Downstream Processing
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Lecture - 31
Chromatography(Continued)

Let us continue with the concept of discreet stage analysis in chromatography.

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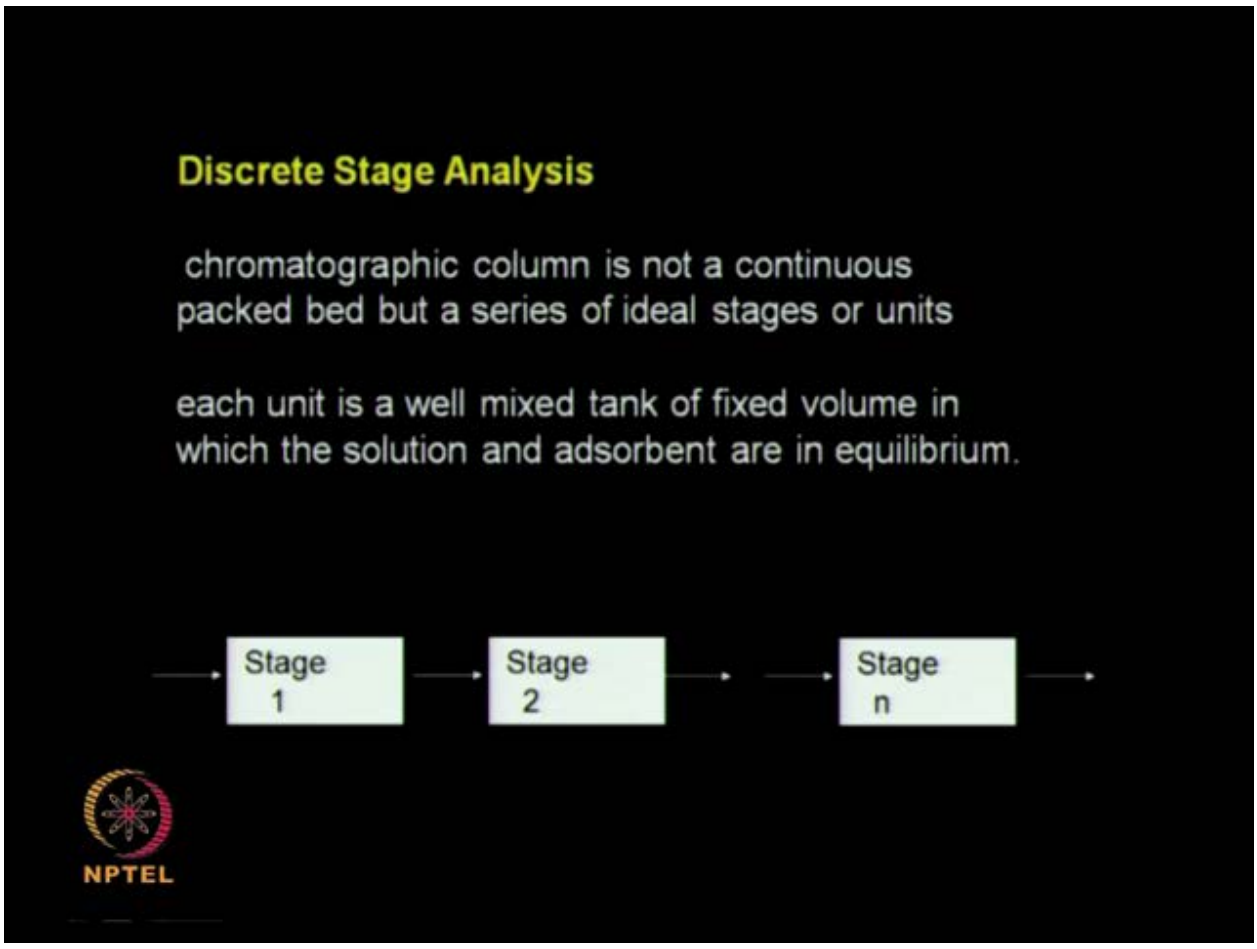

So, what does discreet stage analysis said do the column is divided in two several ideal stages or units and each unit is considered to be totally mixed of fixed volume. So, the solution or the mobile phase, flows and each unit has certain amount of stationary phase and the solute is in equilibrium, the solute that is present in the stationary phase as well as a solute that is present in the mobile phase or a equilibrium.

So, how did we do the solution for this type of a system, we looked at the mass balance of the solute entering and the solute living and then we equated that to the accumulation number solute. Now, the solute gets accumulated in two places, one place is in the stationary phase and the other place is in the continues phase.

So, the accumulation is equated to amount of solute entering is stage minus amount of salute, living is stage that salve, so simple. So, you just have a mass balance and then after that we assume they linear m absorption type of isotherm system; that means, they q that is the amount of solute that is absorbed in the stationary phase is in equilibrium with the solute that is present in the continues space in a linear manner. So, q is equal to k c that is what we have assumed actually.

