Course: Adsorption Science and Technology: Fundamentals and Applications

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Week 07

Lecture 31 | Introduction to Chromatography

Hello everyone, welcome to this week 7 of this course. In this week we are going to focus mostly on chromatography. The chromatography is a very powerful and fine separation technique of very high selectivity. Now chromatography is based on the principles of adsorption science and this is used or practiced widely across the pharmaceutical industries particularly for separation of high purity compounds or separating compounds with very high purity producing compounds also with high purity detecting compounds of biomolecular nature as well as you know various complex molecules and this chromatographic process is actually performed both liquid as well as gaseous phase. Now, before we delve into this chromatography, its details, its mass transfer design, I would like to introduce to the different types of chromatography that are available first and then we talk about its background and how does it operate, what are the key components, the equipment related to this chromatography and everything. So, chromatography is possible in liquid and gas states as I said.

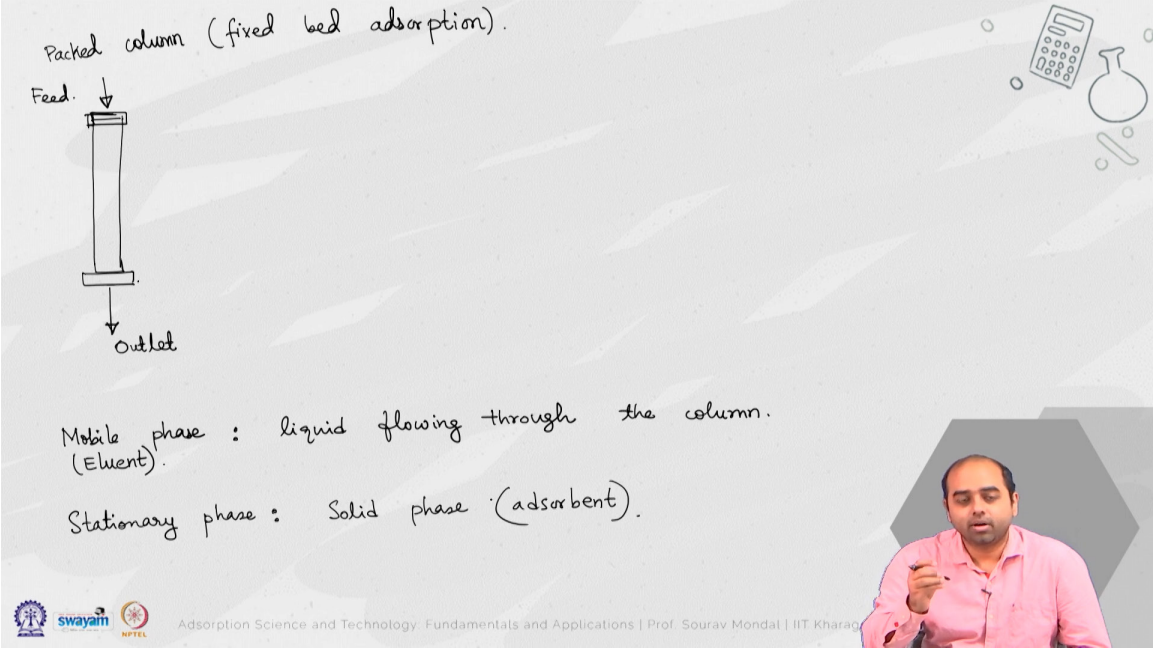
So, normally the liquid based chromatographies are known as high performance liquid chromatography. So, you already have a equipment in the name of HPLC high performance liquid chromatography. Then there is gas chromatography, then there is something known as ion chromatography which is again a liquid based chromatography system, but then it is particularly designed for separation of ions or ionic compounds. Then there is gel permeation chromatography which is based on the principles of you know the formation of this gel and to some extent on electrophoresis also.

Then, there is thin layer chromatography. So, thin layer chromatography is also popularly known as TLC. So, this TLC is actually a paper chromatography based systems where it provides at least qualitative results for this chromatographic separation and

application. Now coming to this background of this chromatography what is normally happens here is that let us consider this to be a packed column or a fixed bed column. So let us consider this to be a packed column, column or a very similar to fixed bed adsorption.

