Course: Adsorption Science and Technology: Fundamentals and Applications

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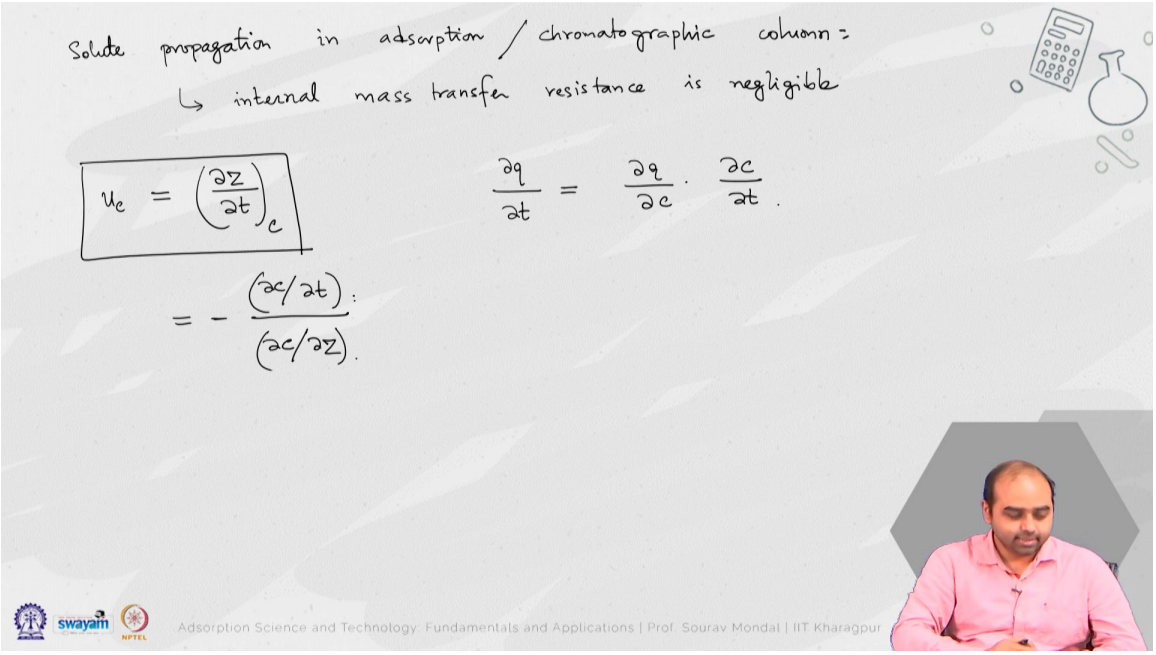
Week 07

Lecture 32 | Chromatography: Principles

Hello everyone, welcome to this second lecture on this chromatography. As you have seen in the last class we have discussed in detail about the basics or the foundations of the chromatographic principle or this how does this chromatographic this operation is performed and what are the different modes of chromatographic operation. So, in this lecture today we will talk about the different principles of this chromatography and how that is important to understand and that can be used for the calculations and to design chromatographic separation in a much better way. So, first let us try to understand what do you mean by the solute propagation or the solute front velocity and from there can we relate the retention time of this species. So, this solute propagation in adsorption or this chromatography column is based on this idea that or this assumptions and that this internal mass transfer resistance is negligible. So, this is generally the idea and the solute you know propagation front or the solute wave front can be related as del z / del t at constant concentration.

