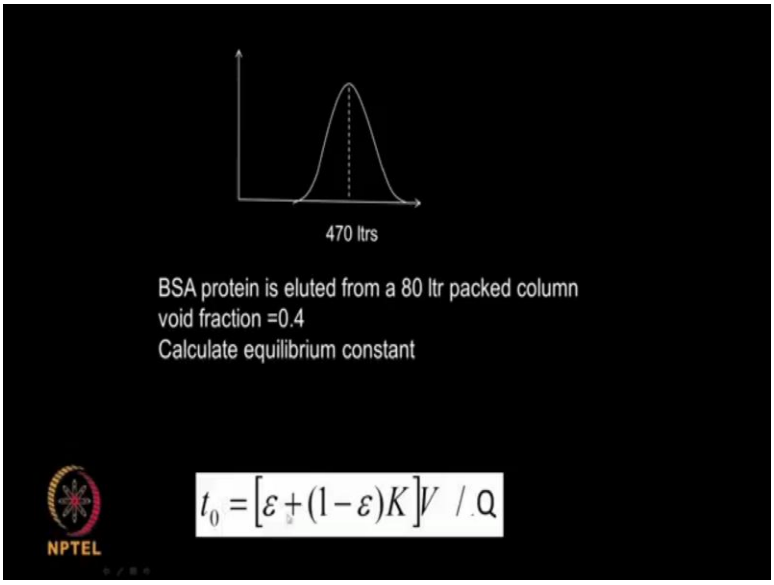
Principles of downstream techniques in Bioprocess – a short course

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

Lecture-19
Chromatography [Continued 3]

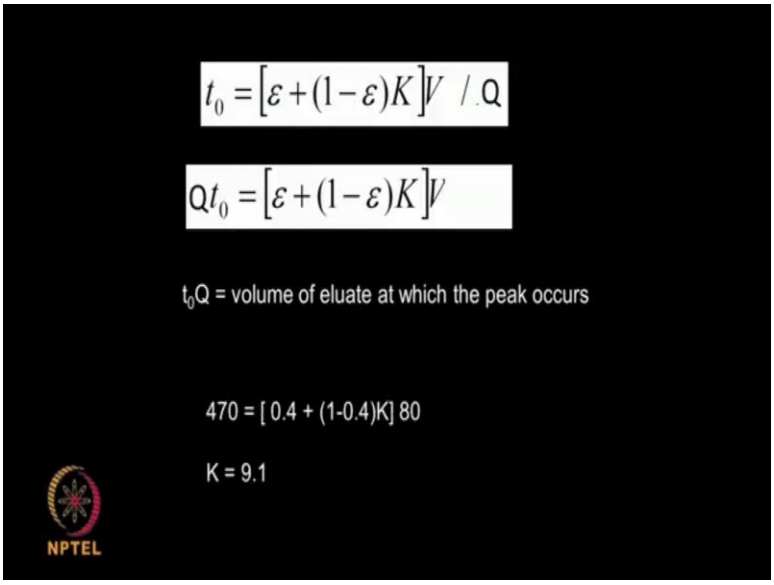
We will continue looking at problems in chromatography. Let's look at a problem. So there is a peak that is eluting out, the maximum is observed after 470 litres of a collection of a liquid. Now this is a BSA protein and the column size is 80 litres and the void fraction is 0.4.

(Refer Slide Time: 0:46)



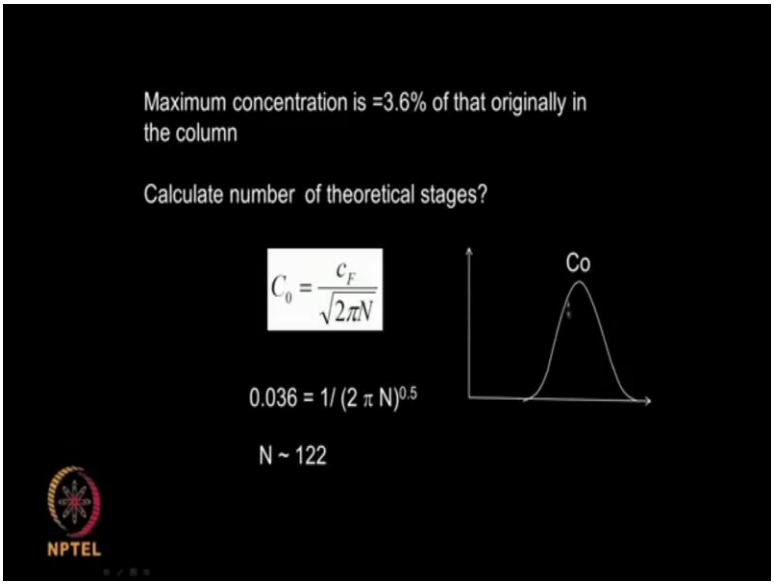
You are asked to calculate equilibrium constant that is the k assuming that there is a linear, So if you remember recall your old lecture, the equation is t0, that is the elution time, is a function of voidage k volume of the packed column and Q the flow rate. So if I multiply t0 by Q. Q t0 will become the volume okay, so that will become 470 epsilon that the void fraction is given 0.4, the volume of the packed column is 80 and all you have to do is, calculate k so simple.

(Refer Slide Time: 1:20)



Okay so you just have to recall the equation and that is what we are doing here. Okay. So we take the equation and multiply on the left hand side by Qt0, Q is 80, so when I substitute all these, so we end up with k= 9.1. That is the equilibrium constant.

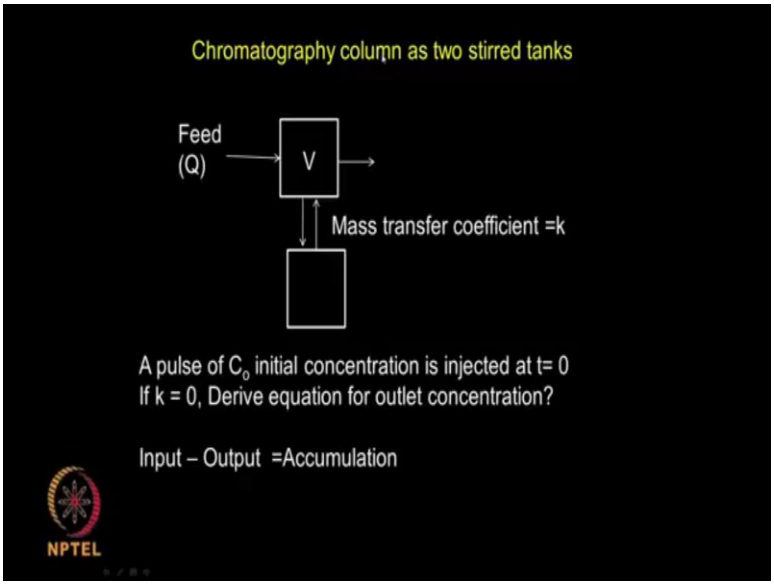
(Refer Slide Time: 1:39)



Let us look at another problem, so the maximum concentration that is C0 here, now you are reaching 3.6 % of that okay. Calculate the number of theoretical stages okay, now you have to recall an equation which connects cF with C0 and the number of equilibrium stages, okay, so that is the equation C0 = cF square root of 2piN.

Okay so the maximum concentration C0 is 3.6 % of the originally that is present in the column, the originally that is present in the column is cF, the C0 is 3.6, 7, 3.6 %. So when you do that, you bring it on the left hand side, it is 0.036. So we can calculate n the number of theoretical stages that comes out to be 122. So if you recall the original set of equations, so you have some concentration cF and then it starts going down and it reaches the distribution of sought in the exit side.

(Refer Slide Time: 02:50)



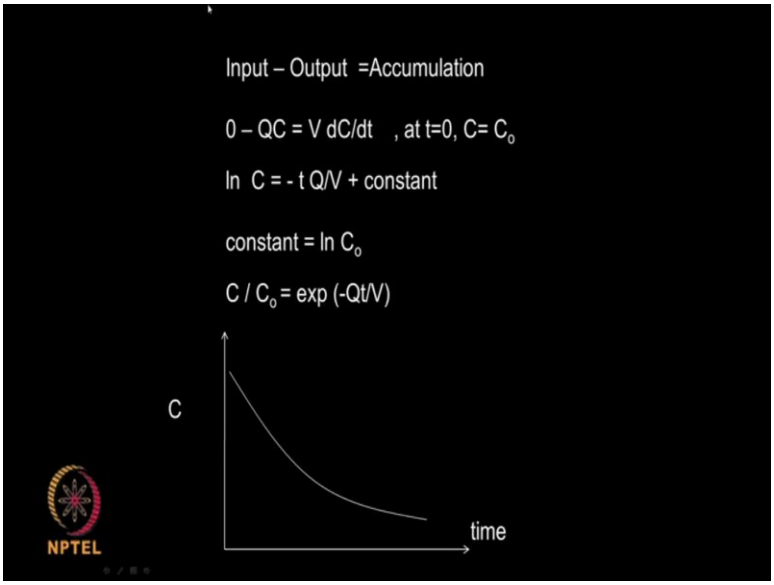
Now let's look at the chromatography as 2 stirred tanks, suppose you have stirred tanks here, where you are introducing the feed and the product going out and there is another tank, which Is not stirred. It is connected to the first tank and there is a mass transfer taking place here. So this is almost like a dead space and this is well mixed and the product is the react the feed is entering here and it is going out here and there is mass transfer taking place okay here.