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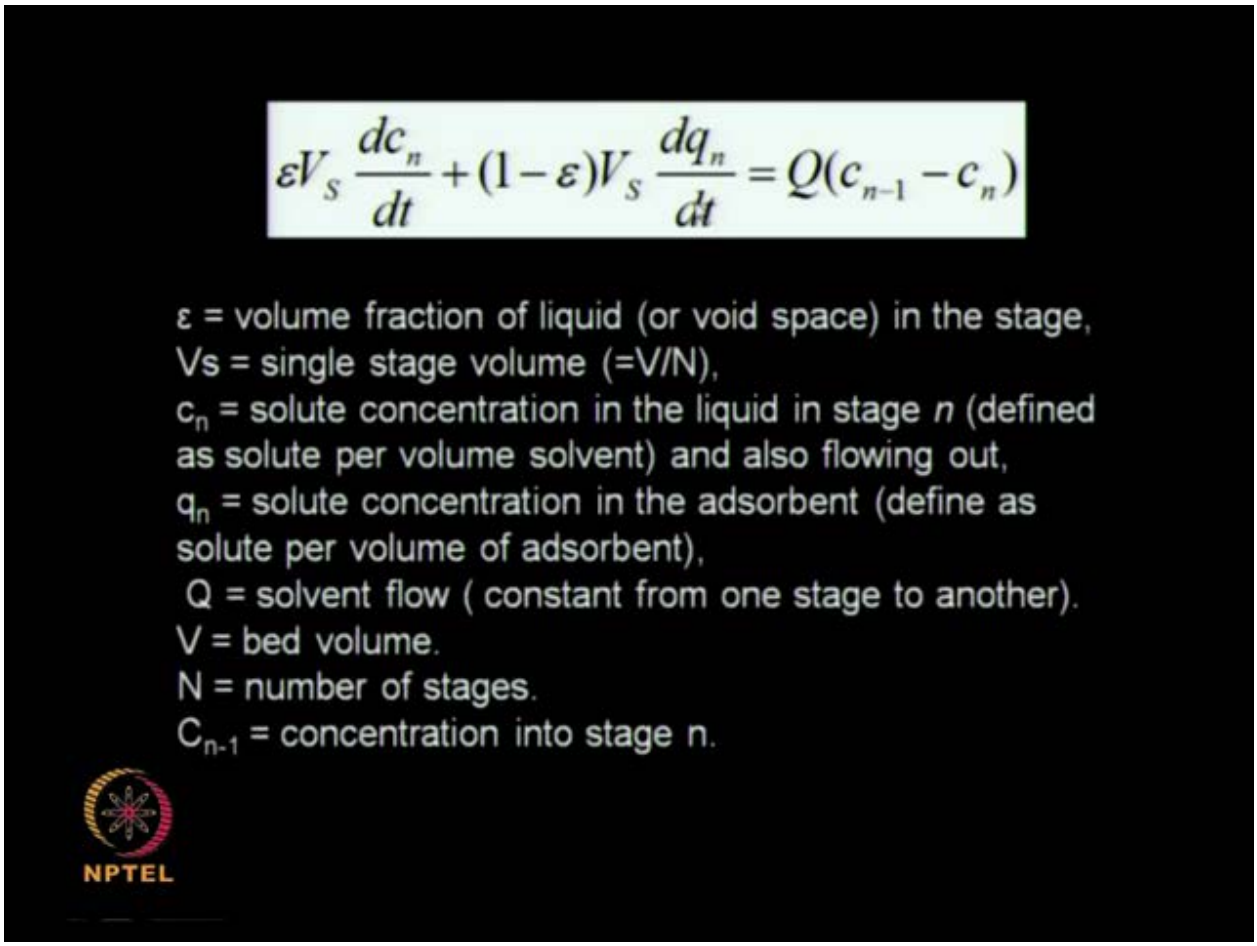

When you do that, we end up with this type of differentially equation all those is equation looks very complicated did not, so actually. Now this Q into C n minus 1, that is the solute entering the stage N from stage n minus 1 and Q into C n is the amount of

soluteleavingthe stage N. This is equated to whatever is getting accumulated, now there are 2 terms for accumulation, one is this solute accumulated in this continues phase, that is the mobile phase.

The other one is the solute accumulated in the stationary phase or fixed phase. So, whatever is accumulated in the fixed phase, we call it Q, whatever is getting accumulated in the mobile phase we call it C interesting point, you need to note that the concentration of the solute in said the stage N is same as the concentration of the solute living the stage N.

That is why here we have put C n, as well as here we have put c n, so please note that. So, in ideal stage process, we assume when there is a equilibrium between 2 streams or phasesthe concentrations a living is same as whatever is the concentration in said the stage.