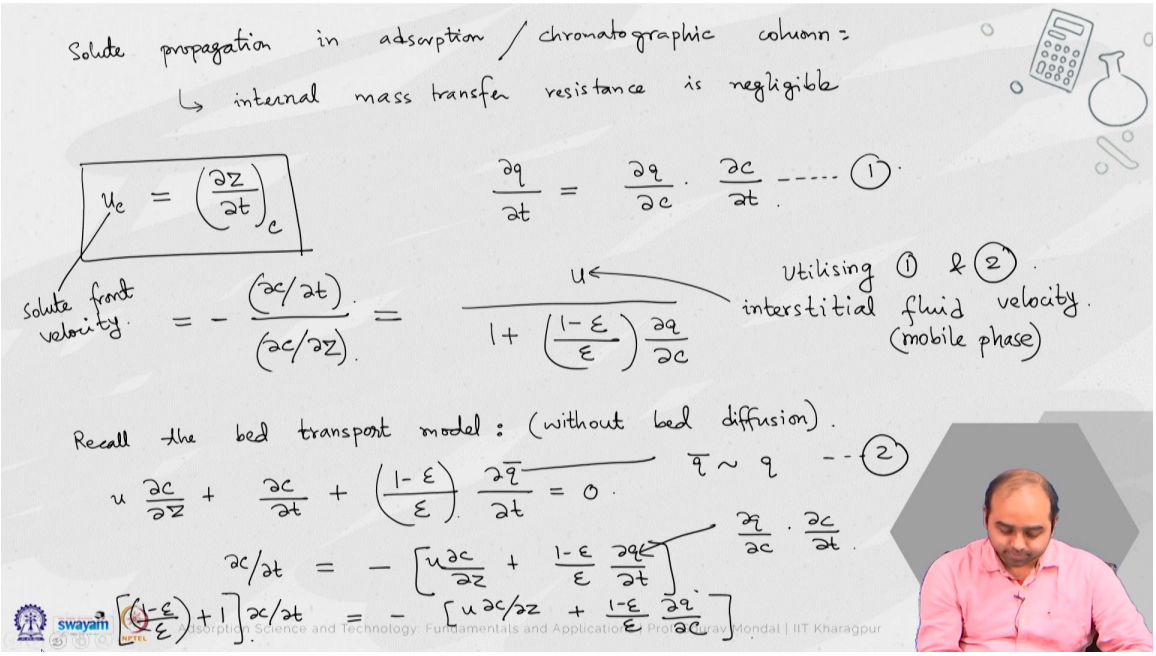
This is what represents the you know the solute propagation velocity. So, very similar to the analysis of the mass transfer zone or the what we have already looked into before which is the rate of the adsorbate concentration in the adsorbent phase or in the solid phase can be you know expanded with the help of this chain rule as something like dq/dc multiplied with dc/dt right. So, this solute propagation velocity instead of dz/dt can be represented as minus del c divided by del C / del Z. Now, this del C / del t can be related to this you know something that we have already discussed before that that can be related to as del q / del t.



So this if you recall the bed transport model you already have u dc/dz plus del c /del t plus. So, without any you know this diffusion in the bed without bed diffusion. This is something which can be written down and without you know this internal mass transfer resistance q bar can be written down as q. So, q bar is the averaged intra particle you know this solute concentration this can be written down. And without any bed diffusion we can ignore the diffusion terms and this is something which can be written down here.

So, with this relation del C / del t, del C / del t can be further you know exploited here to write down in terms of this with the help of this bottom equation. One can write down and taking into account of this del c / del z you can do this further simplification here and this can be written down as u by 1 plus 1 minus epsilon by epsilon del q / del c and this is some algebraic manipulation so utilizing this equation and this equation. So, 1 and 2 you can write down this relation of course, u represents here the interstitial velocity not the super special superficial velocity it is the interstitial fluid or in this case the mobile phase. I will try to explain this little bit in detail that this particular expression was derived from two important assumptions. One is that we ignore the internal mass transfer resistance, we also ignore the external diffusion or the external you know this effects related to diffusion at the bed scale.

In that case the bottom equation from u del c / del z del c / del t. So, del c / del t is actually could be written down. So, if I try to expand this here. So, del c / del t or rewrite this can be written down as minus u del c / del z plus 1 minus epsilon by epsilon del q / del t this is something which is which could be substituted at the top and if you divide this with del c / del z then dq/dt can also be written down as dq / dc and dc into dt and further this can be simplified and you can get or arrive at this expression right. So, this dq / dt can be substituted as dq / dc into del c / del t.

