Downstream Processing
Prof. Mukesh Doble
Department of Biotechnology
Indian Institute of Technology, Madras

Lecture - 32
Chromatography Continued

So, let us continue with the topic of chromatography and how to model this particular pack bed system and what are the issues while modeling. So, typically if you took at a chromatography you are going to get your Gaussian type of peak leaving the column and this is called the retention time, the t naught and this is the maximum concentration it reaches.

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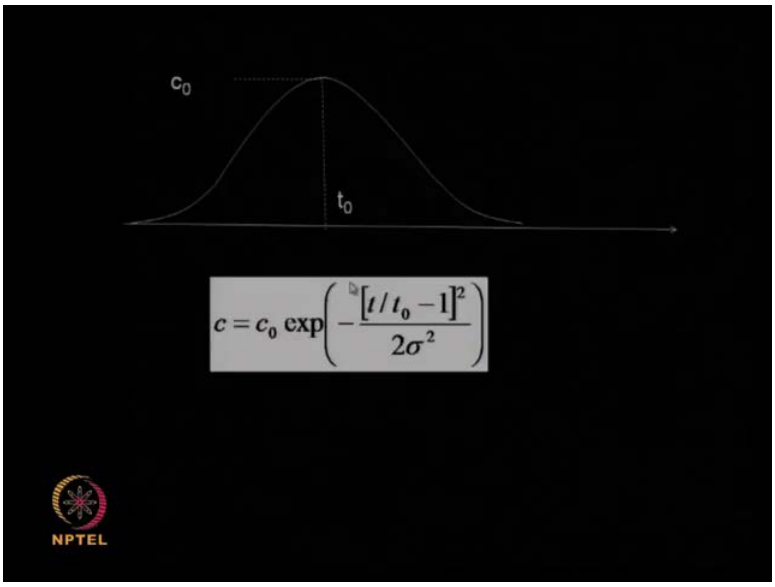

And which normal distribution or which Gaussian distribution then we said this is the equation for a Gaussian distribution, you have a c equal to c naught exponent minus t by t naught minus 1 whole square divided by 2 sigma square. Now, t naught is your retention time and the standard deviation for this Gaussian distribution will be t naught sigma.

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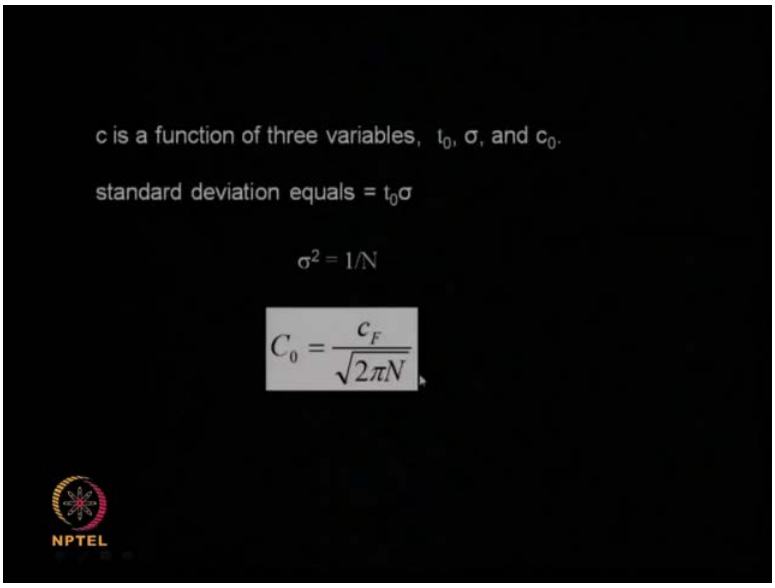

So, making use of that we brought in the concept of number of theoretical stages or theoretical plates. And so the theoretical plates or theoretical stages are, is connected to sigma in this particular fashion. Sigma square is equal to 1 by N. So, if the sigma becomes smaller and smaller, N becomes larger and larger. So, sigma is related to standard deviation by this equation t naught into sigma is equal to standard deviation of the Gaussian curve.

So, if the, if the Gaussian curve is very short then given number of theoretical plates is going to be very large and if in the Gaussian distribution is very broad then the number of theoretical plates you can say is less. And then you also have a relationship between c naught and c F by this equation.

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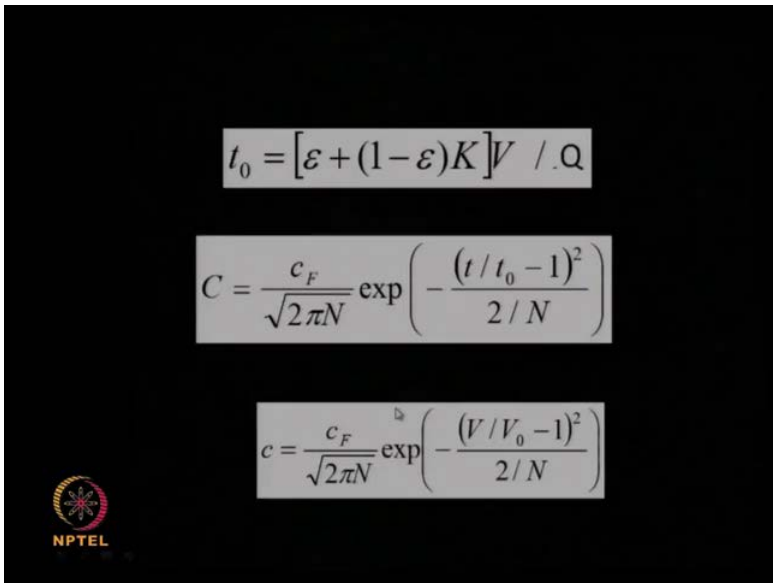

And then later on we also looked at another equation which connects the retention time t naught, look the several parameters, the operating parameters as well as the chromatographic column parameters like epsilon. Epsilon is the porosity of the packed column and V in the volume of the pack column. Q is the flow rate of the continues phase. Now, K is the equilibrium constant. If it is a liquid liquid system we used to call it partition coefficient, otherwise you can call it an equilibrium constant.

So, if we incorporate all these then equation for c becomes like this. So, you have, here you have the number of theoretical stages and then here you have the, and t naught which is the retention time. And similarly, we can have an equation for retention volume also and where we have v naught is the quantity of the liquid that needs to be collected downstream of the pack column.

So, that it reaches the maximum of the p. So, we use these equations quite a lot and we did lot of mathematical derivations and design calculations. We will look at them again once more in some of them because this is a very important set of equation which describes the performance of a chromatography.