So, by doing that, we end of the this type of differential deference equation, this is difference, this differential equations. Now, here epsilon is the wide fraction V s is a volume of a single stage, so they assumption is V is the volume of the entire chromatography column V by n, n is the total number of the stages m that is equal to V s.

So, epsilon is the volume fraction of the liquid, so 1 minus epsilon will be the volume fraction of the solute. So, 1 minus epsilon into V s will give you the total volume of the solute and epsilon in to V s will give you the total volume of the liquid. So, now, we introduced the equilibrium, we are if you assume linear absorption equilibrium that means, there is a linear relationship between C n and Q n when life becomes very very simple.

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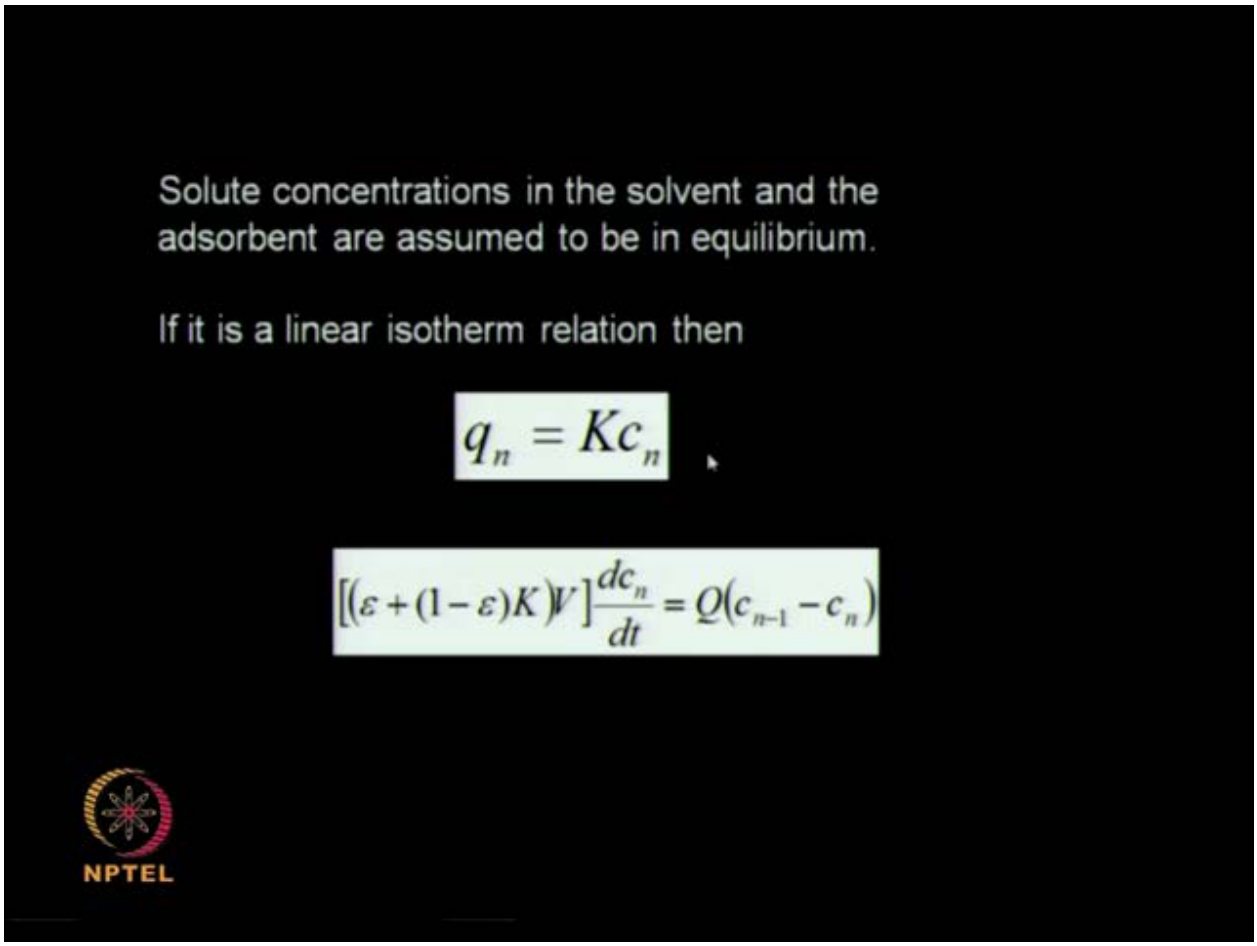

So, if you assume Q n is equal to K C n. So, please note that the concentration solute in stay in the particular stage in the stationary phase and the concentration of the solute in that same stage in the mobile phase or in equilibrium. So, Q n is equal to K C n Q n minus 1 will be is equal to K C n minus 1 Q n minus 2 will be is equal to K C n minus 2, so like that actually.