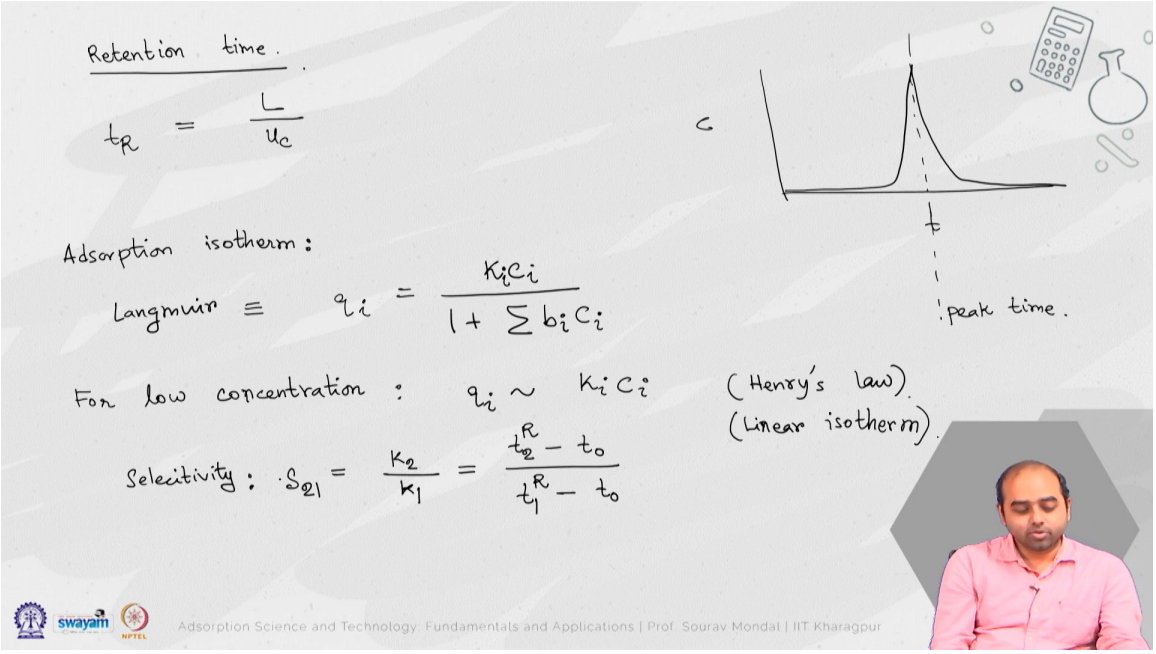


So, then del c / del t turns out to be minus this u del c / del z plus 1 minus epsilon by epsilon this del q / del c and this is also multiplied with 1 minus epsilon by epsilon plus 1 del c / del t. So, this is something that can be utilized in this expression to arrive at this expression of this solute front velocity. So, this is like the solute front velocity within this column. Now, this retention time is an important characteristic and that you know gives a important information about you know how how long does it take for the particular species to be you know present within this bed higher is the retention better is the separation efficiency. So, of course, this retention time or this peak time is is referred as what when the concentration reaches the peak.

So, assuming that there is no dispersion or no diffusion this would again come as a you know pulse output. So, considering that the peak location. So, if you trying to plot the concentration of time. So, even though this is a Gaussian or a distributed you know system this peak time is referred as the time if this is like ideal you know solute front

movement. In that case one would expect this peak time if you recall this you know analysis of this mass transfer zone.

So, the width of the mass transfer zone would be reduced as the mass transfer effects are particularly diffusion and dispersion effects are reduced. So, in that case the you know this this breakthrough curve would actually look like a you know step inputs. In this case for a pulse input in the this solution or within this column the output would also be like a pulse output in this case if this is an ideal scenario if there is no dispersion or no diffusion, but since there is dispersion in the reality. So, this is like a Gaussian output or a bell shaped curve and the peak time reflects the like the residence time or the solute you know propagation velocity is related to the solute propagation velocity. So, now the retention time tR refers to the length of the column divided by the solute propagation velocity u c.