So, in this packed column these are like the two end caps there is a feed and there is a whatever you get at the exit or the outlet. Let us consider top as inlet, bottom as outlet. So, there is something known as this mobile phase and something known as the stationary phase. So, the mobile phase is generally referred to as the liquid or the fluid which is flowing through this you know this column, liquid flowing through the column. It is also known as the eluent mobile phase is also known as the eluent because you are eluting your you know your column or this elution is done in the column with the help of this mobile phase.

There is also known as this stationary phase which is the solid phase, essentially the adsorbent right. So, there are two I mean these are the common terminologies which is used in the you know this chromatography applications and its process. So, normally you the mobile phase contains the adsorbate species that you want to separate or that you want to you know remove from this stream. Now, as you are elevating your column these adsorbate species get bound or bind in the column at with different levels of affinity. So, let us say you have a particular species let us say market with green which is fed along with the, this mobile phase are injected along with the mobile phase are introduced into the column.

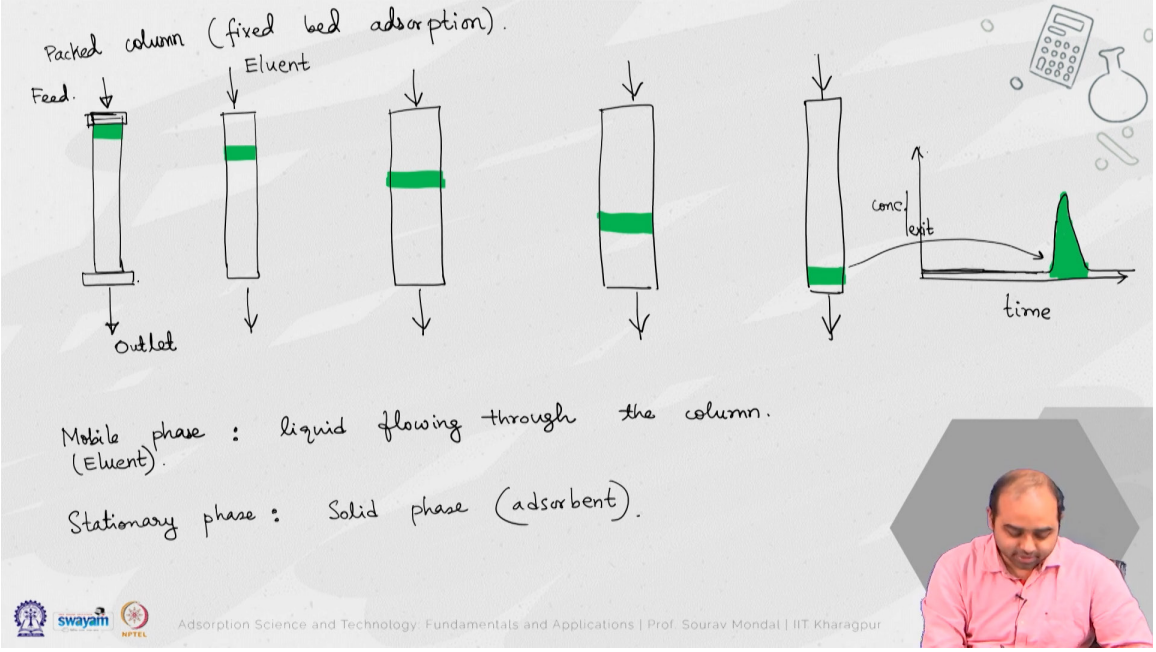


This will get bound or the column will get partially saturated by that particular you know this component or this adsorbate species. Now, if this is a pulse input that means, I inject that adsorbate species as a pulse not continuously it is something that we are talking different from the normal fixed bed adsorption, where to achieve a breakthrough profile you continuously inject or introduce the adsorbate species through your feed. But here instead what you do is that you introduce this adsorbate species or the target molecule into your system you know with as a pulse or at a fixed point in time a certain amount is injected. It is not like I am injecting that continuously. So, the mobile phase is injected continuously, but the adsorbate species which is of course a mixture of the particular component in the mobile phase.

For example, let us say that the mobile phase is water. And the adsorbate species that we want to selectively remove or you know get introduced into the system separate or whatever is like glucose. Now, I am so the water is running continuously in this column and the stationary phase or this adsorbent phase does not have any affinity towards this water. So, there is a continuous solution of this column with water.