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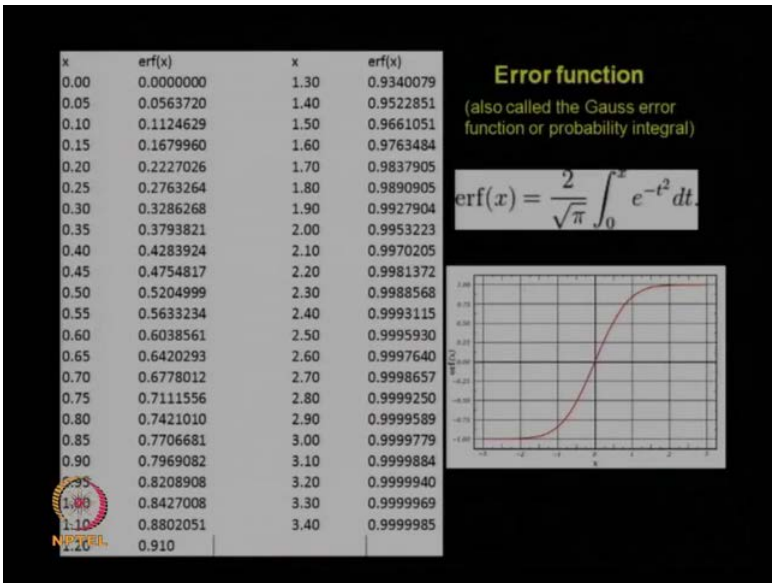

x	erf(x)	x	erf(x)
0.00	0.0000000	1.30	0.9340079
0.05	0.0563720	1.40	0.9522851
0.10	0.1124629	1.50	0.9661051
0.15	0.1679960	1.60	0.9763484
0.20	0.2227026	1.70	0.9837905
0.25	0.2763264	1.80	0.9890905
0.30	0.3286268	1.90	0.9927904
0.35	0.3793821	2.00	0.9953223
0.40	0.4283924	2.10	0.9970205
0.45	0.4754817	2.20	0.9981372
0.50	0.5204999	2.30	0.9988568
0.55	0.5633234	2.40	0.9993115
0.60	0.6038561	2.50	0.9995930
0.65	0.6420293	2.60	0.9997640
0.70	0.6778012	2.70	0.9998657
0.75	0.7111556	2.80	0.9999250
0.80	0.7421010	2.90	0.9999589
0.85	0.7706681	3.00	0.9999779
0.90	0.7969082	3.10	0.9999884
0.95	0.8208908	3.20	0.9999940
1.00	0.8427008	3.30	0.9999969
1.10	0.8802051	3.40	0.9999985
1.20	0.910		

We later introduced something called a error function. This is also called a Gauss error function or probability integral because it is an integral between limits, between two limits. So, erf x or the error function of x is given by 2 divided by square root of pi integral 0 to x e power minus t square d t. So, if you look at the function it looks like this, know it looks like a sigma ideal curve. So, between 0 and 3 it starts from the value of 0 and then its goes up and saturates at 1.

And in the negative direction again it starts from 0, goes down in the negative direction and saturates at minus 1. So, there is a table which gives a numerically integrated values for erf x for different values of x. We can see that when x is equal to 0 the error function of x is equal to 0 and it keeps going up.

Initially, sharply as a representation of this, but then later on it sort of stabilizes, peters out. And then it reaches a value of 1. So, beyond point you around 0.15 or 16 1.6 or 1.7 you can see the error function of x is almost close to 1. Now, this particular table was taken from Wikipedia website. So, for any value of x we can calculate the error function from this particular table. So, this equation, this table is going to be very very useful in calculating eves.

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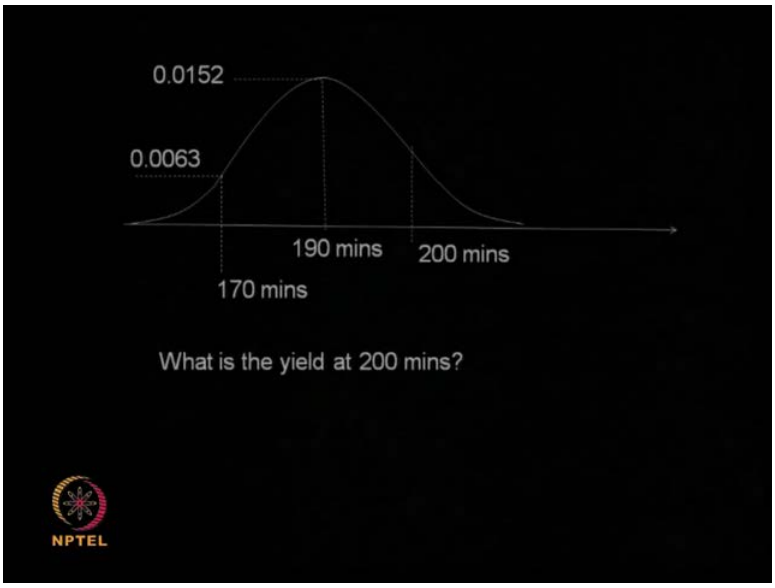

Now, imagine a situation, in fact this problem we did that in the previous lecture as well. So, imagine a chromatography peak like this at 170 minutes you have a concentration like this and the retention time is 190 minutes. Now, we want to know what is the yield at 200 minutes that means if I collect the sample up to 200 minutes, what is the yield of this particular protein. Now, many things are given here.

The team out, that is the retention time is given and then you are also given some concentration value c at a particular time t. So, making use of this what can we do? We can calculate sigma and then using sigma we calculate the yield at two hundredth minute. So, if you look at this equation we know c that is 0.0063, we know c naught it is 0.0152 and we know t naught which is 190 and you know t which is 170. So, by substituent all this, we can get a value for sigma.

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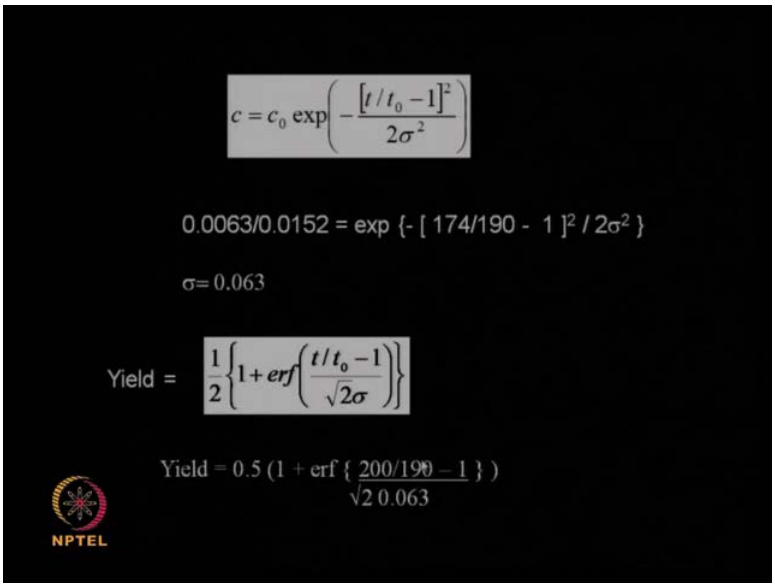

So, that is how you do that. By substituting all this into the equation we get sigma. Now, we go to the yield equation. I hope you remember the yield equation. Yield is equal to 1 by half multiplied by 1 plus error function of t by t naught minus 1 by square root of 2 sigma. Now, why does this 1 comes in? Because we are going to collect sample up to 200 minute which is greater than the retention time 190 minute. So, if you keep collecting up to 190 minute, the yield will be 50 percent, that is 0.5.

So, if you are going to collect beyond 190 minutes obviously, the yield is going to be greater than 50 percent. That is why this particular terms comes in here. Now, we know sigma calculated from the previous case, we know t naught which is 190, t will be your 200. So, you can substitute here, so that will be your x. So, from the table you can calculate f x, substitute here and you get the value for the yield. So, easy it is. So, by plugging in all these numbers we get the x.

Hence, the erf x. So, straight forward, very simple actually. So, it comes to yield is equal 0.8 that means 80 percentage. So, if I keep collecting sample up to 200 minute the yield of the particular product is going to be 80 percent. This is not taking anything about the purity, please note that. We are not talking about purity because there could be other components also coming in which may reduce the purity. So, if there are severally components and if I keep on collecting one component then the purity may be going

down, whereas if I want very high pure material obviously I am going to sacrifice on the yield.