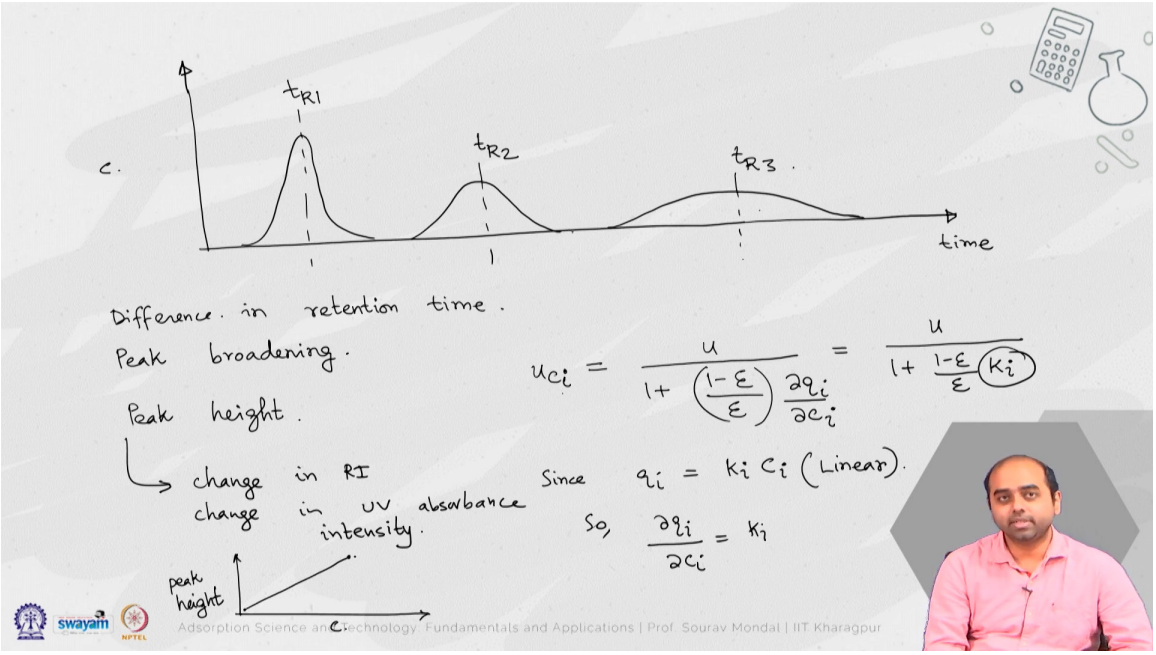


Now, typically the selectivity I mean before defining the selectivity I must also talk about this adsorption isotherm adsorption isotherm. For most systems I mean in this case of you know this adsorption within this column and particularly for highly selective such kind of adsorption this Langmuir type model is used. So, the Langmuir type model is where you have this you know this adsorbed phase concentration in the solid q i is related to, let us say k into c plus 1 plus, this is competitive adsorption. So, I will write this b i c i k i c i. Now of course, selectivity is the dependent on both of these parameters, but for low concentrations which is generally the case and all of these chromatographic application is particularly dealt in low concentration.

Because most pharmaceutical compounds are present in low concentration biomolecules are all of these are generally in very low concentration scenarios. Because for high concentrations you have other modes of separation. So, typically when the mixtures are present in low concentrations that that is where chromatography particularly comes to be very useful and with high selectivity as it is a equilibrium driven process. So, at low concentration, this q, I mean is approximately follows the linear you know adsorption theory this Henry's law type behaviour or linear isotherm. So, now for this case the linear isotherm model is widely used in chromatographic applications and here the selectivity you know is generally defined like selectivity of 2 components S of 2 comma 1 is like K of 2 by K of 1 which is related to the retention time minus some you know base value or reference value with respect to the retention time of this other compound one.