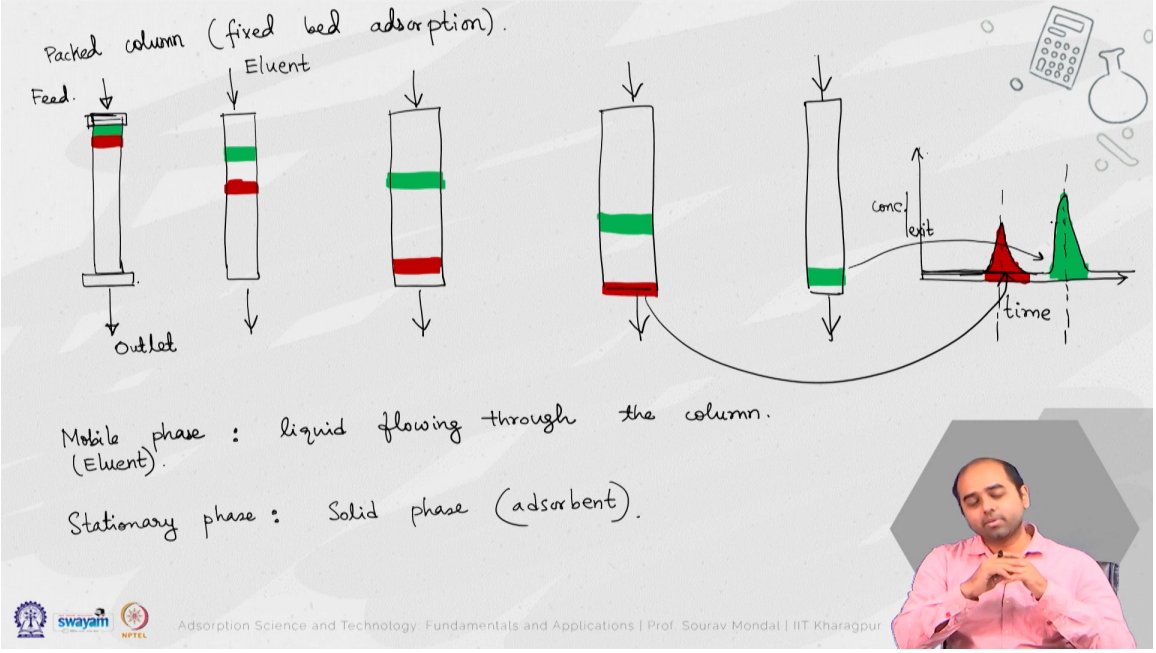
Now, at a certain point let us say t = 0. I inject a pulse of glucose of a particular concentration into this column along with the mobile phase. Now, when once this in this glucose is injected as a pulse what will happen is this since the column is particularly selective towards this mobile phase it will bound or it will bind or get absorbed in this column. So, let us I am call this portion of this green region as like the glucose that is absorbed in this fixed bed column. Now slowly as this you know this column is

continuously muted with this mobile phase where now once the glucose is introduced further glucose is not introduced is only water or the only the element phase or the mobile phase is introduced what happens is that after some time or slowly with this operation let us see the next instant this in this column as continuously you are having this element I will draw some more pictures to get a very clear view. So, this glucose that is already or whatever this particular adsorbate species that is introduced, goes or now slowly shifts it into this column something at towards the bottom region.



So, what happens is this with continuously with the pollutant since the chemical potential in the pollutant is much less or is very low compared to the potential in the column. This there will be desorption or this back diffusion or whatever back mass transport from the column phase to the eluent phase, and then as the eluent moves to little bit lower or to the next step this glucose will be again attached to that particular zone or particular region so like that this glucose even though it is present initially with the continuous flow of the mutant this will be continuously get dissolved and adsorbed dissolved and adsorbed disappear and absorb unless it moves throughout the column So, there is something like a continuous band or this continuous movement of the solute front within this column right. So, like this it will continue to propagate till a time is reached when it will go out or it will be actually removed from this column. So, it is not like that column will be remain saturated for infinitely. Since there is an eluent phase flowing continuously in this system, the adsorb adsorbed molecules or the adsorbate species will be continuously getting adsorbed, desorbed, adsorbed, desorbed across various zones.

Zones means across different you know cross sections or across the length of this column till the point it flows out. So, it is not like this is bounded you know completely or it is like a there is a there binding of these molecules to this bed or the bed is completely adsorbed and it cannot be you know desorbed or regenerated whatever. The same element helps in desorption of this bed simply because this attachment and detachment is based on the principle of chemical potential or its particular selectivity and affinity of this particular solution or this adsorbate species with respect to this. or the mobile phase. So, of course, the choice of the mobile phase is also very crucial.