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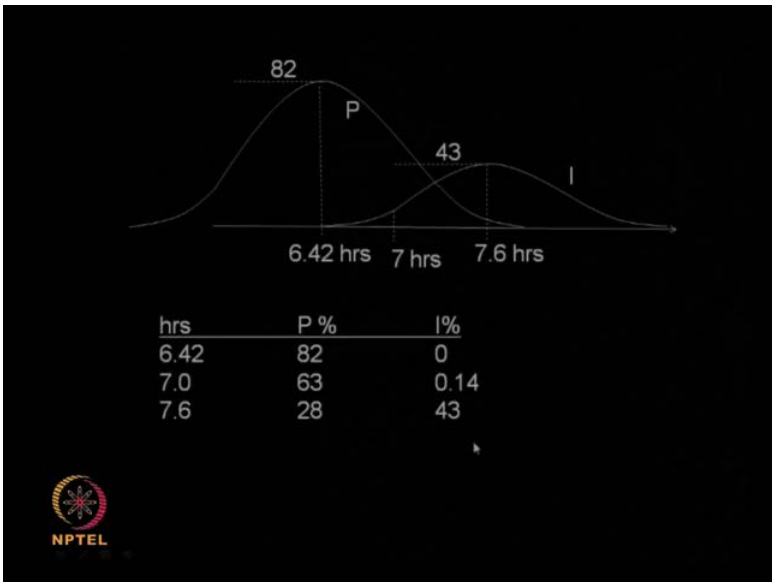

Now, let us look at a two component system. You have a protein P, you have an impurity I. I did introduce this problem in the previous class also. I am repeating it again, so that you are well conversant with this particular system. Now, imagine you have Gaussian type of distribution for protein P, the t naught or the retention time for the protein is 6.42 hours. Now, the impurity also has a Gaussian distribution, but it is coming at 7.6 hours. So, at 6.42 hours you have only the protein whereas the impurity is 0.

At 7.6 hours and you have some amount of protein and you have impurity. At 7 hours you have very little of the impurity and predominantly more of the protein. Now, how do you solve this problem? Now, you will have two different Gaussian distributions so and you have two different retention times. So, obviously you are going to have two different sigma's. So, how do you calculate those two sigma's? We can calculate from these places. We know that t naught for the protein, we know the t naught for the impurity, correct? And then we also know the concentration values at different times.

At you know the c naught for the protein, we know the c naught for the impurity, we also know the concentration at some other intermediate time. So, we can again use the equation c is equal to c naught if our minus t by t naught minus 1 whole square divided by 2 sigma square. I hope you remember this. So, for the protein t naught will be 6.42

and c by c naught will be 63 by 82 and t will be 7, so we can calculate sigma. Similarly, for the impurity c by c naught will be same here as this is divided by this. And that is this value by 43 and t naught will be 7.6 hours and t will be 7. Again, we can calculate a sigma for the impurity.

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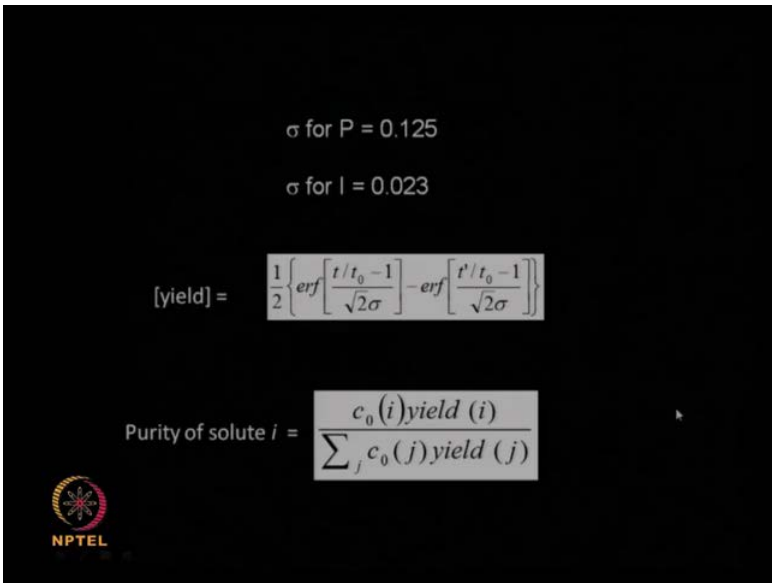

So, you are going to get two different sigma's like this, sigma for the protein, sigma for the impurity. Now, yield is given by this added function equation where half into erf of t by t naught minus 1 divided by square root of 2 sigma minus erf t dash by t naught minus 1 divided by square root of 2 sigma.

So, if you are collecting the product between the time t dash to t then this is the equation for yield. And the purity in this two component system is going to be concentration maximum into yield divided by summation of the concentration yield for component one and concentration yield for component two. So, this is a two component system. Now, you know the sigma for the protein. You can substitute it here and you know the sigma for the impurity we can substitute here. So, you will have two equations, separate separately.

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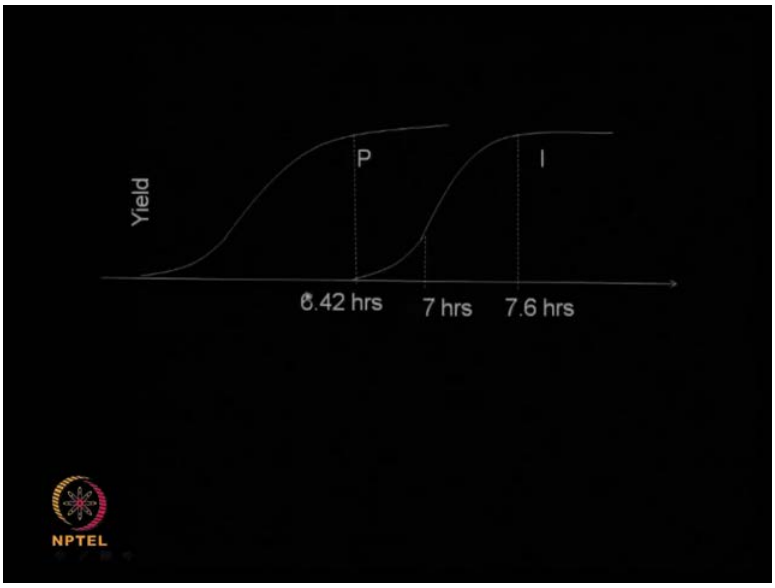

So, we can get yield as a function of time as well. So, the protein yield may build up and then it sort of flattens out. And then the impurity yield may build up and then it sort of flattens out. This is how it look like actually. So, if I go further I will be collecting some amount of impurity as well. Whereas, if I am here I am going to get pure protein whereas if we travel little bit here I am going to bringing some of the impurity also in my product. So, the purity of the protein is going to go down.

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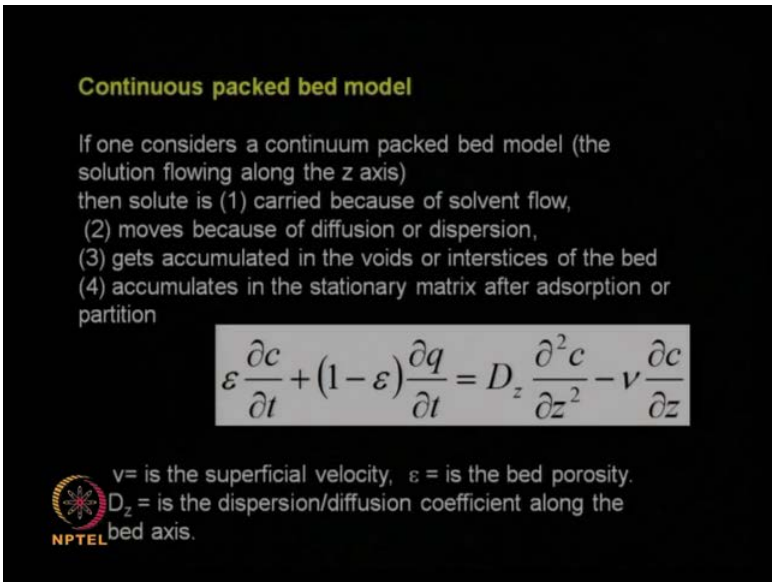

We also started introducing this particular concept which is called the continuous packed bed model. So, if we consider our chromatography column as a pack bed of, consisting of a tubular design, it is a continuous system. So, there is an axial z axis. Now, the solute is moving with the continuous phase from one end and then it just comes out from the other end. You have the packing material or the stationary phase uniformly distributed there is a voidage.