So, the ratio of these two recent retention times is also relates the value of the selectivity for this you know chromatographic applications. Next I want to talk about this you know this band broadening and peak area etcetera on those aspects. Once we see a particular chromatogram, so let us say these are several you know peaks observed here. Now, there is two effects which is seen here in all of this case the velocity v is same or the mobile phase velocity is same, but then in this case we see three different kinds of peak behaviour. So, in one case the difference in.

All of these have different retention times. So this is like tR1, tR2, tR3 difference in retention time that is observed one is peak broadening and another one is this peak area or let us say peak height. So, in these 3 chromatogram profiles or these 3 chromatogram output, there are 3 things that is observed. One is peak broadening, one is the difference in the retention time and one is the peak height. Now, if you recall the solute front velocity is given by this.



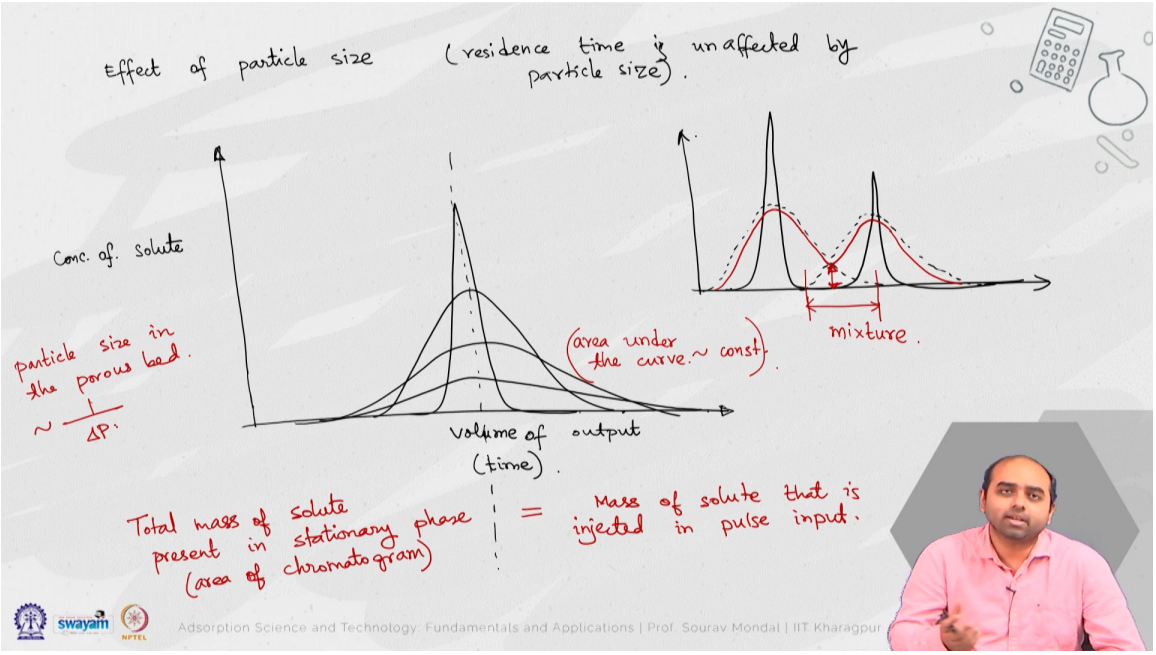
So this is dq by dc. I will write this for i because this is for compound i. So the solute front velocities for different compounds will be different. So for linear isotherm this is Ki since q i is equal to k i c i. So, d q i / d c i is equal to K i linear isotherm model. So the solute velocity for different components depends on the adsorption coefficient Ki and based on the knowledge of the mobile phase velocity or this interstitial velocity of the mobile phase.

So, one can back calculate out from the residence time since the column length is constant for this particular chromatogram you can from the back calculation of the retention time you can one can work out what would be the this particular adsorption kinetics adsorption equilibrium constant Ki. Nevertheless it this difference in the retention time tells us that the relative difference in the affinity of these solutes to the stationary phase so that the the more the retention time the more is the value of this k or the adsorption efficient if in this affinity is more. Lower is the retention time, the affinity is less and it quickly pass through and which also satisfies the physical explanation of this observation. The next important thing is the peak broadening. So, in this case of the peak broadening, this is particularly attributed here to the axial dispersion.