So, as to ensure that there is you know continuous separation or this mass transport between the element phase to the adsorbate phase. So, if you try to draw the profile of how this glucose or whatever this green band looks like in the input. So, you can see that. So this is like time and here you have this concentration at the exit. So, if that is tracked.

So, typically the concentration at the exit is tracked with a detector generally you have a RI detector or a UV detector. So, which this continuously tracks that if there is any change in the absorbance pattern or the refractive index of this mobile phase going out of this column and these are highly sensitive and selective and that is how you can know that where a particular species is coming in the output outlets at any particular time and that intensity or whatever this signal of the absorbance or the change in the refractive index can be related to the concentration that is how the principle of any spectroscopic technique works or any you know imaging technique work that you try to correlate the change in the optical properties either it is absorbance, it could be fluorescence, it could

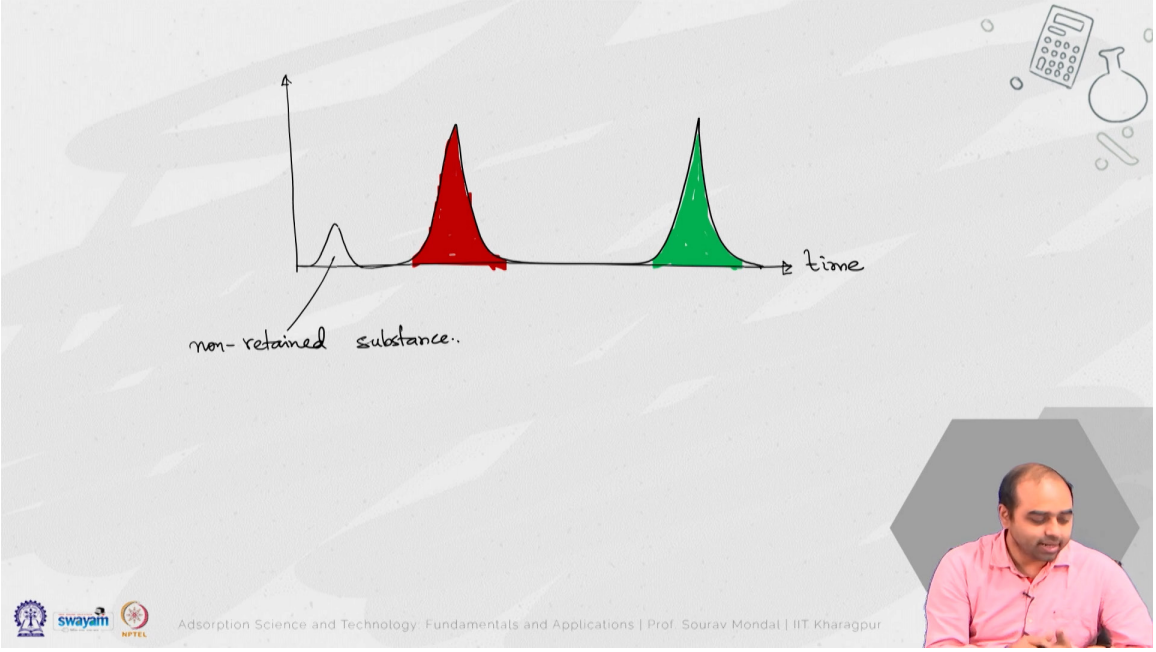
be refractive index and that could be related to the concentration of that solution. So, same happens here continuously as the mobile phase flows out there is a change in the optical property which is tracked by this RI detector refractive index detector or the UV detector. Now, it is I mean you realize that until this the solute band reach the exit right this there will be no change in the concentration and concentration would be like 0. So, there will be 0 value till the point when this particular scenario arrives when the solute band almost reaches the end of this column.

So, the solute velocity or the solute front velocity in the column is very important and it of course, depends on the you know this equilibrium isotherm also as well as the porosity of the bed and the linear flow rate everything or the flow rate of the mobile phase also. you know concentration peak or whatever this peak of this particular solute and again as it goes out this will be tending to 0. So, this particular peak that is obtained is for this particular species this green species. Now as you understand this flow rate or the solute propagation velocity in this column depends on the relative adsorption affinity of that molecule in the stationary phase. Now if there is another particular you know solute which is also mixed along with feed by feed I mean the injection of this particular you know adsorbate species then initially that will be adsorbed.