So, in the wide space you have the liquid, liquid contains your solutes, solute is also absorbed on the packed material and the absorption process could be controlled by several different types of mechanisms. We will talk about these mechanistic aspects later. So, we have the solute moving in with the solvent or the mobile phase from one end to the other. So, solute is getting carried because of solvent flow. The solvent is flowing at certain velocity.

The solute is moving because of diffusion or dispersion because there is always going to be diffusion like your fix law of diffusion. I hope you all remember what is the fix law? And then this solute is going to get accumulated in the voids of interstices of the bed. That means is the voids of interstices of the bed is going to contain your continuous phase solvent. So, solute is going to be present there and you are also going to have solute accumulated or absorbed on to the stationary phase.

That is the solid material. So, you have two accumulations. So, if you combine all these you are going to end up with your partial differential equation. This is a typical partial differential equation. So, on this side that is on the right hand side you can see, you have a diffusion term here. This is called the axial diffusion or dispersion term and then this is going to be a D square c by d z square. This terms comes because of diffusion and then you have a flow term that means you are going to have a solute movement because of the flow. Now, z is the axis, z axis.

So, you have here term coming because of the diffusion of the solute in the, along the axis and you have a term here which talks about the flow of the solute with the bulk solvent. On the left hand side you have two accumulation term, one term corresponds to the accumulation in the void space that is in the continuous phase. Another term is because of the accumulation in the solid phase. So, you have two terms. Now, c is the concentration of the solute in the, in the interstices or c is the concentration of the solute

in the mobile phase, whereas q is the concentration of the solute in the absorbed or in the stationary phase.

Now, epsilon is the void age that is voids and 1 minus epsilon is obviously the amount of solids present. So, there has to be a relationship between c and q and that relationship may depend based on the type of mechanism that is operating. You can have different types of mechanism which will determine what will be the relation between c and q. And so you need to have a very simple mechanism.

Then you will be able to solve this equation analytically. Otherwise, you may have to solve numerically. Now, you can considered several approximations if you want to solve them analytically. So, now what are the approximations?

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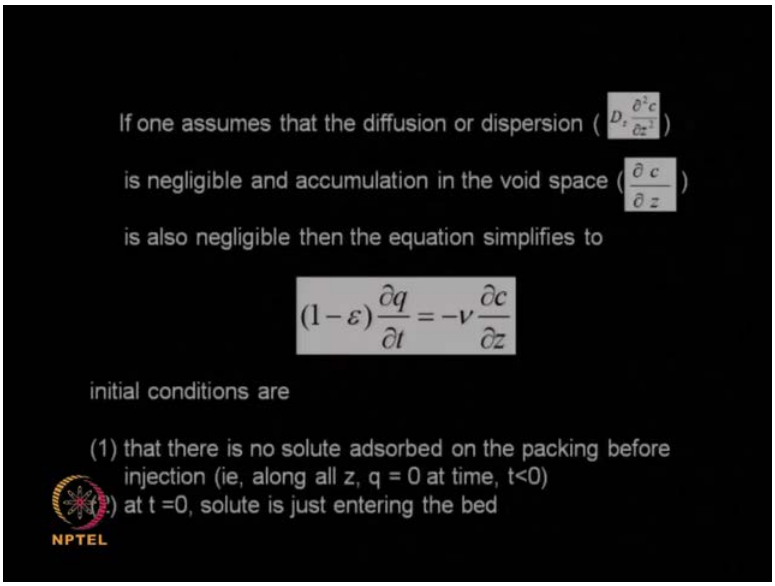

We can assume the diffusion or dispersion is negligible that means d square c by d z square could be omitted. We could assume that the accumulation in the void space is also negligible. Accumulation in the void space is also negligible. So, then what do you end up with? You will end up with the accumulation on the stationary space that is the solid phase and this arises because of the flow of the bulk material, bulk solvent, understand. So, we have neglected the accumulation in the void space or the mobile phase.

We have neglected the dispersion. So, we end up with the simpler looking equation. Now, it needs lot of initial conditions. The initial conditions are there are no solute

absorbed in the packing and when the time is 0 or less than 0 because we have not injected any solute, we inject solute only at time is equal to 0. So, at any place inside the column when time is less than 0 q equal to 0 and at t equal to 0 that is at 0 time the inject the solute at the entrance of the bed because only at the entrance we are injecting. These are the initial conditions. But still it is not enough if you want to solve this particular partial differential equation.

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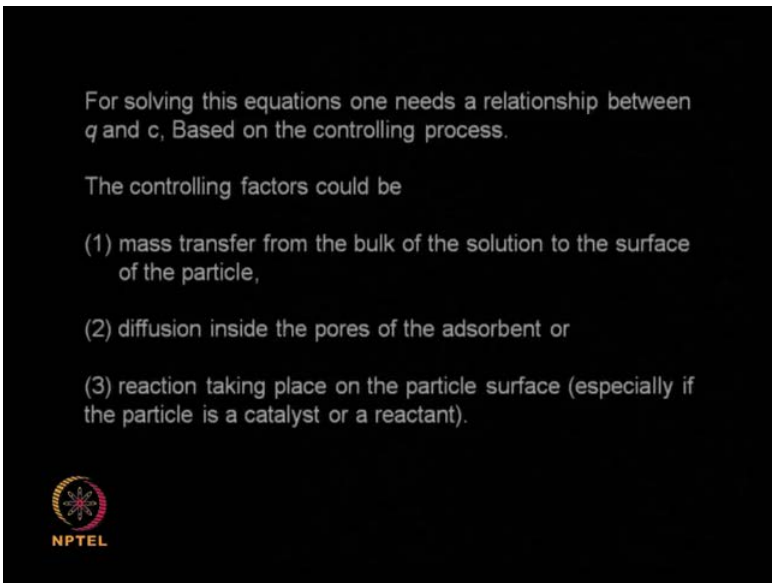

So, we need to have some relationship between q and c. Otherwise, you will not able to solve that. So, that means we need to have what type of mechanism is operating. There could be lot of things happening inside and physical processes happening because it is a chromatography we will assume there is no reaction. So, there, but there are lot of physical process taking place which may be controlling, which determines the relationship between q and c.

So, what are the various physical processes that may be operating here? For example, mass transfer from the bulk of the solution to the surface of the particle. So, you have the continuous phase flowing that is the bulk, that is a liquid whereas your stationary phase is a solid and whenever there is a solid and a liquid interface there is going to be a mass transfer resistance for the solute to diffuse from the bulk on to the surface of the solid or the stationary phase.

So, there is going to be a mass transfer, number one. Number two, if there are pores in your stationary phase there could be diffusion of the solute into the pores. So, that could be controlling. If there is a reaction taking place, then reaction also may be controlling, but in a normal chromatographic system we shall completely omit this. So, these two thing may be happening. One is the mass transfer from the bulk of the solution to the surface of the catalysts or the solid material may be controlling or the diffusion of the solute from the surface of the stationary phase into the pores may be controlling.

So, depending upon which is controlling you may have different types of relationship between q and c. So, we need to understand that depending upon which is the important equation or which is the major controlling factor. We may have different relation between q and c and if you know which is controlling and if you have some sort of a relation between q and c then it will be possible for you to solve the partial differential equation which we talked about in the previous slide.