If k can be some number between zero to infinity that tells you how good the mixing that taking place in the void spa dead space, as well as in the mixed space, so you are having a feed. The problem is, you are having a feed and a pulse of C0 is given at time = 0 okay. If k = 0 derive an equation for the outlet concentration this k = zero, there is no mass transfer taking place. So we can completely ignore this tank, please remember this. We can completely ignore this tank.

So it is like your tank with the volume V and you have a feed you have a stream coming out and there is an input pulse injected with the concentration C0, the time = 0, you would like to know

what will be the outlet . So you have the regular mass balance here, input - output = accumulation okay. So input - output = accumulation. So k = 0, we are ignoring the entire tank 2.

(Refer Slide Time: 04:37)



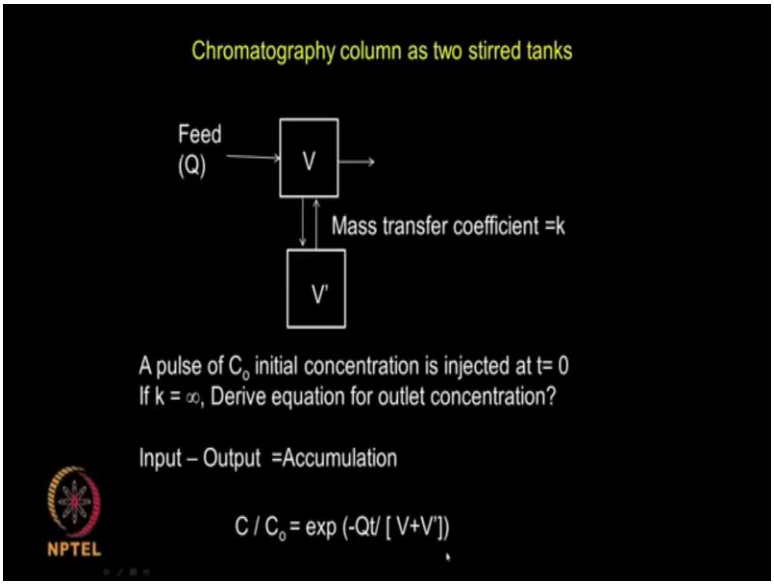
So when we do that input - output = accumulation, so we can say input is 0, output is Q*c that is the concentration of the solute coming out in the stream that = V dc/dt, that is the accumulation inside the vessel. V is the volume of the vessel, dc/dt is the change in concentration as a function of time at t = 0, you c=C0, okay you are injected at t = 0, a pulse okay so this is a first-order differential equation, the initial conditions are given, you must have studied long time back in your first year undergraduate program, so we can solve this.

First we can integrate this, it will end up with log logarithm of c, log c = t Q / V + a constant and a constant can be estimated using the initial condition, so you end up with C = C0 e power - Qt/V okay, so if you bring C0 here, it will be C = C0 e power - Qt /V. So the concentration leaving the tank or the concentration inside the tank will be exponentially decaying at t = 0, the concentration will be C0 and with time, it will be exponentially decaying.

So if you have one single tank and you are injecting a pulse with concentration of C0 at time=0, so as a function of time, the concentration of the solute will decay like this and same thing will happen to the exit stream. The concentration in the exit stream, the time=0 will be C0 and then it will start decaying in exponential manner here, okay as given by this equation. Now V/Q that is

volume by flow rate is called the residence time tow, so we can have the equation as c = C0 exponent - t by tow. Tow is the residence time okay this is the first-order relationship for a stirred tank.

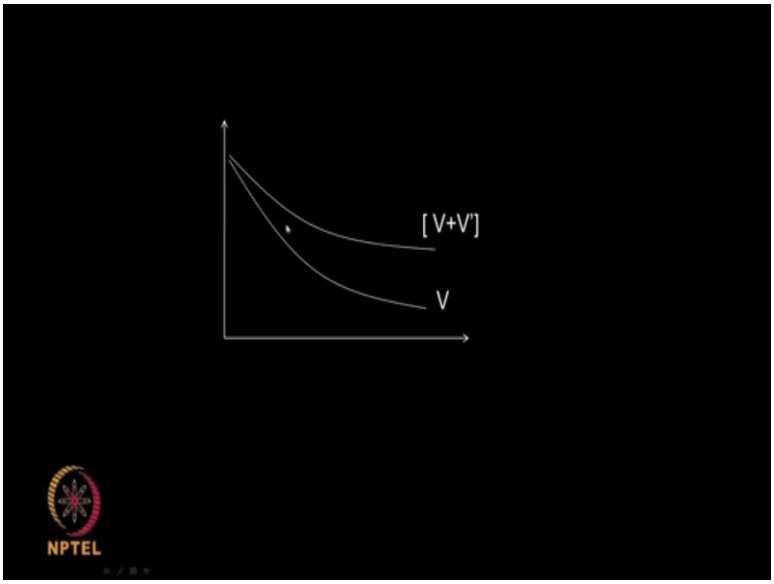
(Refer Slide Time: 6:40)



Now we can have another situation okay where k is infinity that means complete mixing here, okay complete mixing k is infinity, that means whatever there is no resistance at all, offered for mass transfer. So in that case, we can assume the whole system has one single tank with the volume V + V '. Do you understand? So if k = infinity there is absolutely no resistance for the movement of the solute. So this can be considered as a single big tank with the volume V + V '.

So we do the same thing but in this case, the volume will become V + V ' whereas in the previous case, the volume was V okay, so we do the same thing. So you will end up with the equation like C /C0 = e power – Qt/V + V '. So the residence power will be V + V ' / Q. So in the previous case, the residence time is V/Q whereas when k = infinity, the residence time will be V + V '/ Q that means the residence time has increased. Do you understand?

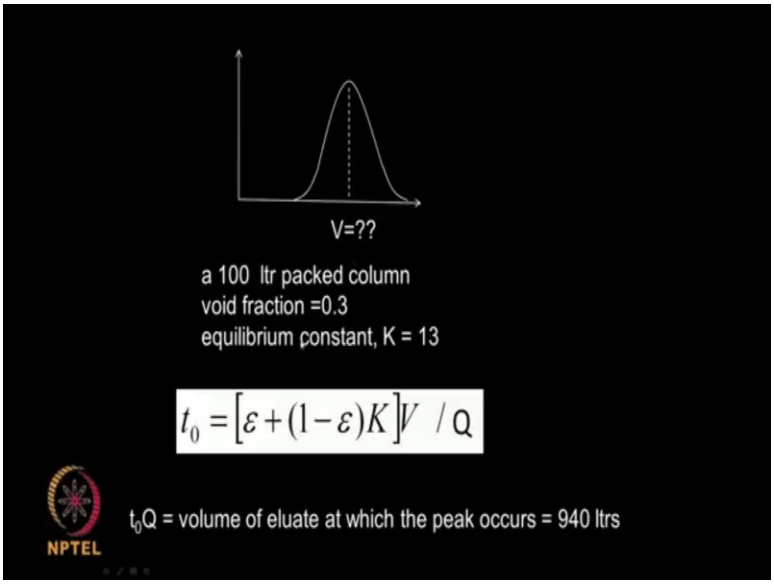
(Refer Slide Time: 07:54)



So how will it decay? It will decay much slower because your residence time has increased. Whereas in the previous case, when k = zero residence time is shorter. So the decaying is faster. When the residence time has increased then k = infinity it will decay slower okay. So for all other values of k, k lying between 0 to infinity, the way it will fall when you give a pulse will be between these two limiting curves okay.

So this curve related to k = zero, that means the second tank is completely not used. We do not have to use in the calculation when k = infinity, there is absolutely no mass transfer resistance. So it is like one single tank, so the residence time has increased and these are the two limits for any value of k, you will have a curve lying between these two okay.