You know just at the same position as this particular thing, but then again relative depending on its relative affinity with adsorbate species this solute velocity can be faster than the green one. And perhaps when the still this you know this green solute is within the column this red solute whatever this other solute can actually go out. So, in this case the there will be another peak. Now this peak height etcetera depends on the concentration of that other species but it will happen at a different time right. So it will happen at a different time than this green solute.

So I can relate this time like here and in this case the red solute is actually leaving the system. So, like this if there are multiple solutes depending on their relative adsorption you know affinity in the column this can be selectively separated at the exit. So, what I mean that if you stop the operation, at a time after the red solute is removed then you know that the red and the green are separated and you will realize that it is possible to separate these two compounds in the mixture present in the mixture with high degree of selectivity or high degree of separation efficiency in this column. So, clearly you see a portion here this portion can be looked into where you know the difference between this time and the difference between this time larger is the difference better is the resolving

power of this unit. Now of course, if the this can be improved by increasing the length of the column, by reducing the flow rate of the eluent or you can also change the adsorbent phase of the solid phase because particularly the this velocity or the solute frontal velocity is dependent on this adsorption coefficient or this adsorption curve sorry the adsorption constants.

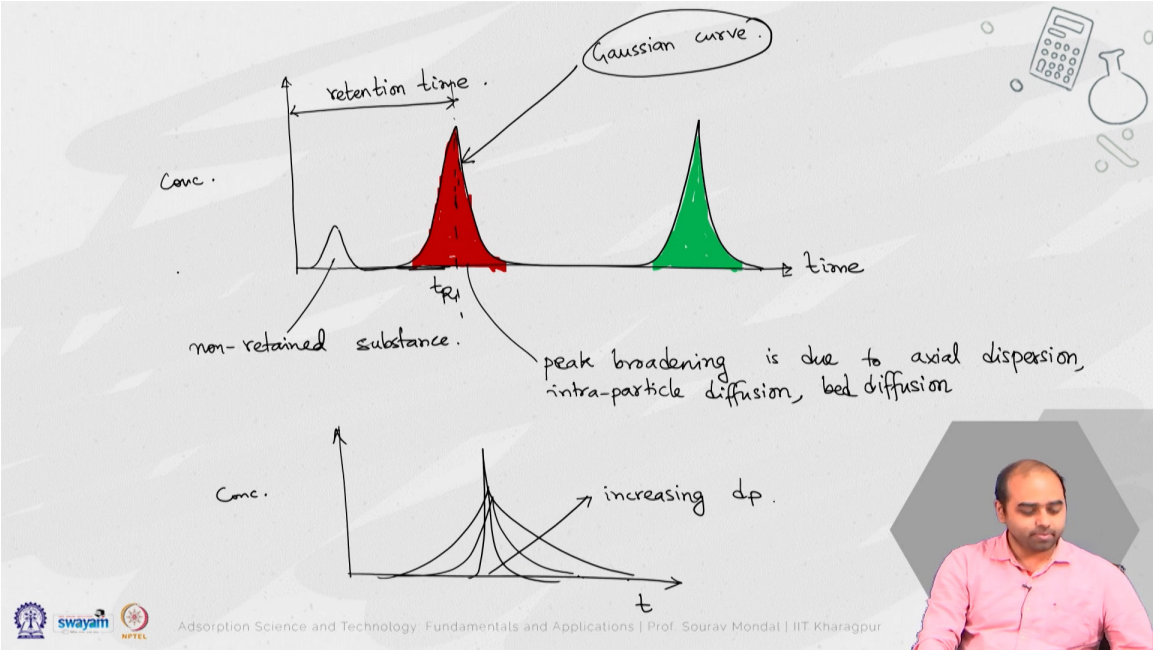


Now, if there is more than 2 species if there are multiple you know species present in this column then they will also can be separated or there could be a possibility that they are not very accurately separated and sometimes you get 2 of them even present in the mixture. So, there is overlap of this peaks we will talk about those something later on in this week. But it is possible to selectively separate this by separating by you know by this chromatography which is essentially nothing but an adsorption principle within this column. So, typically what is important to note here or to understand here is this the retention time. So, for a particular solute the retention time is very important is something similar to the residence time, but then again residence time is more for fluids in a particular column.