So, the axial dispersion for different solutes is different because the diffusivity of the

molecules are different. Since the diffusivity whatever the porous media structure and the porous porosity etcetera constant for all the solutes. So, it is the diffusivity of the molecule right which is determining here that the level of broadening in this case. So, now the peak broadening can be you know handled or the peak broadening can be reduced by altering the flow rate. So, as to reduce the you know this axial effect of the axial dispersion.

The peak height represents peak height or the peak area in some cases, but particularly it is the peak height which represents the intensity of that particular adsorbate species present in the solution. So, by intensity I mean the concentration. So, the peak height relates the concentration, the peak broadening relates the diffusivity of that particular species within this packed bed column. And the retention time reflects the level of affinity or the adsorption equilibrium constants in this column. So, higher is the retention time higher is the value of this k or this isotherm constant because more is the k lower would be the velocity and the retention time would increase.



In the case of peak broadening for broader peaks this dispersion coefficient or the diffusion is more peak height is directly proportional to the concentration. So, in fact, the level of the peak height. So, the typical chromatogram output that is obtained from this equipment actually produces the you know change in the refractive index change in refractive index or change in UV absorbance intensity, and what is generally used is that by changing this you know the concentration of the solute a known concentration of the solute you track what is the peak height change in that particular equipment that is highly

selective to your column to your flow rate and to the mobile phase composition. Because please note that the solutes sorbent or the solute adsorbent you know this equilibrium relation is you know intimately coupled or this linked to this mobile phase composition this polar solvent this polar groups the presence of the solvent hydrophobic groups all of this plays a big role particle size. you know the packing level, porosity, bed length everything affects this you know this peak height.

So, actually for known concentrations what is generally done is this for different concentration this peak height is actually measured and a sort of linear relation is tried to be established and now for the unknown sample using this correlation between the peak height and the concentration from your known previous you know exercise you can relate from your unknown peak height or unknown sample what is the level of concentration. So, this is a sort of a calibration curve, which can be used to relate the peak height with the concentration for known samples or known concentration. Then the same thing is used as an inverse where for an unknown sample you find out the peak height and from there you find out what is the level of this concentration. But please note that this cannot be related for different compounds. So, this is very specific to a particular species and a particular adsorbent and a particular column and all these are like you know specific or highly selective process.

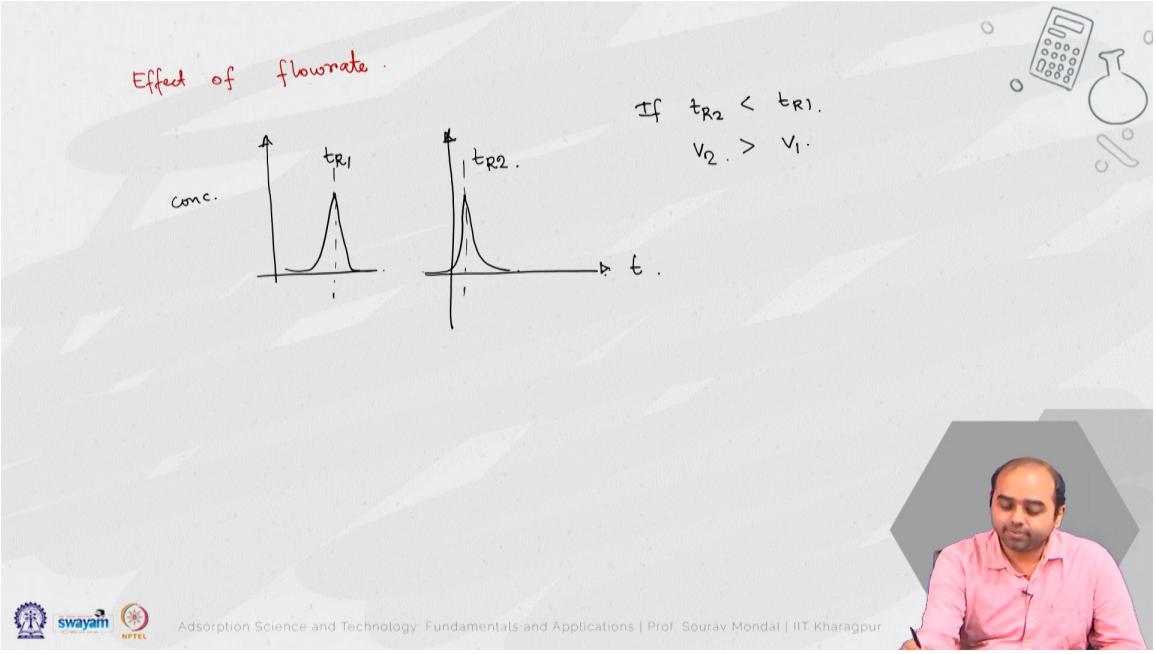
Now, coming to this you know the effect of the particle size. So, the effect of the particle size typically influence, the level of peak broadening. The residence time is unaffected by the particle size. Residence time is unaffected by particle size. And this purely depends on the porosity of the bed as well as the adsorbent and the particular solute equilibrium isotherm constant.