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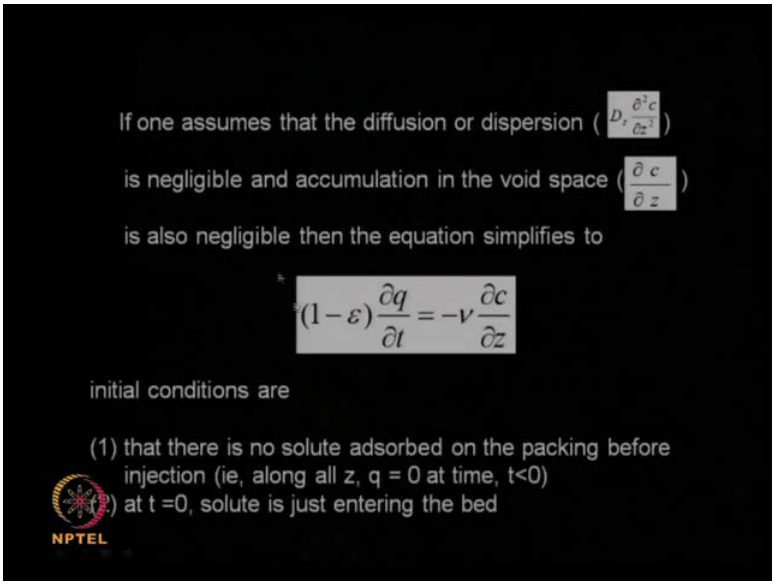

Namely this which is 1 minus epsilon into d q by d t is equal to minus v d c by d z where v is velocity, epsilon is a void age, q is the concentration of the solute which is bound to the stationary phase, c is the concentration of the solute which is present in the mobile phase, t is the time and z is the axis. So, what it means? It means the concentration varies along the axis of the chromatographic column as well as the concentration varies as a function of time.

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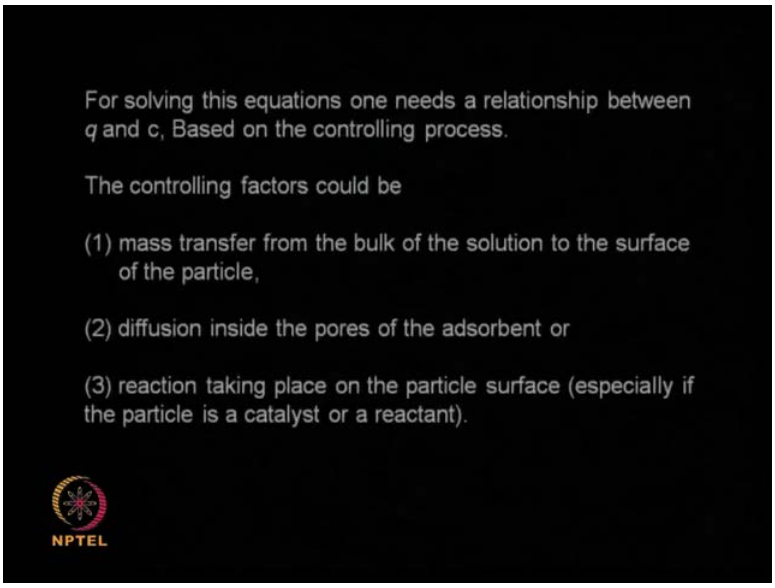

So, if the mass transfer of the solute for the bulk to the surface of the particle is controlling as I mentioned this is a solid particle and the bulk solution is a liquid. So, whenever there is two different phases coming in, in this particular case it is a solid and liquid. If there is a gas then there could be a gas and the solid.

If there is a absorption where you can have a gas and a liquid, again there is a two phase system. Any two phase system will always have an interface. And there will be a resistance for a particular component to move from one phase to another. It may be very slow, it may be fast, but still there is a resistance.

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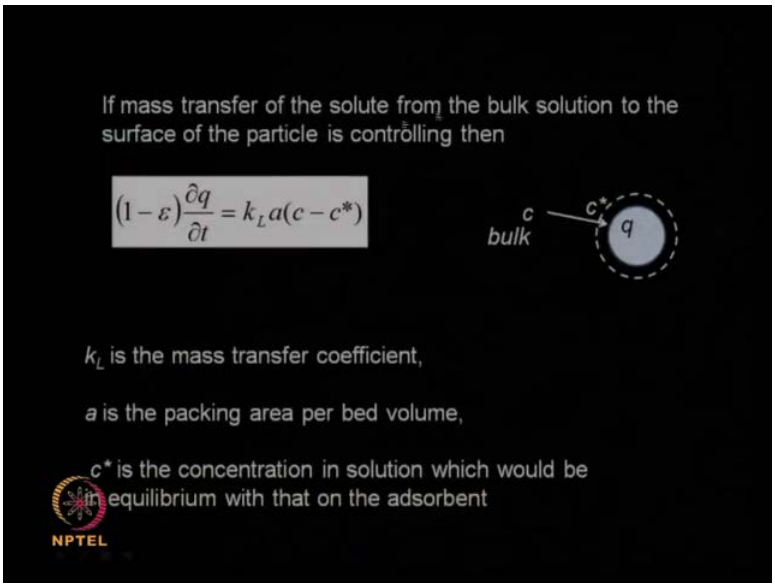

So, in this particular case we call it the mass transfer resistance, the movement of your solute from the bulk to the surface. So, we can assume the concentration of the solute at the surface at c star and the concentration of the solute in the bulk is c. So, what does it mean? The driving force here is c minus c star and the equilibrium interaction is between q and c star not between q and c. Please note, the absorb species is not c, the concentration c, but it is seeing only the concentration c star.

And so the driving force is c minus c star. So, if the mass transfer of the solute from the bulk to the surface is controlling then you can consider equation like this. 1 minus epsilon d q by d t is equal to k L a into c minus c star where c is the concentration of the solute in the bulk, c star is the concentration of the solute on the surface of your particle, k L is the mass transfer coefficient, A is a packing area per bed volume and epsilon is your voidage, q is the concentration of the solute on the stationary phase.

So, if the mass transfer is controlling what this equation tells you is the accumulation of the solute in the solid phase is exactly equal to the amount of material which is moving from the bulk to the surface.

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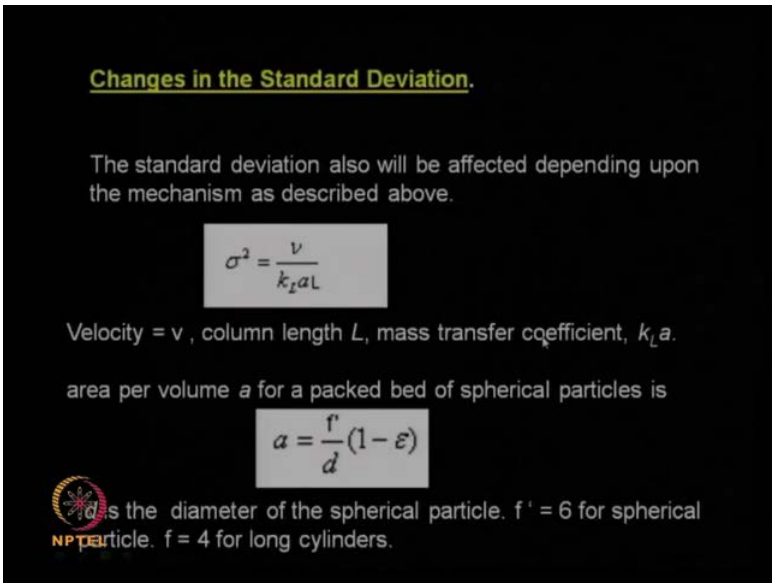

So, with that equation you will be able to solve the partial differential equation. Now, let us again go back to our Gaussian distribution or normal distribution for our chromatographic peak. So, we said the chromatographic peak looks like a Gaussian shape with retention time and a standard deviation. Now, the standard deviation can keep changing depending upon the type of mechanism. For example, the standard deviation in the previous case we talked about mass transfer controlling, we said k, k L a and is the mass transfer rate c, c minus c star is the driving force.