So, they may another assumption is the K is independent of the concentration that means, we assume same irrespective of the concentrations of that solute in this stationary or in a mobile phase. So, when you do that, we end up with the immix simpler locking equation, once again this is the a mass balance, the amount of solute entering amount of solute leaving is equal to the accumulation.

So, this is again cold here differential difference equation, you have the differential we have the differential equation here.Now you have the initial conditions that means, the times a less than 0 there is no solute a tall present the column has only assume only the mobile phase, there is no solute flowing.

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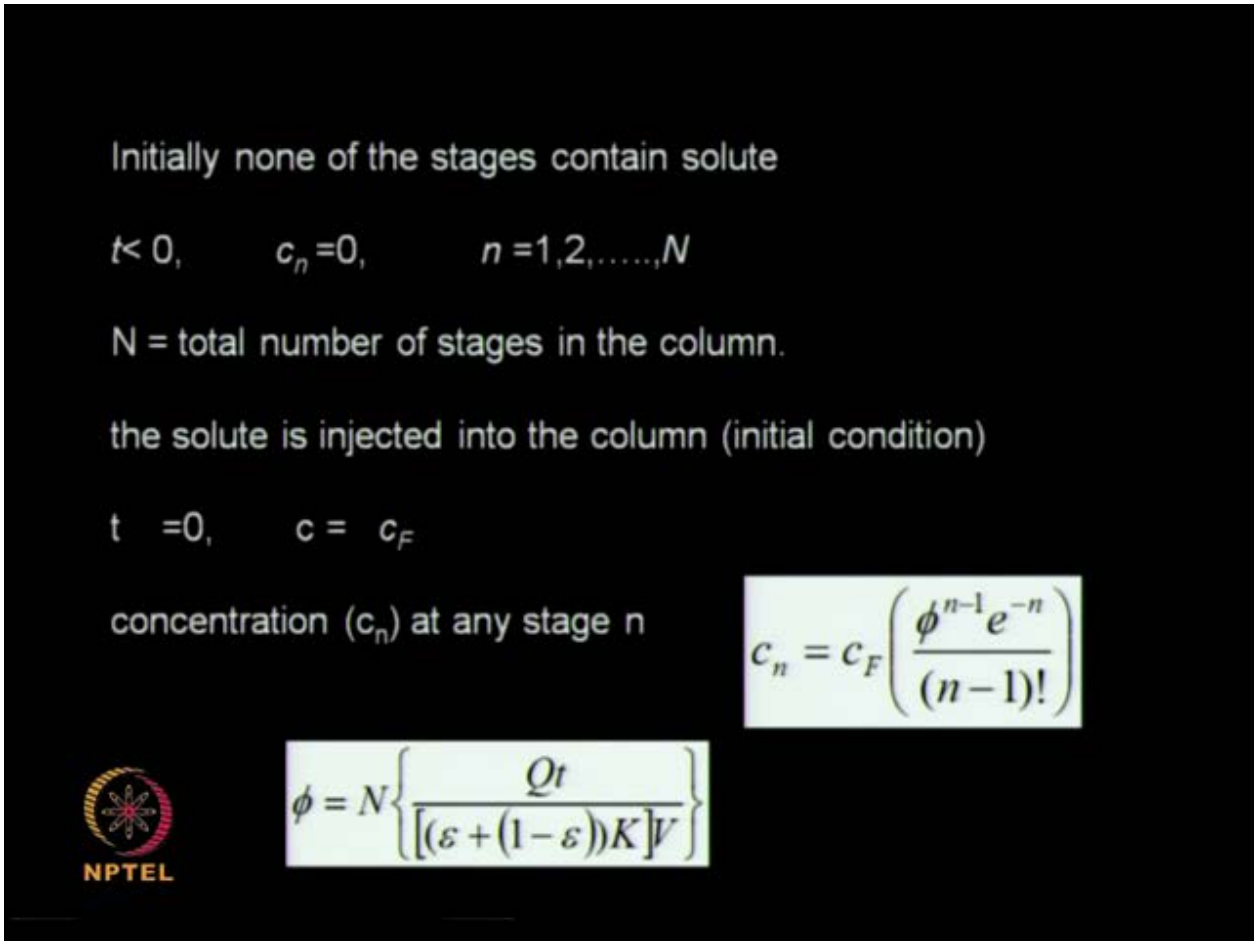

So, all the stages will have concentration of the solute is equal to 0 stage 1 2 stage n. Now the solute is injected at time is equal to 0, that is C is equal to C f than what happens that is the initial condition. So, by using that, we can solve the differential difference equations to end up with the relation like this, C n equal to C f multiplied by 5 raise to the power n minus 1 e minus n divided by n minus 1 factorial, where 5 is given by this a particular term.