So, if you try to relate it with the, this volume and this is like concentration of solute present in the mobile phase. This could be like volume of output or in other words since the, this will be like a constant flow rate. This could also be the time. Now typically as the particle size is increased.

Sorry. Let me try to draw properly. So, it may be noted that since the residence time is unaffected with the particle size. So, for any particular species if the particle size in the you know column is changed the sharpness of the peak is improved. So, that is the reason

why you know columns are prepared with very small sized particles in the micron range. And there is an effort made to reduce increase you know further reduction in the particle size. So, lower is the particle size the better is the you know this peak formation or this pulse type output behavior or the axial dispersion can be reduced as well as the mass transfer effects in the particle can also be reduced.

So, lower is the particle size better is the you know this resolves better is the output peak. Now, you can argue that what is the problem with the broadening of the peak. So, if the peak is broadened or if there is like this kind of a flat peak then the exit concentration or the difference between two solutes can be overlapping. So, if the peak is sharp then there is less chance or or it is possible to segregate two solutes with very close you know retention time because each of these solutes would be coming out at close you know at in close proximity then the solutes can be differentiated very nicely and accurately. So, what I mean for example that let us say that since the residence time is unaffected by the particle size if the peak is sharp let us say we are having this scenario of a sharp peak, two sharp peaks of two different compounds.



Now if this is produced in a you know in a column where the particle size is large then one would see a peak broadening effect something like this and the second one will also look like this the peak height will not the peak location will not change, but the the curves would be like broadened out. Now, in this case if there are these two peaks there is a

chance of overlapping peak. So, the final output profile you will not be able to see where does this profile actually ends rather you will see output like this. So, two peaks if they are not separated this compounds this suggests that the compounds are not separated and there is a portion here where both the compounds are present. So, separation is not complete and essentially you cannot utilize and you will see in the next class probably I will talk about more about this Gaussian type distribution and its feature can be used for resolving power and other you know things.

So, there the separation is not done and essentially you cannot distinguish these two particles or if you are doing it for production then there will be a small portion where both the species I mean this zone could be anywhere from the here to here. So, within this period there is a presence of both of these solutes. So, the exit concentration at this moment of this time or within this period is a mixture and that is highly undesirable because you want a separation of these two you know compounds or two species in your final chromatogram. Now, one important point even though the you know this particle size effect leads to peak broadening, it must be understood that the area under the curve is constant in this case. So, we suggest that the area the area here is to some extent gives an idea about the total mass of the solute that is present and this area under the curve is nothing, but the total amount of the solute.

