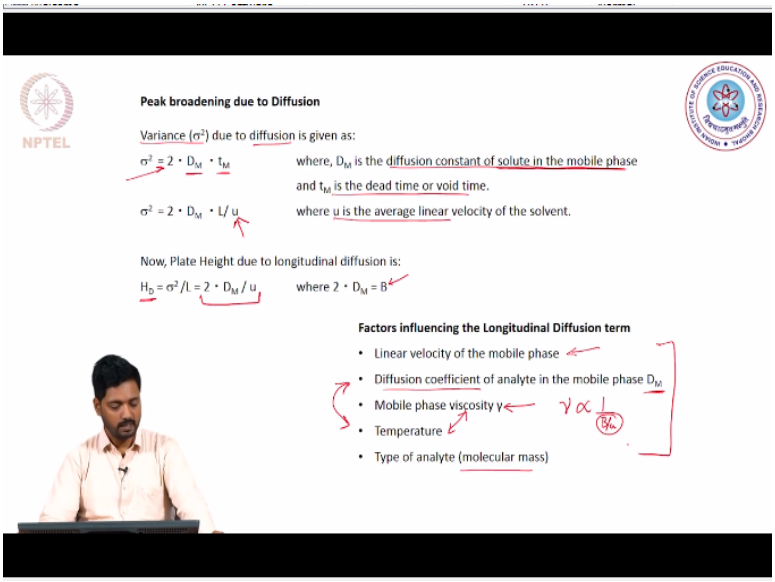
So, what we realize here is that the longitudinal diffusion term arises due to the natural diffusion of the solute out of the band on both sides and that is why it is known as the longitudinal diffusion. Now as we can think about it, the longitudinal diffusion term is definitely much smaller, when we are talking about liquid phase chromatography. However, when we are talking about gas chromatography, this B by u term or the longitudinal diffusion term becomes very dominant term in dictating what is going to be the plate height arising from this column.

Now, that happens because the diffusion of the solute inside the gases is much faster. And on the contrary when we are using liquid chromatography in liquids because of their dense nature, the rate of diffusion is significantly slower and hence the solute will not be able to diffuse out to a significant extent during the short chromatographic separation times that are usually preferred.

Of course, the B by u term or the longitudinal diffusion term is a dominant term at low flow rates, because as we can understand, when the flow rate is small, this was the profile that we had for the B by u term. So, at low flow rates, which is this regime, the longitudinal diffusion term is having a very high value and that is because at low flow rates the solute spends more time inside the column.

And when such a solute gets more time to spend inside the column, it allows greater diffusion out from the band and hence increased band broadening. So, one very straightforward way to reduce the B by u term is to have the chromatographic process happening at high flow rates of the mobile phase, which is this regime where the B by u term is minimal.

(Refer Slide Time: 39:21)



Let us look at what are the peak broadening that happens due to this diffusion term. So, the peak broadening has been explained by us by implying the variance of the peak and this variance due to diffusion is given by this equation here, where we have 2 terms D M and T M. Now, D M is the diffusion constant of the solute in the mobile phase and T M of courses, the dead time or the void time.

Now T M can as well be written as L by u and where u is the average linear velocity of the solvent. When we rearrange all of this, we get the plate height arising due to the longitudinal diffusion as sigma square by L or 2 into D M by u. Now, this 2 into D M by u is actually our longitudinal diffusion term. So, it mainly depends upon the diffusion constant of the solute in the mobile phase.

So, the factors that influence this longitudinal diffusion term will be the factors that govern the diffusion of the solute in the mobile phase. Of course, we have already discussed that the linear velocity of the mobile phase is one of the most important factors that control the longitudinal diffusion term. And we have already seen that we need much higher flow rate to achieve the minimal value for this longitudinal diffusion term.