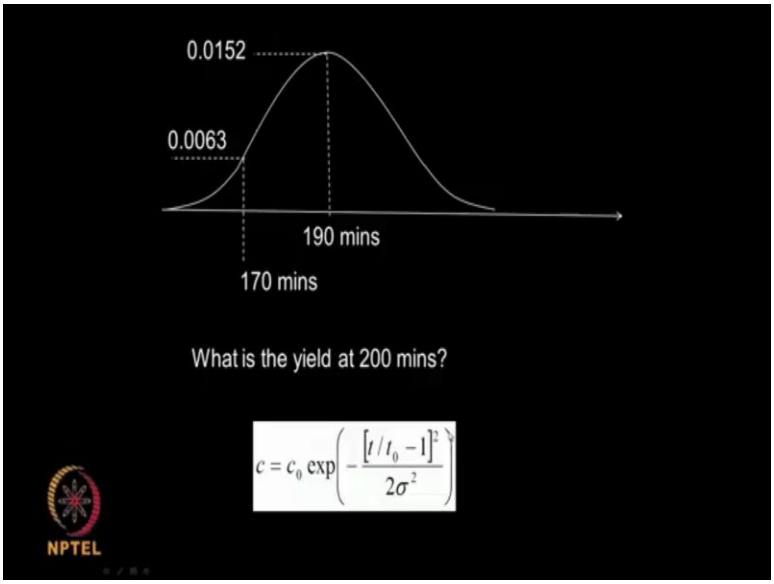
(Refer Slide Time: 8:55)



Okay lets again look at the chromatography. So imagine a 100 litre packed column, okay a void fraction is point through 0.3, the equilibrium constant K is given by 13, now you are asked to calculate what will be the V? That is how much of the liquid I need to collect and so that the peak reaches its maximum that is equivalent to your residence time okay because t0 is your residence time and t0 Q = V. So you need to use the same equation. You remember this equation?

So t0 is the residence time, Q is the flow rate, so t0 Q will be the V, epsilon is given as 0.3, K is given as 13, volume of column is given as 100, so simple okay so you need to calculate t0 Q very simple. So when you do that, you get 940 liters okay. So you need collect 940 litres of the liquid for it to reach the peak and if I know the flow rate I can calculate t0 that is the residence time okay simple. So you need to use this equation to calculate the total volume of the elute at which the peak occurs. So you are again using the same equation.

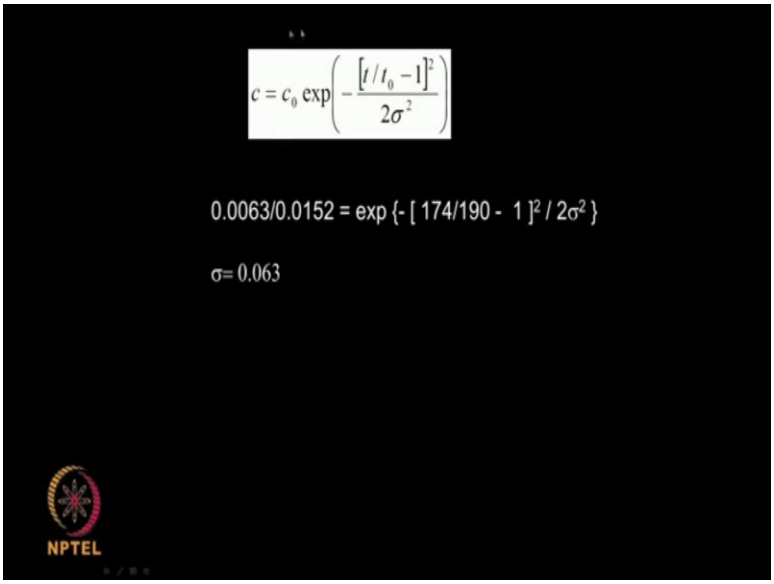
(Refer Slide Time : 10:17)



So let us look at another equation, okay now you have a peak here at 190 minutes and the concentration is 0.05… 0.0152 that is C0 is 0.0152, t0 is 190 minutes, now at170th minute, you have concentration of 0.0063 okay. So concentration along this will be less than 0.0152 right? Now the question is what is the yield at 200th minute? So here what is the yield? That means how much of the material I have collected at 200 minutes. So the equation which you need to use is slightly different.

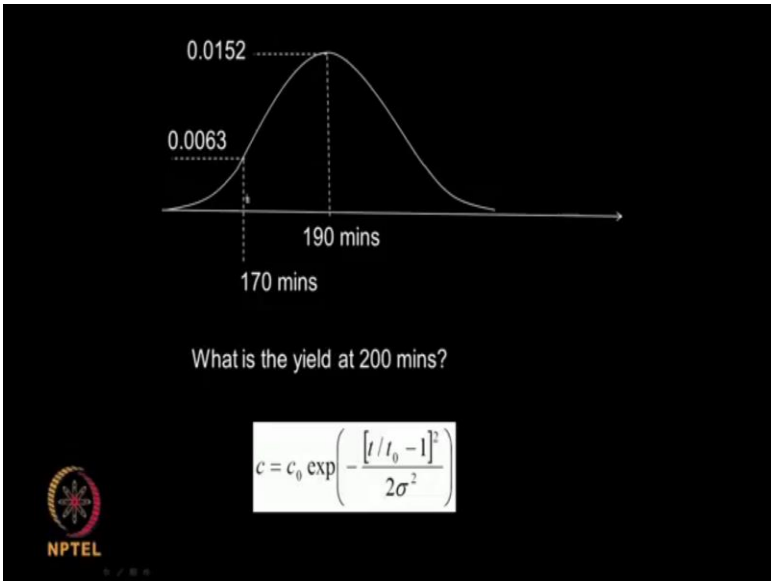
So you are now looking at the original equation which describes this type of Gaussian distribution c = C0 exponent t / t0 - 1 / 2 sigma square. Okay. So all I need to calculate is the sigma because I know that t0, t0 is 190 minutes, I know the C0 which is 0.0152, at t = 170 I know the concentration 0.0063. So I can calculate the sigma. Once I calculate the sigma, I can calculate the yield. If you remember the error function, so using that error function equation, I can calculate the yield. Okay. Let us go step by step. First let us calculate sigma okay.

(Refer Slide Time: 11:49)



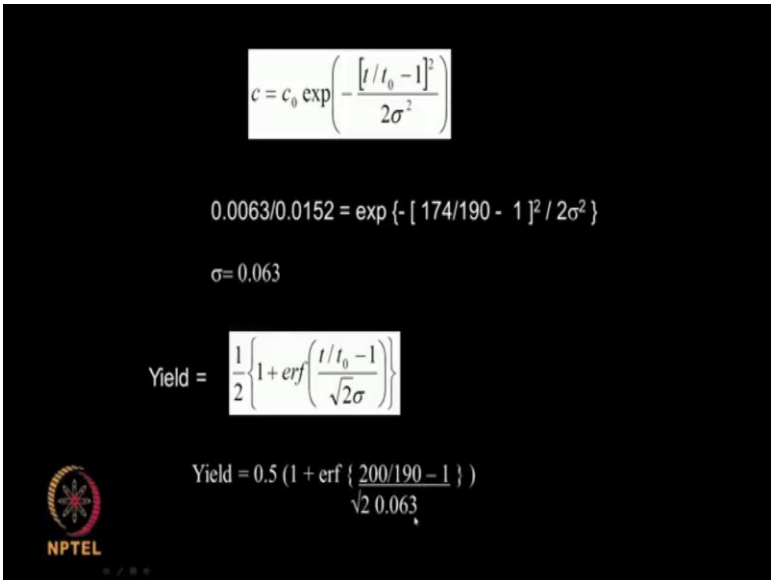
Same equation so I know c and I know t0 which is 190 minutes and I know C0 which is 1.0152 I know concentration c which is 0.0063 at 174th minute and from there I can calculate sigma. Okay. That is the first step I calculate the sigma.

(Refer Slide Time: 12:19)



Because I know t0, I know C0. I know at some time t, what is the concentration c. So I use this equation to calculate the sigma.