So, in such a situation then your sigma square, standard deviation is sigma into t naught where t naught is the retention time. So, if mass transfer is controlling then sigma square could be equal to v divided by k L a in to L. v is the velocity, L is the length, k L a is the mass transfer coefficient. k L is the mass transfer coefficient, a is a area per unit volume. That is interfacial area per volume actually.

Now, a in a packed bed of spherical particle is approximated like that, a is equal to f dash by d into 1 minus epsilon, d is the diameter of the spherical particle and f dash is 6 for, if it is spherical and it is equal to 4 for cylindrical type of particle actually. So, a is the interfacial area between the two phases. In this particular situation it is the bulk liquid or the mobile phase liquid and stationary phase solid. So, if you are decreasing particle size, you are increasing mass transfer coefficient, correct? Because the a comes below.

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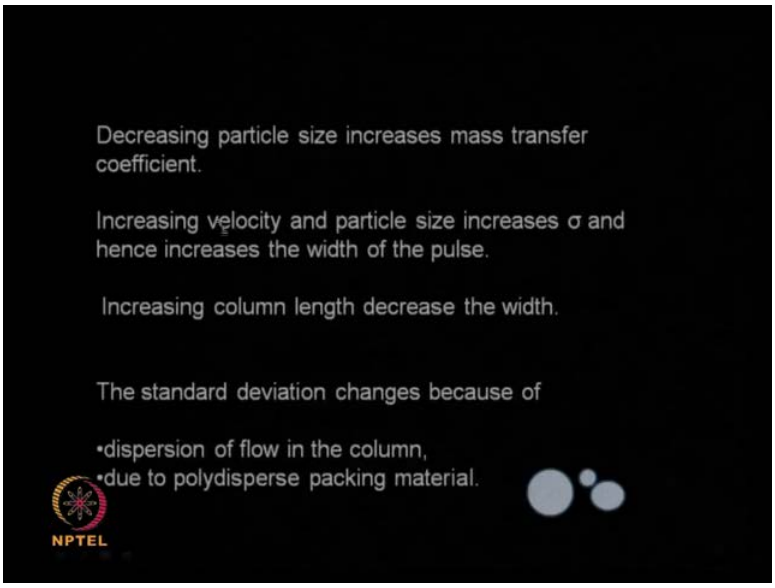

Decreasing particle size increases mass transfer coefficient. So, ideally it will be very nice to have very small sized particle. But then in a packed column if you reduce particle size you are increasing the back pressure. That means you need to have pumps which can deliver very high pressure, that is why if you have HPLC type of systems where the particles size are in micron range the pressure developed by the column is so high that you need very high pressure pumps. Increasing velocity and particle size increases sigma.

So, if the particle size increases sigma increases, if the velocity increases also sigma increases. So, if sigma increases obviously the bit of that Gaussian pulse also increases. Increasing column length decreases the width. So, if I have longer column I am having more number of theoretical plates.

So, the width of the pulse also decreases. Now, in addition the standard deviation changes because of dispersion of flow in the column due to polydisperse packing material because although they say you have uniform size particles packed inside a chromatography column, there will be still a distribution of sizes on the average diameter you know. So, there will be a plus or minus 10 percent of the average diameter. So, the polydispersity also will affect the standard deviation.

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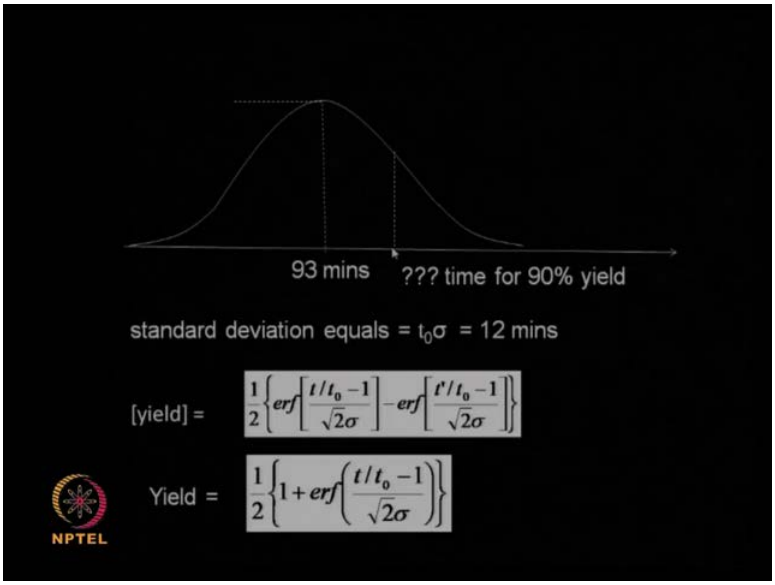

Now, let us again go back to same chromatographic output like this. Now, the retention time is 93 minutes. Now, I want to calculate what is the time required if I want to have 90 percent yield. This is a very important problem, I have a protein in a mixture, I am running a chromatography and I know the retention time, I know the slandered deviation. I want to know how long should I keep collecting this effluent, so that I get an yield of 90 percent.

So, it is a very important problem. Now, the standard deviation for this system is 12 minutes. So, the retention time is 93 minutes, standard deviation is 12 minutes. So, how do you calculate yield?

Now, so I know t naught, I know standard deviation, so I can calculate sigma 12 by 93. Now, taking these I can substitute in to the yield equation. Now, the yield of course, I want 90 percent yield. So, obviously if I am collecting up to the retention time, my yield will be 0.5. So, anything above 93 minutes my yield will be above 0.5. So, what equation do I use? I will, this is a general equation for yield which is half into erf t by t naught minus 1 divided by square root of 2 sigma minus erf error function t dash by t naught minus 1 divided by square root of 2 sigma.

But because my yield is going to be more than 50 percent I will use the simpler equation half into 1 plus error function of t by t naught minus 1 divided by square root of 2 sigma. Now, in this equation yield I substitute as 0.9, t naught is 93, sigma is given by 12 divided by 93. So, if I substitute all of them the only unknown is t. So, from this I can

calculate what should be my time until which I keep collecting the effluent, so that the yield is 90 percent.

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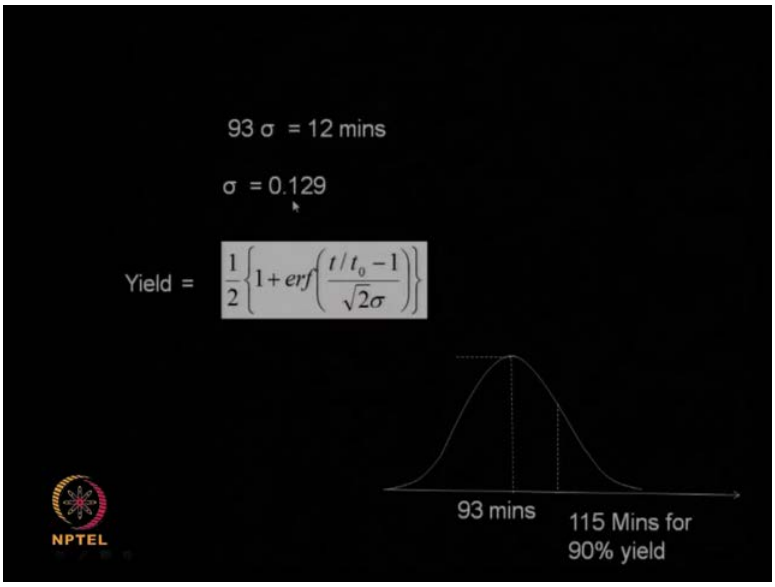

So, first I calculate sigma. Then I substitute that. So, by doing this and how do I calculate erf x? I, if you remember I showed you table where given x, I can tell you what is the error function x.