So, changing the particle diameter will not change the total you know mass of the solute that is absorbed here and in this case the total mass is something that is already given from the feed information. So, the total mass of solute present in stationary phase is equal to the mass of solute that is injected in pulse, right and this area under the curve is nothing, but the total mass of the solute present in the stationary phase. So, this is area of this chromatogram. So, this is essentially same even though the peak broadens. So, now one can ask that why do not we then go for very small sized particles and why we are what causes the restriction in reducing the particle size.

So, the particle size in the porous bed is inversely proportional to the pressure drop. So, which means that lower is the particle size more is the pressure drop and you need a large pumping capacity to push this liquid or this mobile phase through your column of such densely packed, you know adsorbent particles of such smaller size. So, typically the this for particle size in the micron range or 10 microns or something this pressure drop leads to almost like you know 10 bar of pressure 10 to 15 bar of pressure and that is very significant for you know this chromatography operation because you need a higher

pumping capacity in that case. So, that is what determines that you cannot go beyond a certain particle size even though you know that reducing the particle size can improve the you know this shape of this peak or make the peak narrow instead of getting broader. Before I finish today's lecture there is one more curve which I want to talk about which is the van Demeter curve, but before that let us try to understand the effect of the flow rate.

So, the effect of the flow rate directly affects the retention time. So the effect of the flow rate directly does not change the shape of this curve, but it affects the retention time. So it does not affect the shape or the peak height of this you know this particular solute, but this is like in this case whatever the retention time that is obtained and in this case the retention time that is. So, this these are like 2 chromatograms I am talking about. So, it is one should not confuse this with a single chromatogram.

So, in another case you change the flow rate. So, if these are two you know this chromatograms where in the second case the residence time is reduced which simply suggest that the velocity is increased. tR2 is less than tR1 which suggest that the velocity in the second case V2 is greater than V1 and if the shape and since the shape of the peak is unaffected by the flow rate shape and the peak height is an area and the peak height both are unaffected by the flow rate. This residence time directly affects the you know this velocity essentially the sorry the velocity affects the residence time and this leads us to this point of something known as this van Demeter curve. So, here this plot is made

with respect to the this theoretical plate height which is something known as this height equivalent to a theoretical plate. We will talk about in the next class what do you mean by this theoretical plate, number of theoretical plate, height of the theoretical plate.

So, typically this height, so the number of theoretical plates multiplied with height of each theoretical plates gives us as an idea on the this total height of this column. Now, typically the number of plates is again affected by the time, but it is the height of the plate which also determines what is the total you know column height. So, in this case there are you know this depending on this carrier velocity or the fluid or the mobile phase you can also think of velocity. So, this has 3 contributions I mean essentially the this contribution of this theoretical plate this height equivalent to theoretical plate is contributed by inverse of the flow then it is there is this a constant.

And there is a part which is proportional to the flow. So what are these three things? First is the, you know, this resistance to mass transfer. So that is unaffected by the, you know, flow rate. That is the constant part of the height equivalent thing. So this is like resistance to mass transfer. Then there is a part which is proportional to the flow rate and then there is a part which is decreasing with the flow rate.

So, now particularly the portion where it is decreasing with the flow rate is related So, the part which is related to the flow rate is attributed to the this convection and the or the you know this convection due to the solid loading. So, this is like the solute effect of solute velocity and this is the part which is related to the adsorption So all of this which affects the you know height equivalent of this theoretical plate this tells us that there is a optimum you know curve. which exist and the minimum height equivalent can be obtained for a particular carrier velocity. So, it suggest that if you are operating at this particular carrier velocity at the minimum carrier or the minimum you know for this particular velocity the minimum h e t p is obtained. This suggest that for a particular separation if you if you are operating with the particular velocity then that would be like the minimum you know this column length that you would require to achieve this particular separation in that case.

So, I think so this is known as this van Deemter curve and this is very useful for design of chromatographic columns and particularly fixing the carrier velocity in this case. So, I thank you everyone for your attention. I hope all of you found this lecture useful. See you everyone in the next class.