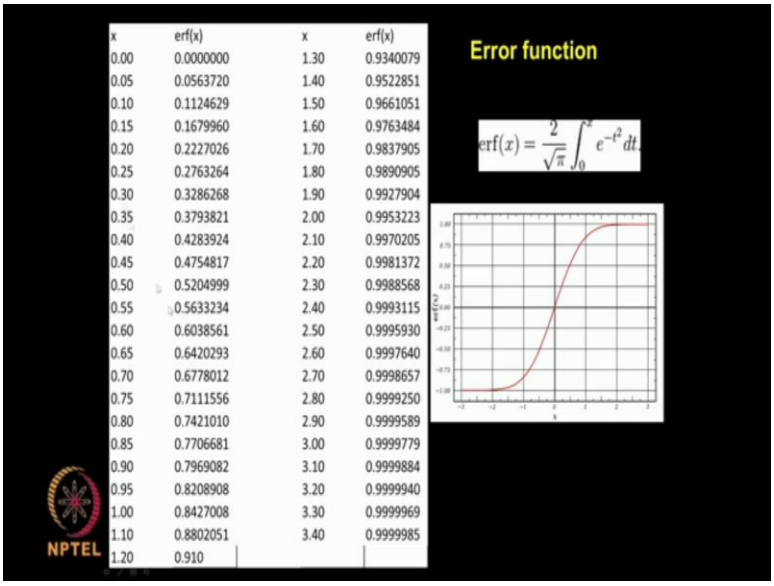
(Refer Slide Time: 12:30)



Now the next step is to calculate the yield. Okay. Now I need to go to this equation, if you remember this, so if I collect sample from the beginning right up to 200th minute, what will be the yield. The equation is half multiplied by 1 + error function t / t0 - 1 / square root of 2sigma. Now I know t okay. t is 200 minutes, so am collecting t the output from the chromatographic column up to 200 minute and t0 is 190 minute, sigma is known, so I need to calculate the yield.

Only hang up is error function and I once showed you the table. Using that table I can calculate the yield. Now let us substitute the terms t0 as 190, t as 200, sigma as 0.063 okay. When I do that I will get error function of 200 by 190 - 1 / square root of 2.063.

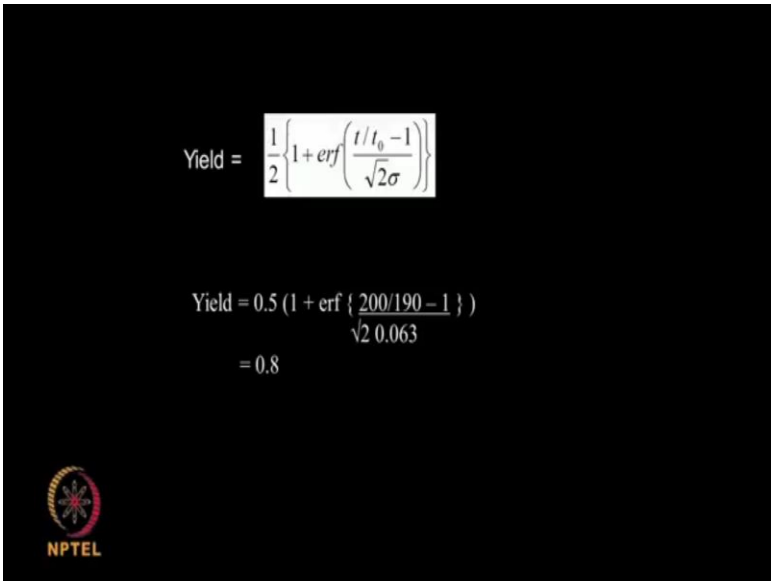
(Refer Slide Time: 13:36)



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		

So I get some number okay I need to calc determine what is the error function. Like I said if I know x, I can calculate error of x, this table is very very useful. So if know x, I need to calculate what is the error of x okay. So I have 200/ 190 - 1 / square root of 2 sigma.

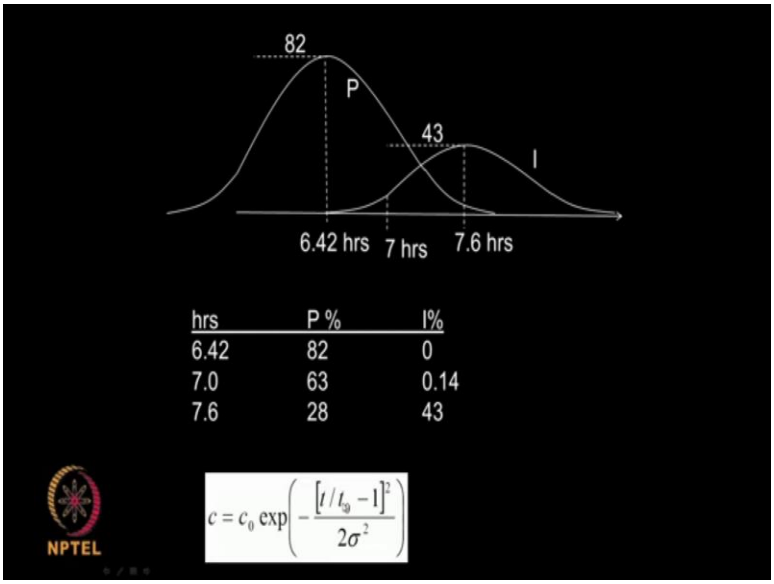
(Refer Slide Time: 13:58)



So when I do that okay so 200 /190 - 1/square root of 2 0.063. So I will get this x value here and from the table I will get error f x. So I will get the yield as um 80 %. So 80 % of the solute could be collected, if I collect sample up to 200 minutes when the retention time is 190 and the sigma is 0.063. How did I calculate sigma I got that from the other concentration at certain time? So it's very very useful equation. Okay. This is a very very useful equation.

So we sort 2 very useful equation, one equation relating the concentration as the function of time. Assuming the Gaussian distribution and then this is the yield relationship, where when I collect the sample for a certain period of time, how much of the solute I will be collecting. Okay. The main assumption in all these is that the curve which we get is a normally distributed output.

(Refer Slide Time: 15:16)



Okay let's look at another problem slightly more complicated. So you have the product p and you also have an impurity I okay. So you have the product and generally these impurities overlap, life is not so easy that the product peak and the impurity peak are far away that whatever you collect will be 100 % pure. No. So if you are collecting sample up to this, there won't be any impurity but if you are collecting beyond this, some impurity will keep coming.

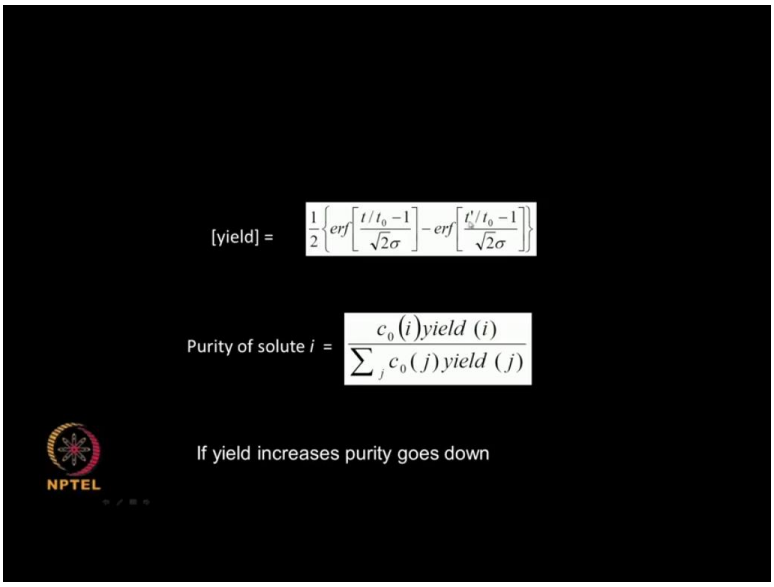
You see this okay and you cannot just collect up to this because your yield of the product will be low. So you need to strike a balance if I want more of the product that means if I collect up to this time, then I will have this much of impurity. If I collect up to this then I will have 0 impurities if you collect up to this, then I will have more of impurities okay. So that is what will happen and these data gives you how these things work.

For example the retention time for the product is 6.42 hours, the retention time for the impurity is 7.6 hours and the time = 6.42, product is 82 % and impurity is 82 %. At 7.6, product is 28 %, impurity is 43 %, at 7 that is here, product is 63 %, the impurity is 0.04 % 0.14 %. So depending

up on where, up to what time I collect, the amount of impurity also will keep increasing but then if it is a very important product, I would like to collect as much as possible. I might like to stop with 82 % because I am losing 18 % of the product.

So I need to collect more of the product but impurity will also keep going up. So how do we do this problem? We have to again use the error function type of things. So first we use this. For the product t0 will be = 6.42 hours okay and C0 will be 82 whereas for the impurity t0 will be 7.6 hours and corresponding C0 will be 43. Okay. So we have 2 equations, we will have 2 sigmas and then from there, we calculate yield. We calculate the purity okay depending upon whatever time I want to collect.