Q is the flow rate of the have mobile phase, V is the overall volume, N is the number of stages, epsilon is your porosity, K is your linear absorption isotroterm.So, concentration at any stage of the solute in the mobile phase are of the solute living that stage can be calculated by this relationship. So, this is very use full relationship because, we can make use of this relationship with the relationship, we have for a normally distributed, Gaussian distributed curve that is living the chromatography column.

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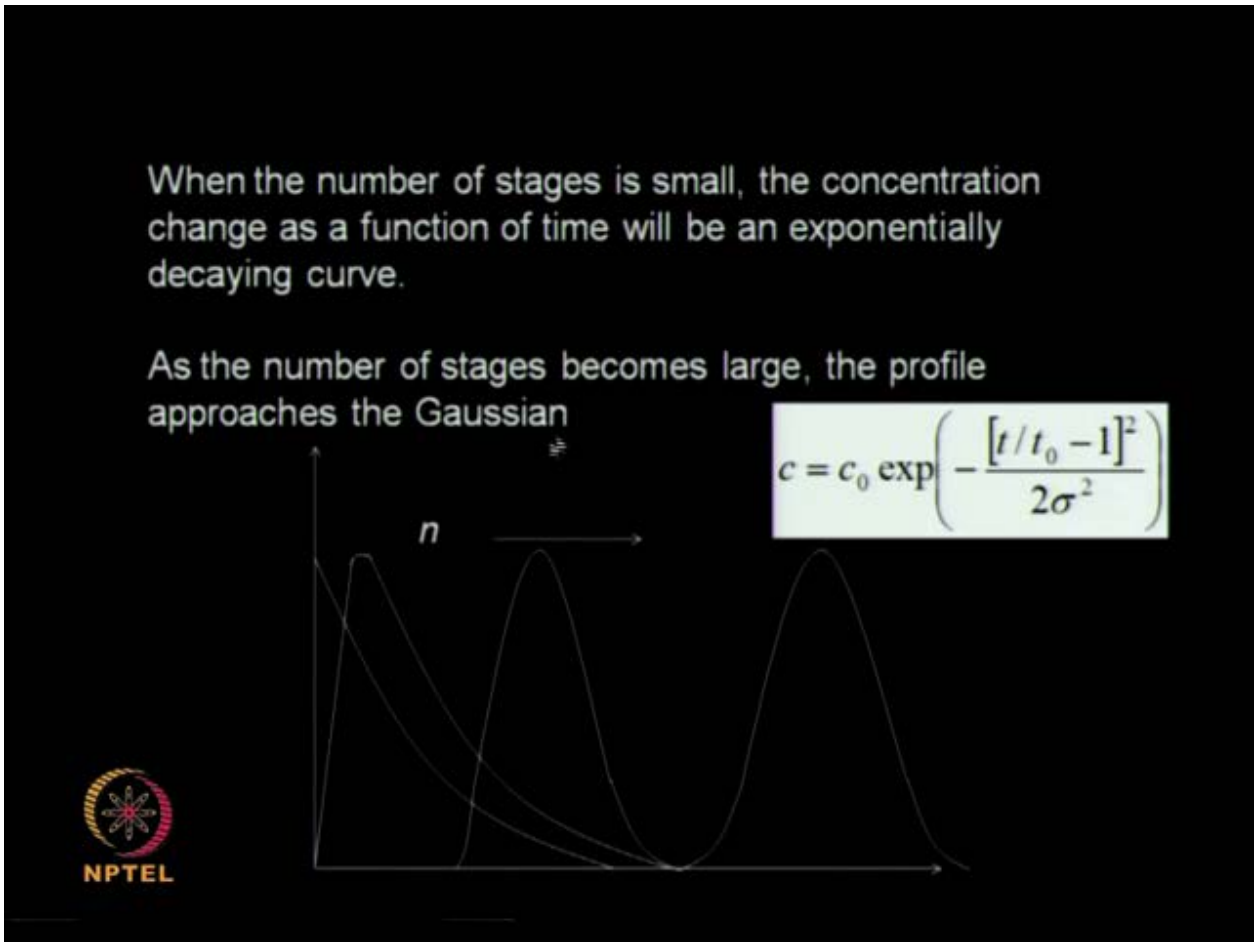

So, before doing that likes lock at effect of the number of stages m when n is very very large than; obviously, the distribution reaches of a gaussian type of distribution where as a number of stages are very very small.

For example f n is one; that means, we assume the entire chromatography column as one single mix stage than; obviously, it is like a stirred tank vassal than a concentration will fall down as a function of time.

So, here the x access is the time. So, concentration you will fall down as a function of a time when number of stage is equal to one. So, as we keep increasing the number of stages the shape of the curve initially may appear like this and slowly slowly it will reach a normally distributed a shape like this.