Now, it also depends of course, on the diffusion coefficient of the analyte in the mobile phase which we have denoted as D M solute that has a higher diffusion coefficient will diffuse out much faster within the same time frame compared to a solute that has a lower diffusion coefficient in the same mobile phase. So, we need to keep these diffusion coefficients into

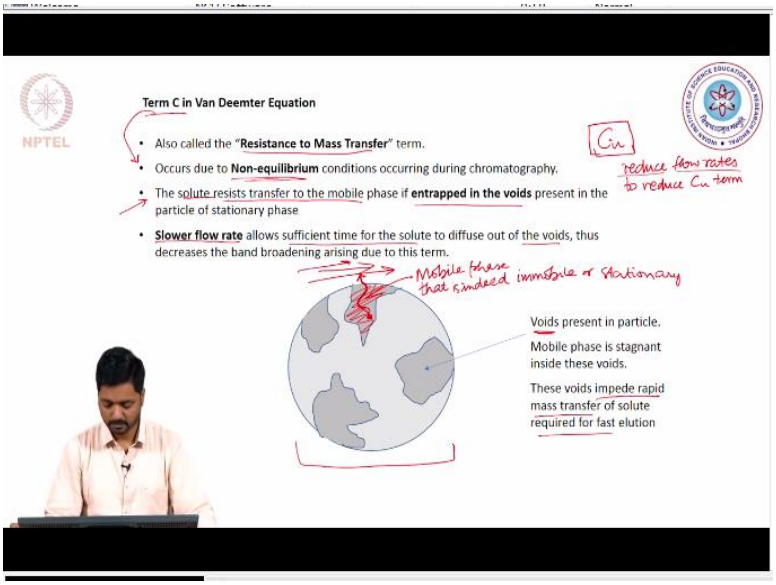
mind when we are undertaking our chromatographic separations, especially in gas chromatographic separations.

The other factor that comes into play is the viscosity of the mobile phase. Now, this viscosity term is in fact, can be considered as a factor that reduces the diffusion into the solvent or the mobile phase and the viscosity value will in this case has negative influence on the B by you term. So what we would prefer is that the mobile phase has a high viscosity, so that the diffusion of the solute out of the band is reduced.

And of course, viscosity and temperature are very correlated and so are the diffusion coefficients and the temperature. So, both of these factors, the change in viscosity or the reduction in viscosity with temperature, as well as the increase in the diffusion with increase of temperature will have it is influence on how the solute diffuses out of the band. And the final factor that we may have to consider is the type of analyte or it is molar mass because molecules which have smaller molecule mass diffuse out much faster than molecules whose molecular mass is much higher.

So, we may want to consider all of these factors to achieve smallest value for the B by u term which is our longitudinal diffusion term.

(Refer Slide Time: 44:30)



Now, in the final week, come to the third term of the Van Deemter equation and this is also known as the resistance to the mass transfer term. Before that it is important to understand that this term arises due to the non equilibrium conditions that are occurring during

chromatographic separations. Now, please note that the presumption in the plate theory was of equilibration of the solute between the mobile and stationary phases.

And it was found that this presumption is quite not true at the fast rates at which elusion occurs through the chromatographic column. And hence, the term C in the Van Deemter equation or the resistance to mass transfer term relies or occurs due to the non equilibrium conditions that are happening due to during the chromatographic separation. Now, this term was proportional to the solvent flow rate and that means we would require much lower flow rates to reduce.

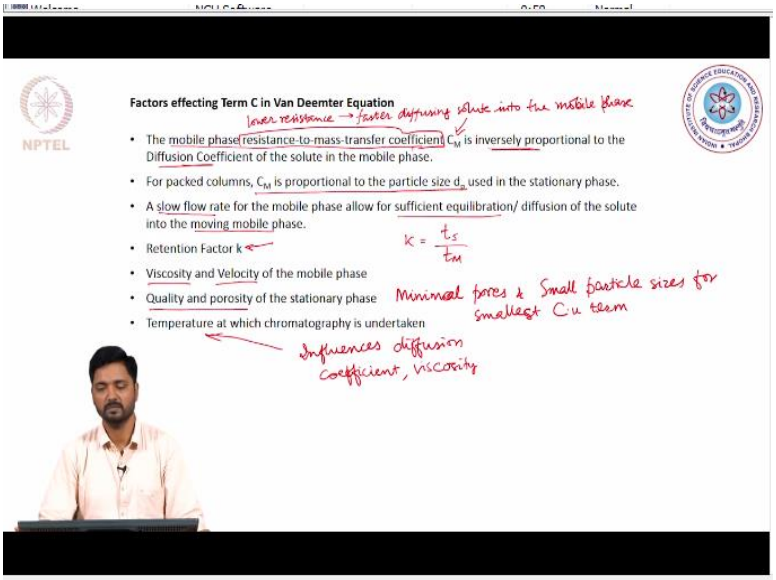
So, we need to reduce flow rates to reduce C into u turn the final term of the Van Deemter equation. Now, this is quite contrary to the previous term related to the longitudinal diffusion, where we wanted to flow the solute out at a very rapid pace, so, that the longitudinal diffusion out of the band could be minimized. Now, why this C term has such a relation with the flow rate is explained in the third point.