(Refer Slide Time: 17:48)

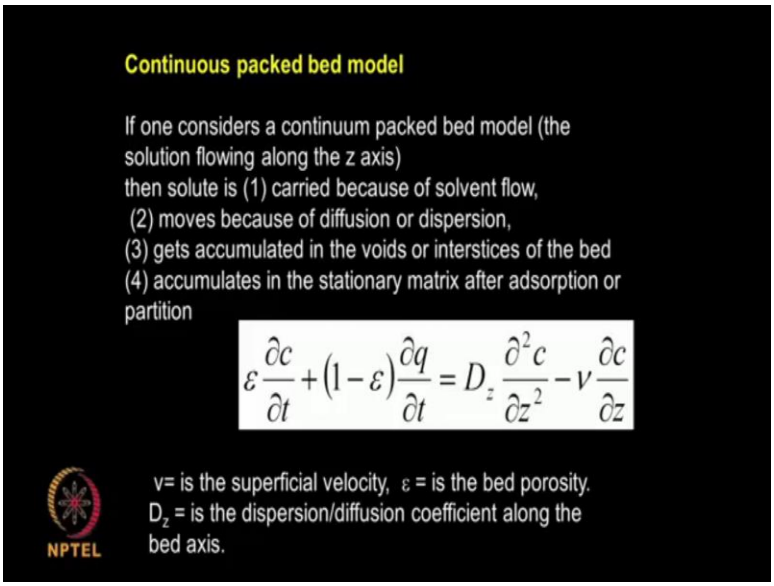


Okay so yield is given by this, if I am collecting sample from t' up to t time, then yield will be given by this. So you have 2 error function terms here. So the purity of solute, I here we have solute and impurity. So this is related to the solute and then the denominator will have 2 terms. The solute related terms that is C0 * yield + C0 * yield for the product okay.

So if the yield increases purity goes down because the impurity also starts coming in. So if the yield increases purity goes down. So it depends on it's a very expensive product, you will like to increase the yield, the purity will go down but doesn't matter. We may have another purification

chromatography and try to purify further. So if the product is not very expensive you may like to stop with lesser yield but higher purity.

(Refer Slide Time: 18:55)

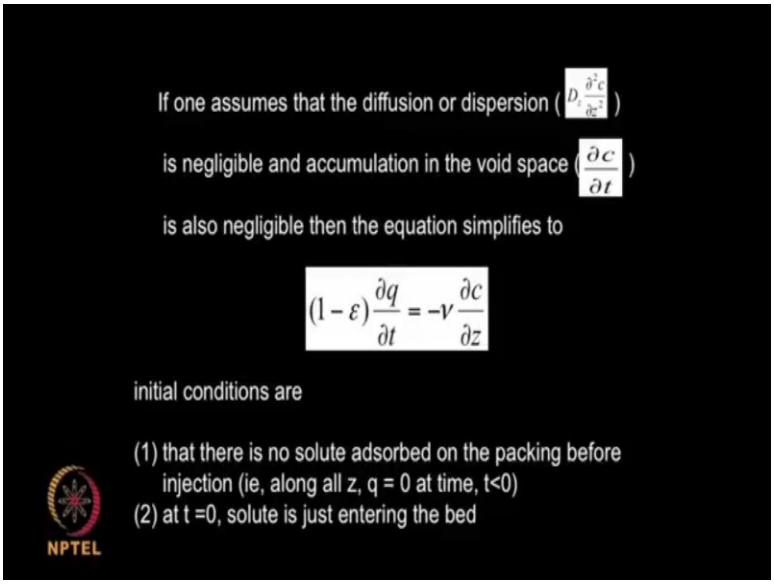


Okay so that is what we need to do in this particular problem actually. Do you understand? That is what we can do. That continuous packed column okay, so if one considers a continuous packed column model for your chromatography because the chromatography column is continuously packed okay. So it the concentration will vary as a distance z, the concentration will vary as a time t. So general equation will look like this okay.

Epsilon dc/dt okay that is the concentration of the solute in the mobile phase, dq/dt that is the concentration of the solute in the stationary phase or the solid phase. Epsilon is the void. So 1 - epsilon will be the packing material, d is the diffusion coefficient or the dispersion coefficient, V is the velocity the superficial velocity. So you have dc /dz d square c/dz square so this term comes because of fixed law of diffusion.

This term comes because of the movement of the solute because of the superficial velocity this terms comes because of the accumulation in the mobile phase. This term comes because of the accumulation in the stationary phase okay. So these equations cannot be easily solved analytically. So we need to use numeric solutions and we need to have some idea about the diffusion coefficient and so on actually okay. That is how we need to do.

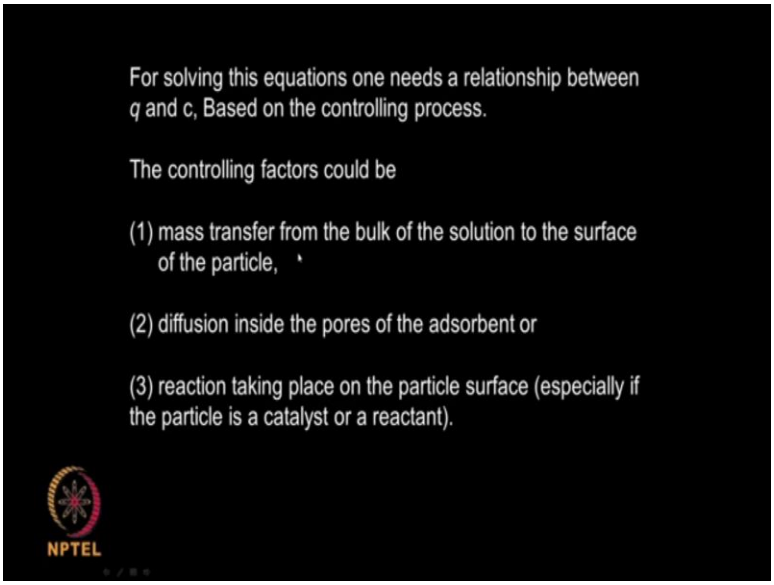
(Refer Slide Time: 20:26)



So if one assumes that the diffusion or dispersion is negligible and accumulation in the void space dc/dz is also negligible, then the equation will go up to be a very simple form, that means we are neglect diffusion coefficient and then we also neglect the accumulation of the solute in the void space that is dc/dz. So we will end up with 1 - epsilon dq/dt = V is the velocity dc/dz is the change of concentration along the distance and this is the accumulation in the solid phase or the stationary phase as a function of time.

So the initial condition, there is no solute adsorbed on the packing before injection obviously at time less than 0. Everywhere there is no solute at all. So at time less than 0 Q will be = 0 at time = 0, you are injecting a sample the solute just entering the bed okay at time = 0 and that is what you are doing actually.

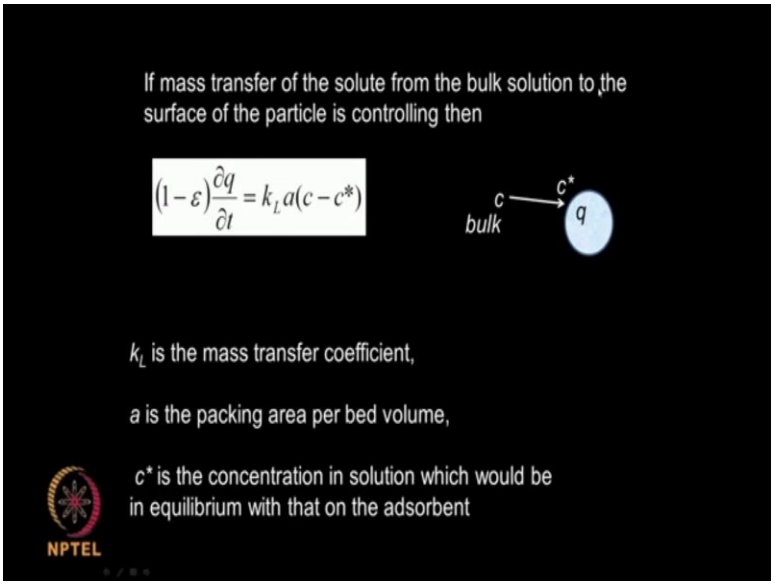
(Refer Slide Time: 21:30)



For solving this equation one needs a relationship between Q and c okay so between Q and c. So you can have a linear adsorption, you can have a nonlinear, you can have a Freundlich, Langmuir and so on actually. Then you also have to have controlling factors okay. There is a mass transfer from the bulk of the solution, to the surface of the particle because the solute has to move from the bulk of the solution, come to the surface of the stationary phase and get adsorbed then there could be a diffusion inside the pore of the adsorbent. If the pores are very porous, then there could be diffusion.

Then if there is a reaction, then of course you will also have a reaction. But in chromatography we are not talking about reaction. So we can completely ignore this. So two things can happen one is the mass transfer of the solute, from the bulk solution to the surface of the particle and then diffusion inside the pores. That is called the pore diffusion. Okay especially for example in gas, if you have the gel permeation chromatography where the solutes diffuse inside the pores. This term may become important also okay. Okay.