So, for very large n this is cold a gaussian distribution are a normal distribution and a equation for a gaussian distribution if it is a remember long time back the introduce it it is cold c is equal to c not experiment minus t by t not minus one hold square divided by two sigma square.

Now, here c not will be the maximum concentration it is reaching m when that when the m solute concentrations lives the chromatography column t not is your retention time that is this time and a t not sigma is standard deviation of this gaussian distribution or normally distribution.

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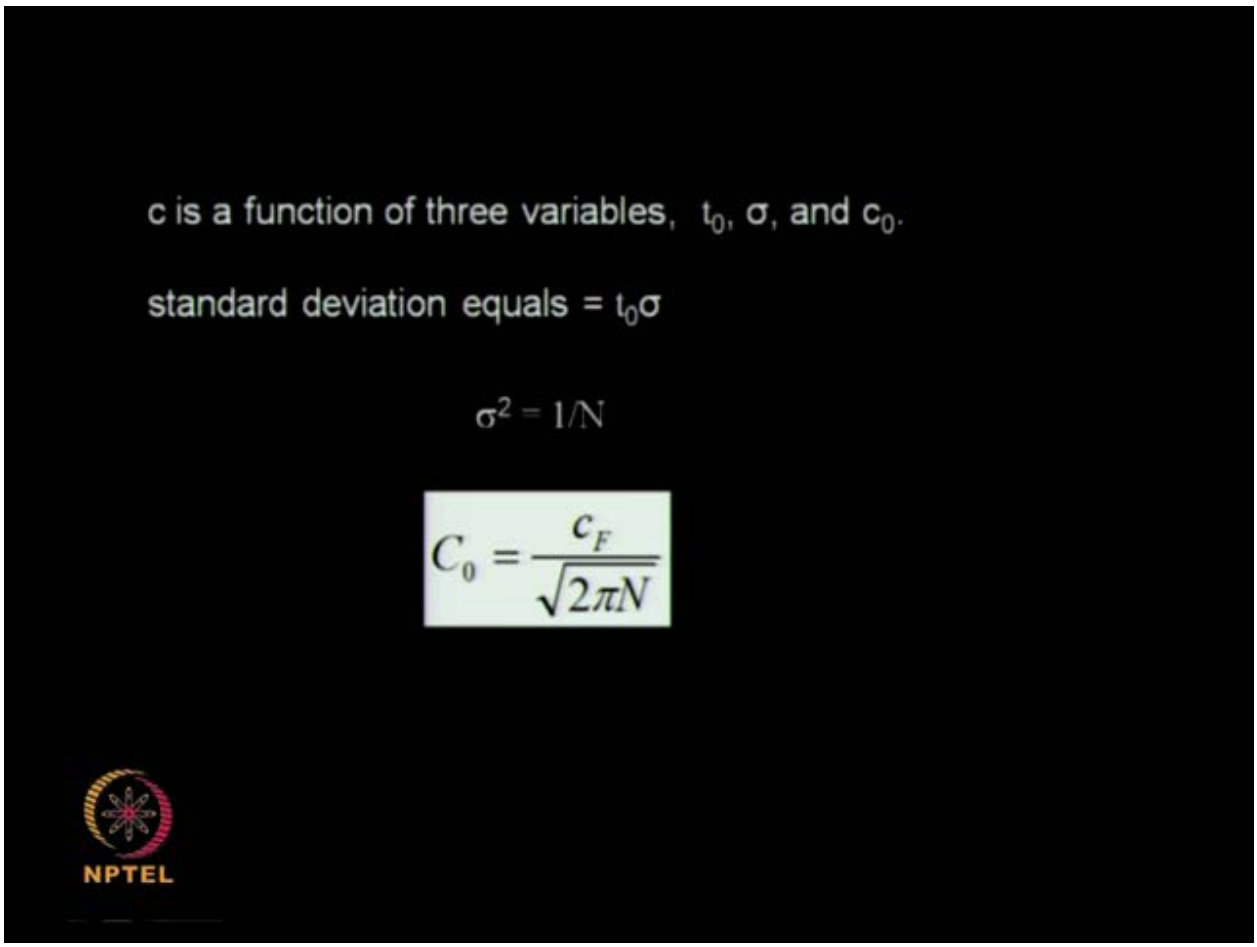

So, this is how a normal distribution equation will look like now we can bring in the analyses which we did for a discreet stage model m where we assumed the chromatography column is divided in to n stages and e stages is a well immixed systemthat means, the times a less than 0 there is no solute a tall present the column has only assume only the mobile phase, there is no solute flowing by doing that we can end up this sum very interesting equations sigma squire will be is equal to one by n n is the number of theoretical stages or theoretical plates c not is equal to c f divided by squire rote of two phi.