So, if I keep collecting up to 115 minutes my yield will be 90 percent. So, you see this is a very important problem especially if you are wanting to collect as much as the metabolite that is present in my broth as possible. I use the same equation and I have t that is only unknown, rest of the things are known.

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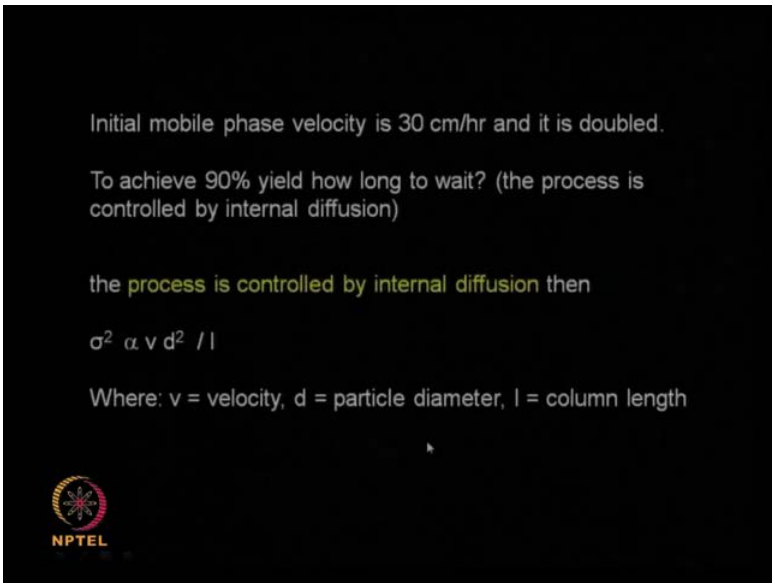

So, t comes out to be 115 minutes. Let us look at another problem where we are looking at the type of controlling mechanism. So, initially mobile phase velocity is 30 centimeter per hour. Now, I am doubling it that means it become 60 centimeter per hour. So, I want to achieve it 90 percent yield.

Now, how long do I wait? Now, the controlling mechanism is internal diffusion. The process is controlled by internal diffusion. So, if the process is control by internal diffusion. Anyway, sigma square is going to change or the standard deviation is going to change or the, width of these peaks are going to change.

Now, if the processes is controlled by internal diffusion that means diffusion of the solute inside the pores of the solid matrix, then sigma square is proportional to v the velocity d square, d the particle size, particle diameter and l the length. So, here I am doubling the velocity from 30 to 60. So, obviously sigma square will get doubled or sigma will be square root of this number.

So, I will get a new sigma, I know the old sigma. So, with the new sigma I substitute. So, I should get a different time. Now, I have doubled, so my retention time was originally 93 minutes for 30 centimeter per hour. Now, I have doubled it, so my retention time becomes half of that 46.5 minutes.

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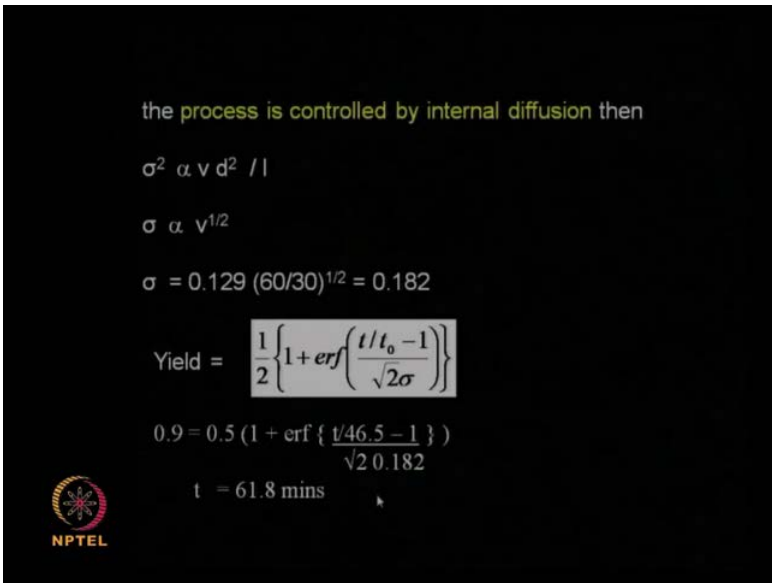

Now, sigma square is proportional to v d square by l that means sigma is proportional to v raised to the power half. So, now originally it was 30 centimeter per hour. Now, the velocity has become 60 centimeter per hour. So, originally my sigma was 0.129. Now, the sigma will be 0.182 because sigma is proportional to v raised to the power half. Velocity has doubled, so you have to multiply by square root of 2. So, your sigma has gone up from 0.129 to 0.182.

Now, I go back again to the yield equation, I take yield equal to half 1 plus erf t by t naught minus 1 divided by square root of 2 sigma. Now, t naught has gone down. Remember, I have doubled by flow rate. So, t naught has gone down from 93 to 93 by 2 and sigma has gone up from 0.129 to 0.182. So, I will substitute new sigma, I will substitute new t naught and again I calculate this error function and yield I want to know the 90 percent yield or point time, so I will calculate what should be my time?

So, my new retention time is 46.5, my new sigma is 0.182, my yield is 0.9, so t has become now 61.8 minutes. So, if we remember the previous case, so t has become 61.8 minutes in the internal diffusion. So, the internal diffusion is controlling factor, then sigma will be proportion to v raised to the power half and when I am doubling from 30 centimeter per hour to 60 centimeter per hour my time required to collect has become 61.8 minutes. Now, let us consider another situation.

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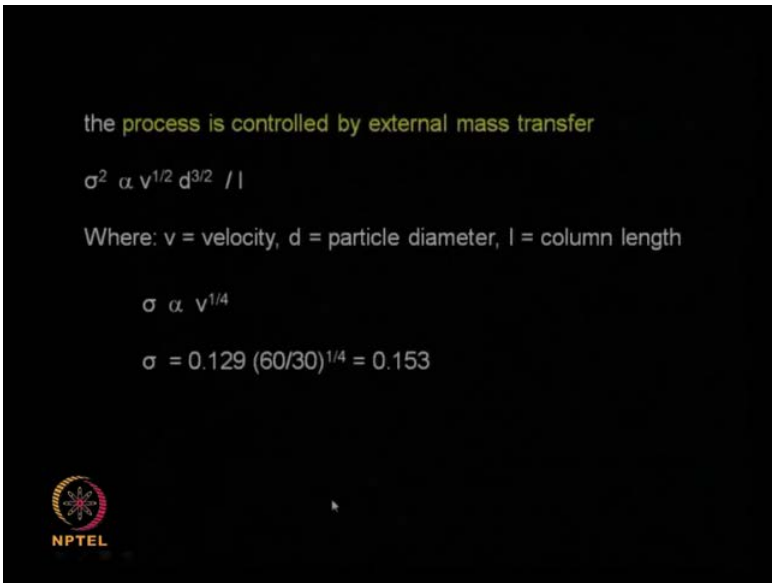

The process is controlled by external mass transfer that means the movement of the solute from the bulk to the surface because of the two phase system is controlling. So, in such a situation it has been observed that sigma square is proportional to v raised to the power half where v is your velocity, d is the particle diameter, d raised to the power 3 by 2 divided by l, l is the column length.