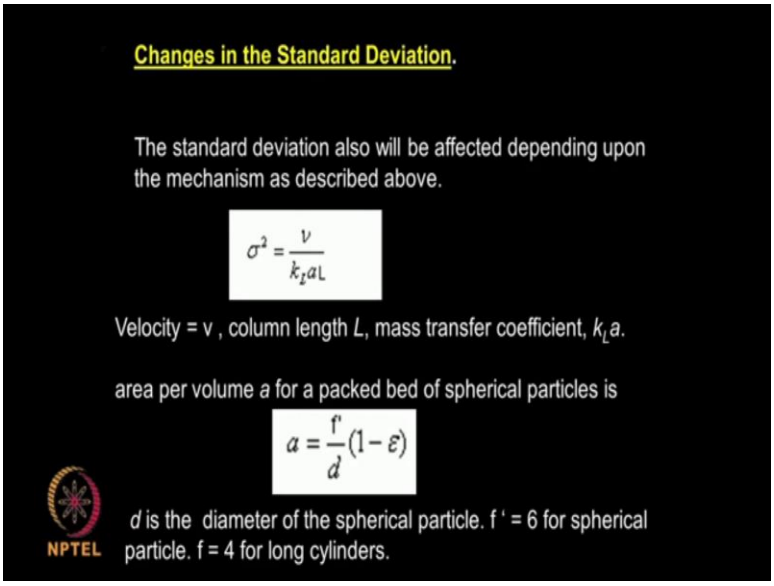
(Refer Slide Time: 22:53)



If so the mass transfer of the solute from the bulk to the surface of the particle is controlling, that means concentration in the bulk could be c but concentration on the surface could be c*. Then c* and Q will be in equilibrium, not c and q c*. So you need to remember c* and Q will be in equilibrium, not c and q okay. Then your equation could be 1 - epsilon dq/dt that is concentration of the solute on the stationary phase as a function of time, this will be = kLa.

kL is the mass transfer coefficient, a is the packing area per volume c - c*, this is the driving force. So you see that c* is the concentration in solution which could be in equilibrium with the Q. So the relationship between Q and c* could be linear or nonlinear but0 between Q and c, so between c and c* will be based on the mass transfer. So this is the equation and we can substitute for c* in terms of Q, if we know what type of adsorption taking place. Okay. So if it is like if you remember linear Q could be = k c*. So we can replace c* with okay Q by k.

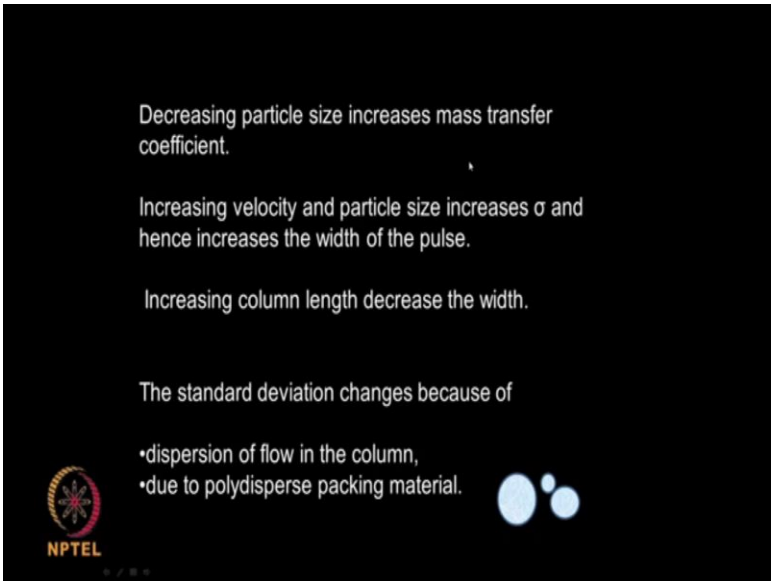
(Refer Slide Time: 24:13)



Okay so if we replace you can have changes in the standard deviation, if we look at, if you remember the equation for c in terms of C0 and t0 you also have a sigma coming in and the standard deviation I said is sigma into t0. So there the sigma could be dependent on so many of these terms like your mass transfer coefficient, the a, the area per volume and so on actually. So the standard deviation will be affected depending up on the type of mechanism.

For example sigma square could be the velocity kLa, that is the overall mass transfer coefficient and length of the column okay area per volume okay is given by a = f ' /d into 1 - epsilon f ' is 6 for a spherical particle and it = 4 for long cylinders actually and d is the diameter of the spherical particle okay. So a is generally of this equation or if you assume spherical then this will be = 6 for long cylindrical this could become 4

(Refer Slide Time: 25:28)

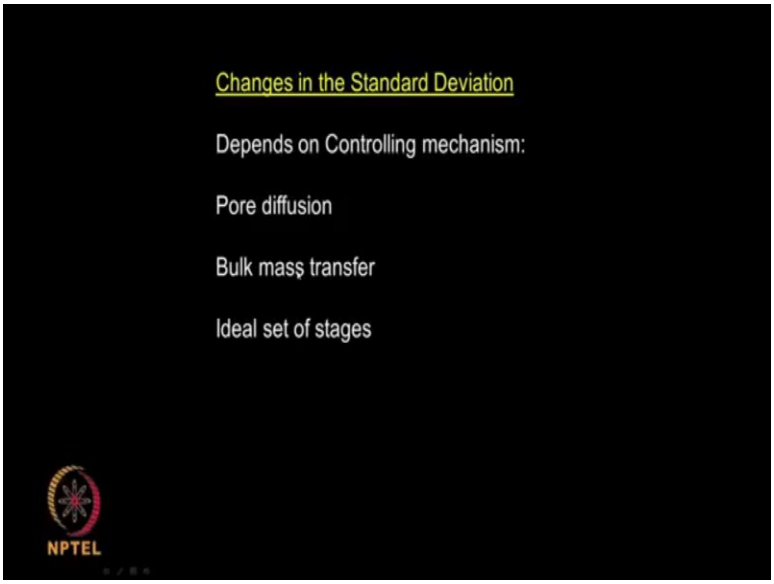


So decreasing particle size increases mass transfer coefficient okay because a comes in the denominator increasing velocity and particle size okay increases sigma and hence increases the width of the pulse. So if I increase velocity and if I increase particle size sigma becomes larger okay. If I increase velocity and if I increase and particle size, it increases sigma and hence increases with the width of the pulse increases column length decreases the width okay.

So if I have a longer column, I may be having a smaller standard deviation. If I am decreasing particle size, am increasing mass transfer coefficient. Increasing velocity increases sigma, increasing particle size increases sigma because my mass transfer coefficient decreases there. So the standard deviation changes because of dispersion of flow in the column due to polydisperse of packing material. So although I say the particle size is say d but then you will not have a very uniform particle size. The particle size may be having a small distribution.

So because of that polydisperse nature, the standard deviation could be also changing actually. So standard deviation is never a constant but it could be a small distribution because depending upon the polydisperse nature of the packing material.

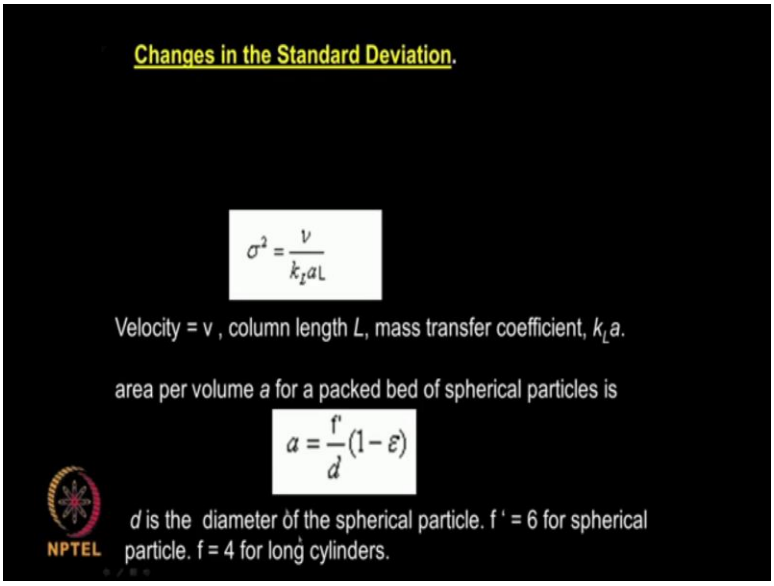
(Refer Slide Time: 27:03)



So changes in standard deviation depends on the type of mechanism. Is it pore diffusion, is it park bulk mass transfer like I showed you and it depends on the stages also. Generally we the n the number of stages or number of plates, it is an ideal but it is never ideal because the distribution of the solute coming out of the column never follows a normal or a Gaussian or a bell shaped distribution.