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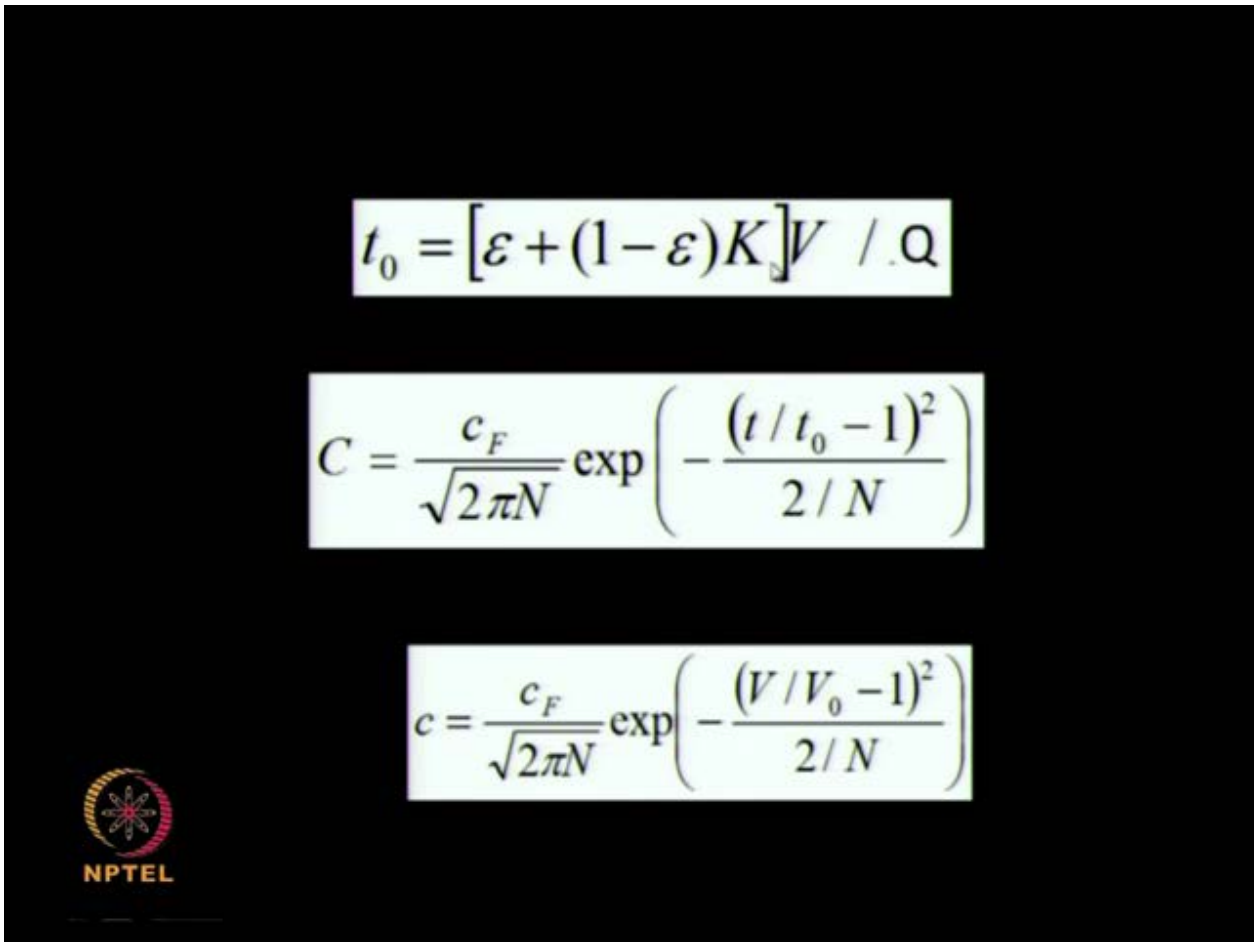

t not is also related to epsilon and k in this fashion actually.

And by substituting all the terms we end up a equation for c x the concentration a profile of the solute as it lived the chromatography column as a function of time is given like this where n is the number of theoretical stages m c f is your feed concentration t not is here retention time just like time we can also have a we coming in to the picture where we not is the retention volume. So, analogue as to this equation we can i have another equation exactly similar looking t by t not we replace it by v volume

So, these equations are very very useful. So, if i have a column i want to know how many theoretical stages are there in the column all i do is i inject a sample and i look add the retention time of the peak living the column and i look add the spread or the slandered deviation and i can tell

What is the number of theoretical plats in the column. In fact, a wanders when the sell that chromatography the mention how many theoretical plates thus this particular column

have. So, a larger the number of theoretical plats you know the column is very efficient in separating a smaller the number column is not as efficient relatively

So, when we by chromatography column you always look for that number of theoretical plats and then make a decision on how good the separation efficiency of the column is or not as good and. So, on actually so, will look that five problems in the privies a lecture on how to make use of these a equation to calculate the number of theoretical plats or stages if i now at the standard deviation of my a normal distributed curve as well as the they retention time ok.