Now if you consider the particle that is often used to make the stationary phase inside chromatographic columns. These particles are having voids present in them and these voids are filled with mobile phase that is indeed immobile or stationary. So, these voids impede the rapid mass transfer of the solute that is required to the fast elusion. So, if I have a solute particle which is sitting inside these voids, it will have a difficult time traversing this distance.

And reaching a place where the fluid flow or the mobile phases flowing freely. So, this solute which is entrapped in the voids present on the particles of stationary phase resist transfer to the mobile phase which is flowing freely outside the particle. So, this is again indicated here. So, this particle will take much longer to come out of these voids and get transferred into the flowing mobile phase.

Now, we can reduce the flow rate of the mobile phase. So that we allow sufficient time for the solute to diffuse out of these voids and that will result in a reduction of the band broadening arising due to this term and that is why we have C into u profile for this third term. So, if we need to reduce this term, we would have to reduce the flow rate.

(Refer Slide Time: 49:44)



So, again considering the factors that affect this C term or the third term in the Van Deemter equation. So, we have what is known as the mobile phase resistance to the mass transfer coefficient C M, which we already eluded to previously. And this is inversely proportional to the diffusion coefficient of the solute in the mobile phase that means a solute that can defuse out of into the mobile phase much faster will have a lower resistance.

So, a lower resistance is found for faster diffusing solute into the mobile phase and consequently, if the diffusion coefficient is smaller we will have a greater resistance coming for this mass transfer to happen from the void to the free flowing mobile phase. Now, also for packed columns, it has been found that the larger the particles, the bigger will be the voids present in them.

And hence, C M is found to be proportional to the particle size used in the stationary phase. So, indeed we would want to use smaller particles, so, that the C term can be minimized. And we have already seen that a slow flow rate of the mobile phase will allow for sufficient equilibration or diffusion of the solute into the moving mobile phase. So, indeed here we would want to reduce the flow rates to achieve low values for the C into you term.

The other factor that needs to be considered is this retention factor. And as you can recollect, the retention factor was the time the solute spends on the stationary phase over the time it spends on the mobile phase. So, a solute that has a higher retention factor will also exhibit greater resistance to the mass transfer into the mobile phase. And, indeed the velocity and viscosity of the mobile phase will also alter or influence the diffusion coefficient terms.

And hence, will have an influence on the C term of the Van Deemter equation. Finally, the quality and porosity of the stationary phase becomes important, we would want to have minimal pores and small particle sizes for smallest see in pewter and of course temperature it influences the viscosity of the medium and both of these terms has their own influence on this resistance to the mass transfer coefficient.

This brings us to the end of this week's lectures, where we have established a deep theoretical understanding of how solute passes through the chromatographic columns, and we got introduced to the terms like the plate height, plate count and the Van Deemter equation, which all are used to explain the movement of solute inside the chromatographic column. Now, in the subsequent week, we will be dealing with some practical chromatographic techniques that people employ in their laboratory.

These include the high performance liquid chromatography, gas chromatography, and supercritical fluid chromatography. We will also have time to discuss the detectors that are used in detecting the analyte that is eluting out of the chromatographic column. So, with this, we come to an end to this week's lecture and we will pick up for the next week's lecture. So, thank you.