So, here it is a slightly different, sigma is proportional to v raised to the power 1 by 4 whereas if it is a pore diffusion, it was like sigma proportional to v raised to the power half whereas if it is external mass transfer sigma has become proportional to v raised to the power 1 by 4.

So, same thing, original sigma was 0.129, double the velocity, but the exponent is 1 by 4. So, my sigma has become 0.153. Now, again I make you some the yield equation, 0.9 is the yield I would like to have. I know the t naught, t naught is the, reduced from 93 to 46.5, my sigma has changed.

Sigma has increased to 0.153. So, I substitute them inside this same yield equation to get a new time t is equal to 59.4 minutes. See, you see depending upon the controlling mechanism you can, you will require different times for collecting you effluent to achieve an yield of 90 percent. Let us consider another situation.

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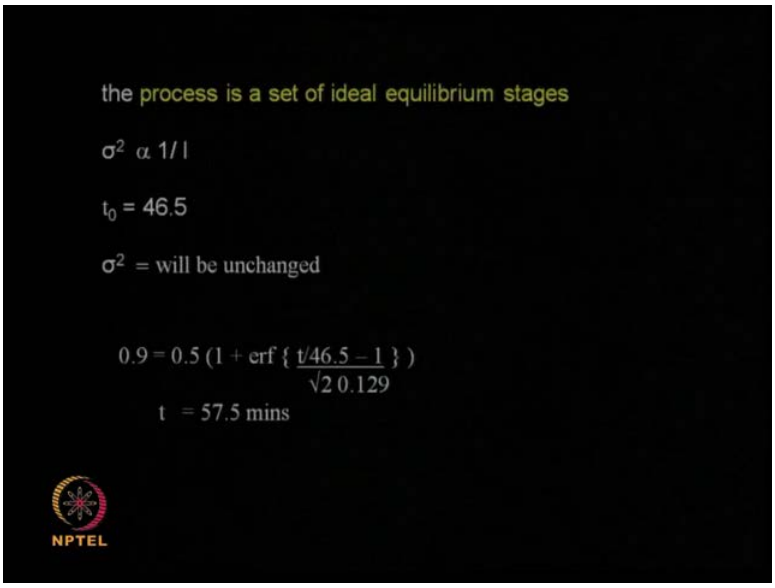

Now, if the process is a set of ideal equilibrium stages. If you remember long time back may be two three class back I talked about ideal equilibrium stages that means the chromatography's entire column is divided into n number of ideal stages. Each stage has the stationary phase equally distributed.

Now, the continuous phase is entering from and that, say from the left hand side and then it is leaving on the right hand side into each stage. So, from the stage one output goes into stage two and from stage two output it goes into stage three and so on.

So, there is an interaction between the solute in each stage with the corresponding stationary phase. So, there is an equilibrium in each stage taking place between the salute in the continuous phase as well as the salute in the stationary phase in each stage. So, that is called an ideal equilibrium stage process. So, the entire column is divided into n number of ideal stages, large number of ideal stages. So, in that situation sigma square will be proportional to 1 by l that is the column length.

So, if the column length is very, very large you have obviously large number of ideal stages then your sigma will become small. That means the peak width will become very small. Obviously, the efficiency of the column is extremely good. If the column length is short your sigma will be large that means peak width also is large. That means the efficiency of the column is not so good. Now, in such a situation, in an ideal equilibrium stage sigma square is proportional to 1 by l.

So, velocity you can see is not coming into the picture. Now, t naught is once again a half of 93 that because you have doubled the, from 30 to 60, but sigma square will be unchanged because velocity is not coming into this place. So, you take this t naught, you take the old sigma and then put it into the yield equation and you should be able to calculate the time required to achieve the 90 percent yield. So, when, if you do that, so you take your old sigma. So, the sigma is the same whether it is n number of stages where n could be anything. Sigma remains unchanged here because sigma is only a function of l, it is not a function of the velocity.

So, the old sigma is taken that is 0.129, but the t naught is of course, gone down from 93 to 46.5 because you have doubled the velocity. So, when you substitute that into the yield equation. If you remember this yield equation, yield is equal to 0.5 into 1 plus error function t by t naught minus 1 divided by square root of 2 into sigma, you will get a new time. So, we, see we saw three different situations depending upon what is controlling, we said if the bulk mass transport is controlling that means the solute movement from the bulk of the solution to the surface of your stationary phase or if the pore diffusion that is the movement of the solute molecules inside the solid matrix is controlling.

That is one situation. Third one we looked that is ideal situation where we are dividing the entire column into n number of ideal stages and then trying to see whether that is controlling. So, depending upon the type of controlling mechanism you may have different sigma values. And then you use them all in your yield equation and when you use them all in your yield equation and you will be getting different time until which you need to keep collecting the effluent, so that you achieve the desired 90 percent yield.

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So, a chromatography column can have different types of mechanism because you have a packed system and you have a liquid flowing from one end to the another end. So, you may be having bulk mass transfer taking place from the bulk of the liquid to the surface of the particles or the stationary phase. Then this particular solute gets absorbed on the stationary phase and then they start diffusing inside the pores. So, that could be controlling and if there is a reaction taking place between the solute and the stationary phase material or if there are any ligands present in the stationary phase material which is acting as a catalyst when there could be a reaction taking place.

So, all these could be controlling and what does controlling means? When we say controlling, all these three may be acting in series, but one of them is the most important. Rest of them may be very, very fast. So, if out of these three, if two processes are very fast and if one process is slow, so we call that as the controlling. Because the other two processes we can assume takes place within instant of a time whereas the third process takes much longer time to take.

So, relatively it takes much longer time to take with respect to the other two. So, we call that as the controlling. So, in this particular chromatographic situation we are saying that the mechanism are varied, the bulk flow, the bulk mass transfer and then mass transfer from the bulk liquid to at the surface of the stationary phase and then there could be pore

movement inside the pores. And then there could be reactions taking place and so on actually.

So, depending upon various situations we say that the interaction between the solute molecule and stationary materials will change. And depending upon these interactions or the strength of these interactions the spread of these Gaussian peaks also change.

So, when we talk about, when we mention about spread we are also talking about the standard deviation or we are talking about the sigma. So, the sigma also will change depending upon the mechanism and if the sigma changes obliviously the peaks may be becoming broader or the peak may be becoming shorter or very tight actually. So, we looked at large number of mathematical equations and we also looked at various types of situations which help you in designing the type of a process operations which we need to carry out.

These operations could be the amount of time required to achieve 90 percent yield or if I collect for a certain period of time what is the yield of my product and if I have two or more different types of solutes, if I am collecting at different periods of time what will be the impurity levels and so on actually. So, these are very, very useful especially if you are running a chromatography and if especially, you would like to know how to run a chromatography efficiently and try to get maximum purity and as well as the maximum yield.

Now, in the next class we will continue with the same chromatography. And we will, let us look at use of chromatography in analytical purposes like you must have all heard about the HPLC the high pressure liquid chromatography or high performance liquid chromatography which is pre dominantly used for only analytical purposes, where if you are interested in identifying a particular metabolite that may be present in your mixtures, it could be a protein, it could be a bio molecule, it could be a small molecule or if you want look at the quantity of that metabolite in the mixture, we generally go for HPLC.

And it is very useful if the component is not vaporizable that means it may have a very high boiling point or if I heat it up it may lose its activity. Then ideally this type of analytical instrument predominantly the HPLC is extremely useful. So, that is what we will be talking about in the next class.