The retention time means that how long does this particular adsorbate species. stays within this column of the specified dimension and for this particular flow rate is very important. So, the higher is the difference of this retention time between the solutes more is the resolving power or the resolution of this chromatography system. Now of course, many a time during this chromatographic you know this application there is something known as this if I try to analyze the peak information many a times it is seen that

immediately at the start there is a small peak. So let us say that this is the green peak or for that green solute that we discussed in just now.

And this is let us say for the other solute. Now this peak height and area is something that we will talk about later on, but it of course depends on the concentration of that particular species present in the pulse introduction. But there is this small you know this peak which is many a times present in the start of this chromatography. So, these peak suggest that there is a presence of non retained, non retained substance present in your mixture which is of course detected but then again this is not separated because it is not even you know staying within this column and it comes out instantaneously along with the relevant phase so it is not bounded or you know the affinity of this stationary phase towards this non-written substance is not there so this non-written substance suggests that it does not have any affinity with the stationary phase and it actually goes out along with the mobile phase So, typically in the case of only adsorption to be dominant the ideal you know shape of this curve would look like a like a pulse output. So, very similar to how you have injected a pulse input.



You would get a pulse input sorry pulse output in the chromatographic or the chromatogram that you analyze at the exit. But normally this is known as a perfect pulse, but normally that is not achieved. So, this whatever the output that is seen is generally broadened because of axial dispersion or diffusion. So, this peak broadening, is due to axial dispersion, intra particle diffusion, then you have this axial dispersion or bed

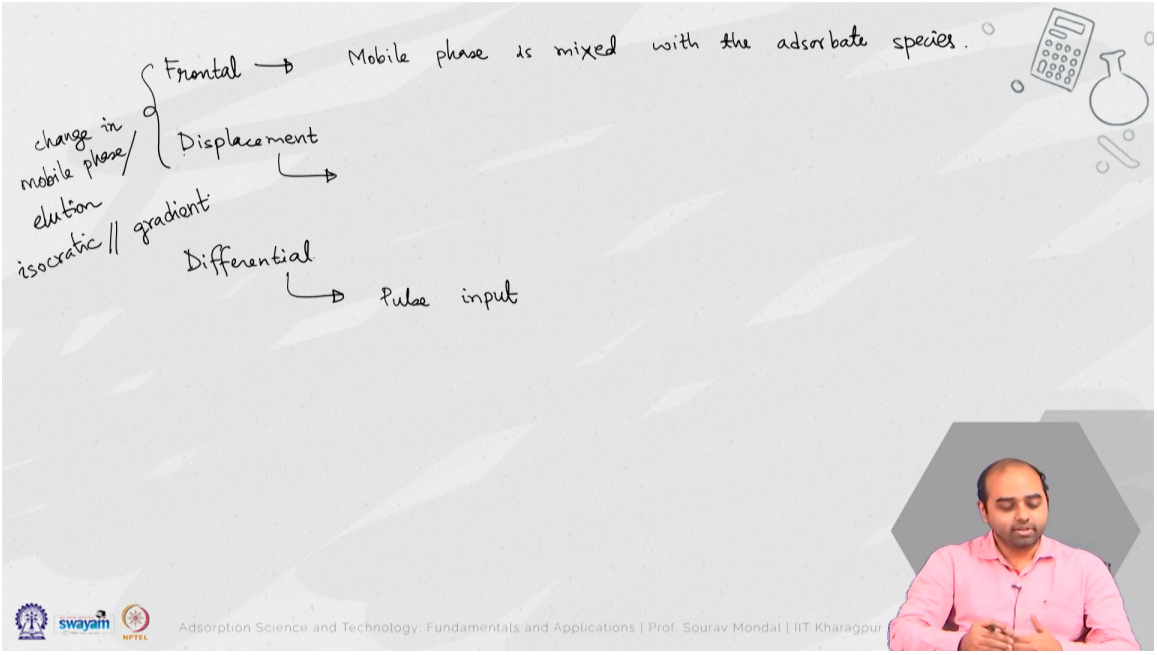
diffusion. So, all this diffusion and diffusion related mass transfer effects leads to broadening of the peak.