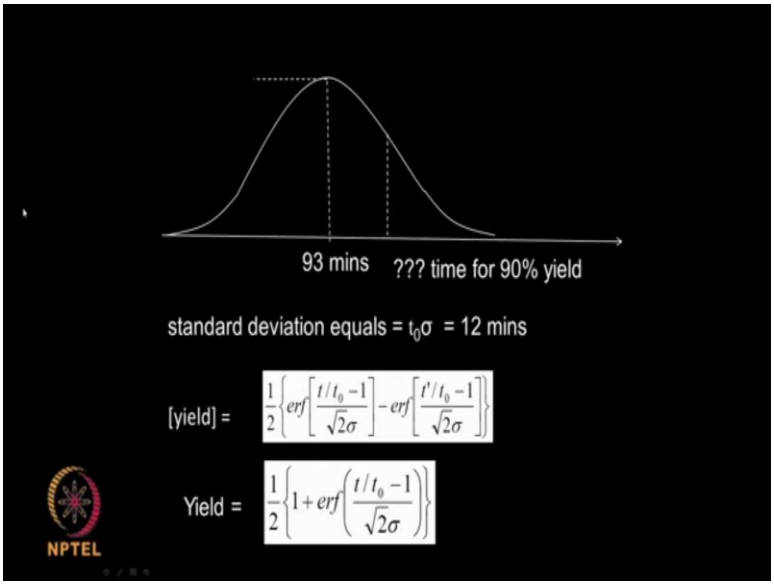
So all these reasons affect your standard deviation. So all these reasons affect your calculations. So you have to but these equations are very very important because they help you to get some feel about the purity yield, number of stages. We can use it for comparing two different columns. We can even simulate some outputs and so on actually.

(Refer Slide Time: 28:00)



So changes in standard deviation like I said this these are very very useful equations to have actually okay.

(Refer Slide Time: 28:04)

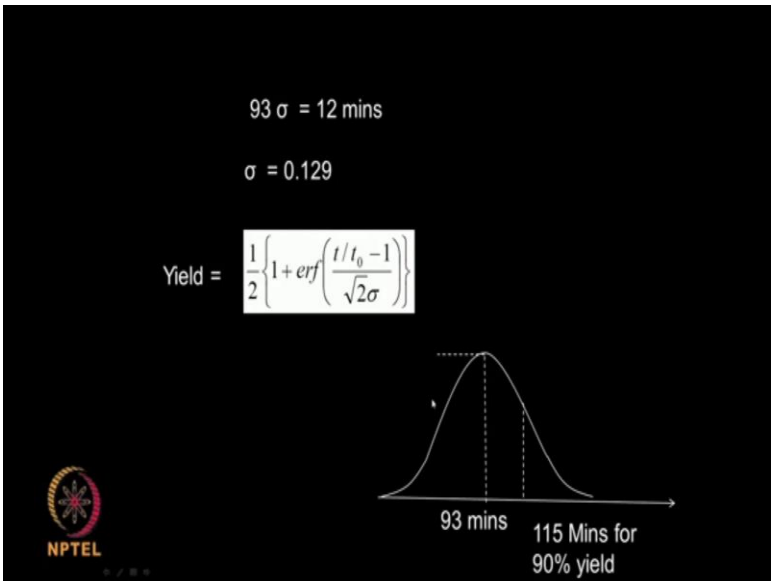


So we have an equation, for example standard deviation as you know is t0 sigma and that = 12 minutes and the retention time is 93 minutes. So what will be the time if I want to have a 90 % yield. So we can do that calculation okay. Not difficult to do okay yield, you remember this equation, I showed you few minutes back. Half * error function t/t0 - 1 /square root of 2 sigma. So I know t0 okay 93 minutes, I know t0 sigma 12 minutes. So I can calculate sigma.

So I can substitute all these, so am running up to sometime so that my yield is 90 %. So I need to know what is the yield. Simple okay very simple. I can substitute and I can use error function through the calculation and yield if am collecting. So this equation boils down, if I am collecting from 0 time to this. So if I am collecting from t' to t, this is the equation but if I am collecting from 0 time, okay this equation will boil down to this.

So from 0 time, what should be my t to get a 90 % yield. So I know t0 is 93 minutes, sigma can be calculated from here because t0 sigma is 12 minutes, I substitute here. So I will get a error of x. As I showed you before from the table when I know x, I can calculate what is the error of x and…

(Refer Slide Time: 29:47)



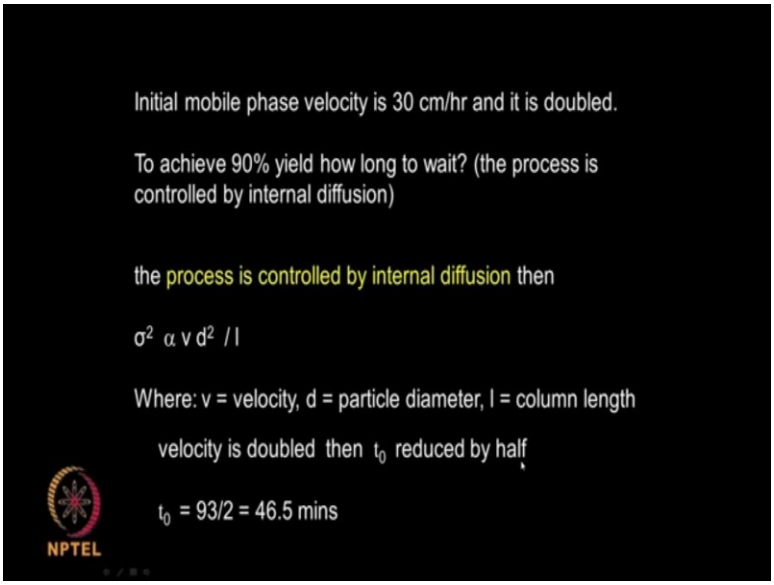
I can get the t okay. So 93 sigma = 12 minutes, so sigma is point 0.129 So I substitute here. So that will be error of x. So yield is given by 90 minutes. So what should be the time. So 115 minutes. So if I start collecting sample from 0 to 115 minutes, I will be able to collect 90 % of the sample. You see this is a very very useful type of calculations in chromatography. So because we need to know how long I need to collect for getting a 90 % of the solute 95 % of the solute.

Knowing certain data knowing things like standard deviation knowing things like retention time and so on actually. Because otherwise you will be just collecting too long and there could be impurity peaks coming downstream which may be getting added up okay and this type of

calculation is very useful when you are going in large scale industrial scale plots and of course there are some assumptions which are the underlying in this calculations.

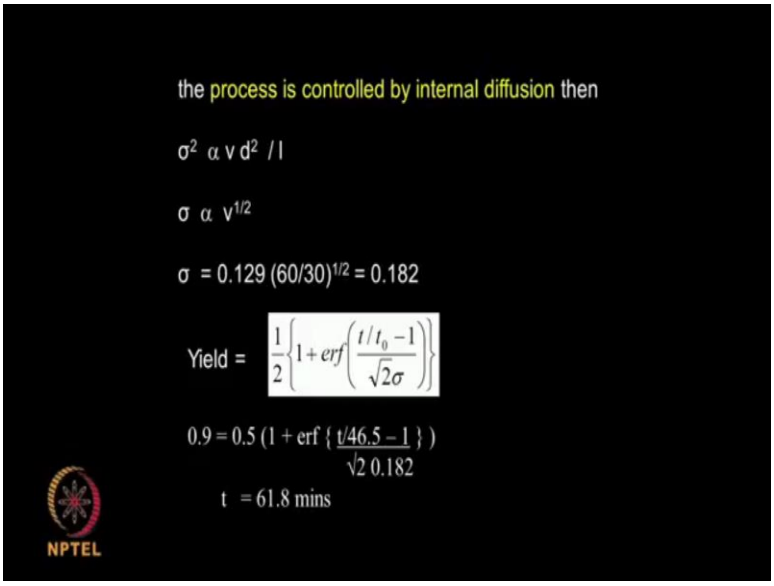
And you cannot help them but these equations are very very useful for you to use okay so t is 115 minutes. So initial mobile phase velocity is 30 centimeter per hour and it is doubled. So to achieve 90 % yield, how long it will wait. Now the process is controlled by internal diffusion. So the process is controlled by internal diffusion then sigma square is getting affected.