So, more is the diffusion. So, you can clearly understand that more is the diffusion more is the peak broadening. So, if you increase the particle size in your column for the same solute one can expect something like this. So this is like increasing particle size. So the retention time would not change because the retention time depends on the particularly on the this affinity and the flow rate of the mobile phase. But this broadening of the peak is because of this actual dispersion and that is the non-ideal situation I would say in this respect.

So, the sorry the retention time is generally referred to as the this peak position. So, this is like the tr, or the retention time. Now, this curve that is obtained in the chromatogram is a Gaussian curve. So, essentially the chromatographic system is based on this idea there is a mathematical relation that the solution or this you know diffusion of a peak sorry of a pulse input in a adsorption column produces a Gaussian type distribution at the outlet of this peak of this pulse input and that is mathematically established something that will talk again in the later. But please understand that this nature of this curve or this output curve that is observed here is a Gaussian curve and all the properties or the statistical properties of a Gaussian curve can be you know leveraged to understand or to relate different you know properties and you know this analyzing the separation efficiency and other aspects of this system.

One more thing I would like to highlight here that typically this column separation in this chromatographic systems are of three types. One is known as this frontal, another one is this displacement type and another one is this differential type. So in the case of the frontal you know chromatography or this frontal absorption profile this is generally used for purification of small molecules or biomolecules mostly in biosystems and the mobile phase is actually fade continuously. So all these things are same but the important aspect is that this the mobile phase is mixed with the adsorbate species, that instead of introducing as a pulse input of the adsorbate molecules along with the mobile phase, the mobile phase itself is mixed with the adsorbate. So, this is very similar to the kind of fixed bed operations that we have seen in the last, last to last week where you are introducing your feed into your system which also contains this adsorbate.

So, here also the adsorbate species is present in the mobile phase or you are continuously injecting the adsorbate species, till a point unless the bed is completely saturated or reached equilibrium. So, how do you detect? So, you start to get the adsorbate species at the exit. So, it is something like you achieve a breakthrough curve. Now, once this breakthrough is obtained, next what is done is that you try to introduce another mobile phase which facilitates the desorption from this particular bed. So, in this way now if your particular bed is more selective towards a compound Y or towards a particular compound between a mixture, then that will be adsorbed within this bed and the other compound will actually flow through this bed.



Now, in the second step when you use a another you know medium to another mobile phase something another event phase to actually desorb this molecules that is already adsorbed by this phase. You you know remove or you do this desorption from the adsorbate from the adsorbent and then that is actually recovered separately from the mixture and you get high quality separation of that compound from the mixture which in other techniques may not be possible. Because please understand that in all of these processes the selectivity of the adsorbent phase towards a particular you know adsorbate molecule is actually what is exploited and that gives very high selectivity. This displacement method is also very similar to this you know this frontal technique except the difference that in this case that in this case instead of having you know one column you generally try to, you try to use you know more than one column or particularly multi column arrangements where one column is in a you know kind of regeneration or

recovery mode another column is in you know this generation mode instead of you know doing this batch wise there is more of a continuous application using multiple columns probably is something we will talk about in much more detail in the forthcoming lectures. Differential is something very similar to what we just discussed that you introduced a small pulse input and that pulse input actually flows or you know this solute front moves through this adsorbent bed.

So, here you use a pulse input that flows through this bed. So, rather than saturating the entire bed similar to frontal or displacement modes you try to elute the bed with the mobile phase without the adsorbent and slowly this pulse input propagates through this bed and at the exit you get a this Gaussian type distribution of the solid concentration. So, both the frontal and the displacement you know modes use a change in the solute change in mobile phase or change in this elution properties either by changing the pH changing the you know this ionic strength of the solution it could be like an isocratic mode or it could be an gradient mode by gradient and isocratic mode I mean isocratic means the properties or the concentrations whatever this mobile phase that is used is constant. Gradient mode is something that where you try to increase the particular property. Let us say if you are using the ionic strength, so the ionic strength is continuously increased in a gradient way or you can increase the, you know this flow rate through this column.

So, that maximum separation can be achieved with minimum time. So, all of in all of these cases mass transfer does play a big role along with adsorption, but these are generally the different types of chromatographic operations that is used for specific applications either for separation, for detection, for production or in sometimes for even for you know this purification of certain compounds. So, I hope all of you got a fair glimpse about the basics of this chromatographic. In the next class we will talk about more on the fundamental principles and the you know this mathematical framework on how these processes can be described. Thank you and see you everyone in the next lecture.