(Refer Slide Time: 31:28)



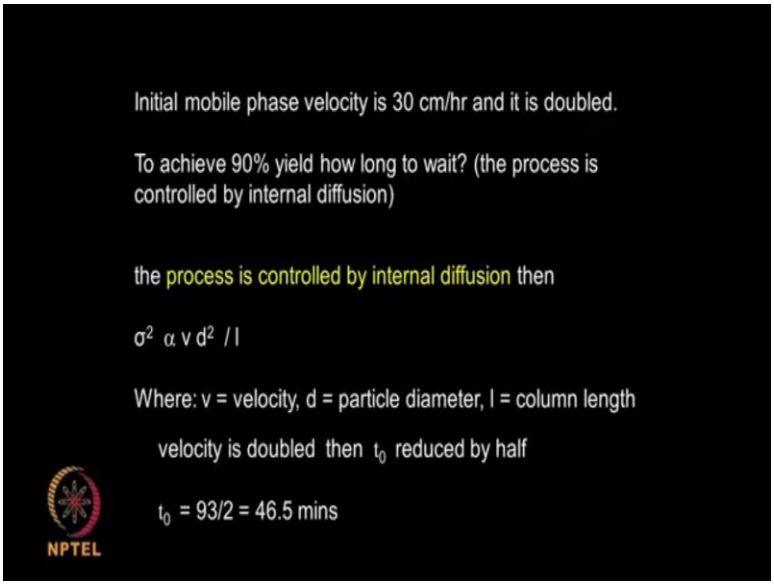
Sigma square is proportional to V velocity d square particle diameter /l okay. So the velocity is doubled okay the velocity is doubled, so your sigma square is going to be square root of that double term. So obviously the sigma is getting trigger okay. So obviously your dispersion is trigger. So velocity is doubled then t0 is reduced by half that is t0 = 93 by 2 46.5 okay.

(Refer Slide Time: 32:06)



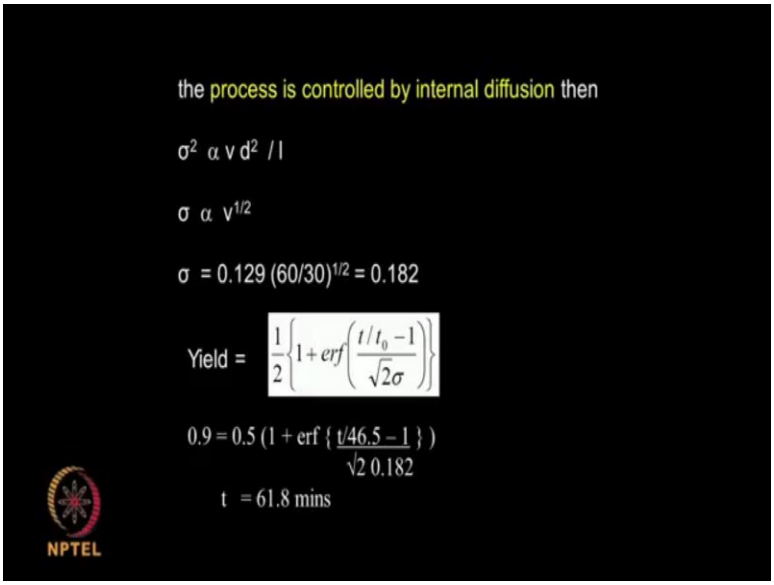
Then the sigma square = Vd square / l. So velocity is doubled okay sig velocity at sigma is rated by this relationship. So your sigma will become 0.182 okay your standard deviation okay. So sigma will become here your t0 has reduced. So I need to use this equation to calculate the t which comes out to be 61.8 minutes. So get to get the same 90 % yield, when I doubled my velocity okay when I doubled my velocity, my retention time decreased by half my sigma changed in this fashion that is square root. So I will get a new sigma, so I will get a new t0 became 46.5. So when I substitute and then I calculate now it's become 61.8 minutes okay.

(Refer Slide Time: 33:10)



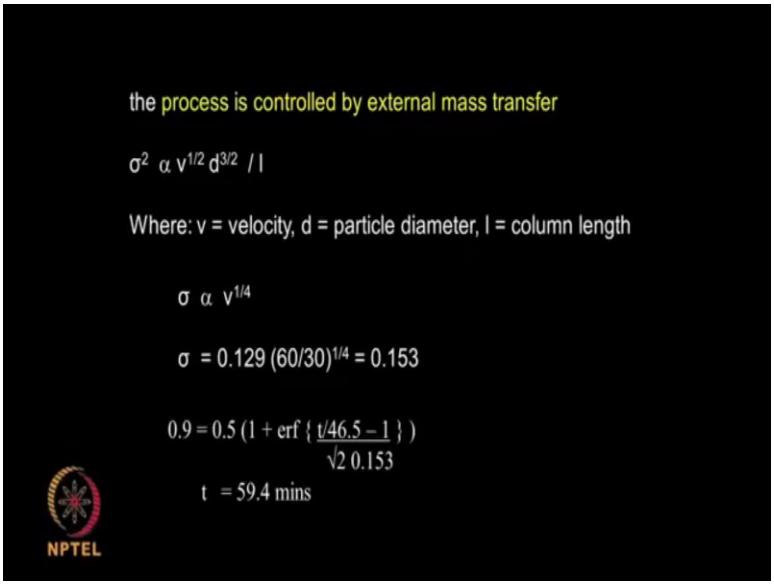
So to get a initial mobile phase is 30 centimeter per hour and when I doubled it to achieve the 90, 90 % yield the t0 reduced from 93 minutes to 46.5minutes.

(Refer Slide Time: 33:27)



My sigma changed quite a lot became 0.182 minutes and then I need to wait 61.8 minutes to collect 90 % of my solute actually.

(Refer Slide Time: 33:31)

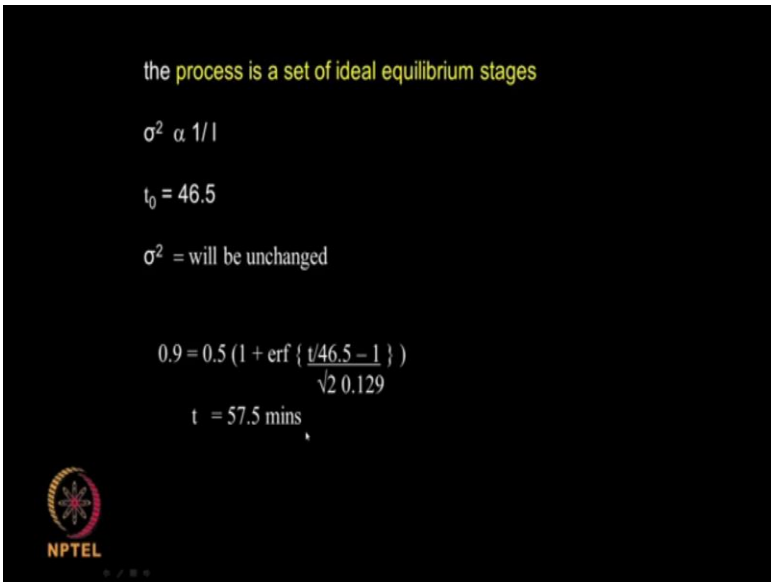


Now the process is controlled by external mass transfer, okay previous case it was a pore diffusion, then the equation for sigma square is this. Okay. Please note the equation changes between the previous and this one here there is a change in the diameter raised to the power 3/2. Okay. So the velocity diameter of the particle, so sigma will become square root of V raised to the power of 1 by 4 whereas if it is pore diffusion it is V raised to the power half.

So the sigma has changed to 0.153 whereas in the previous case it was 0.18 okay. So t becomes 59.4 minutes. So you see that if it is a internal pore diffusion, you have different sigma but your retention time is same. Of course when you double the velocity but the sigma has changed when the process is controlled by external mass transfer, your sigma is a function of t raised to the power 1/4. So you get a new sigma.

So the time required to collect is 59.4 minutes. So depending up on the type of mechanism whether it is a external mass transfer bulk to surfaces controlling or the pore diffusion is controlling you are going to have different sigma values. So you will have different collection time to achieve 90 % yield okay very interesting problem these are and they are very practical useful problem.

(Refer Slide Time: 35:02)



So the if the process is a set of ideal equilibrium stages okay, sigma square will be unchanged because sigma square will be function of only length of the column. if you remember number of stages will be directly proportional to the length of the column. So t0 is reduced by half but sigma square will be unchanged. So we you do that then t becomes 57.5 minutes okay. So we looked at 3 different situation okay we looked at 3 situations, where one is the bulk mass transfer is controlling.

The second one is the pore diffusion is controlling, third one is ideal equilibrium stages. So when we do these three different situations. So when I double the velocity through my chromatographic column, my retention time goes by half but my sigma changes depending up on the type of mechanism. So my collection time changes for each of these changes to achieve 90 % yield. So we looked at chromatography in very in detail in the past 4 lectures.

We looked at various principles involved in it and also we looked at mechanisms of these different types of chromatography and we did large number of problems which connects the various principles of chromatography, which are very useful for scale up, which are very useful in the industrial applications as well.

Thank you very much.