Now, let us keep doing some work problem. So, that these concepts become very very clear.

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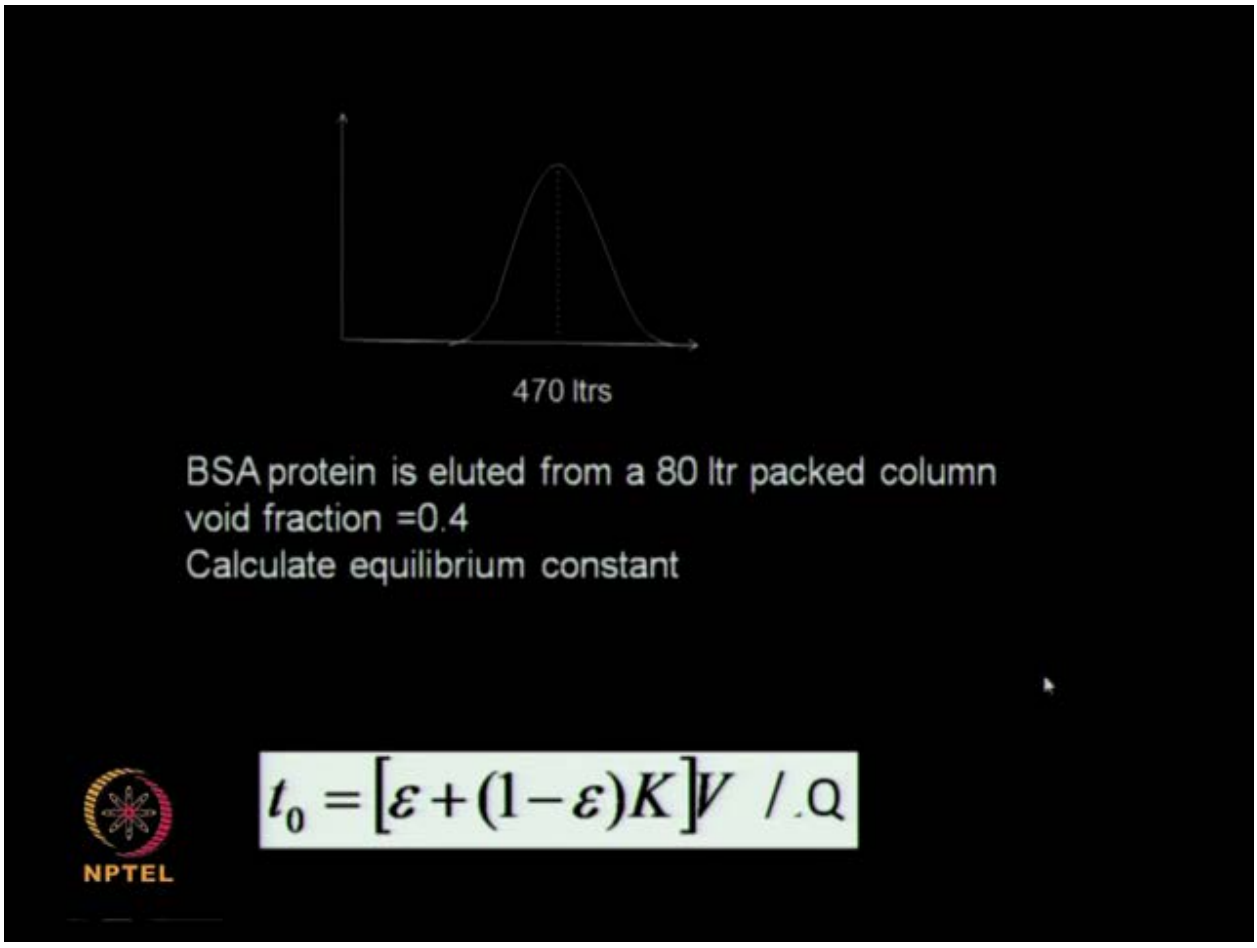

now imagine I am using a chromatography column to purify b s a ovine siren all beam in a protein ok

So, when I use that now the column is about eighty liters it is a packed column of a eighty liters with the void fraction of point four. So, is god a void fraction of pint four; that means, one minus point four will be the amount of solute presenting seize now this particular peak comes add act a retention volume of four hundred and seventy liters ok.

They as a set just like a retention time we can also qualid retention volume. So, this maximum happens add four hundred and seventy liters now calculate the equilibrium constant. So, if we remember the equation which relates equilibrium constant divide age the column size as well as a retention volume ok

This is the equation t not that is the retention time is equal to epsilon that is the void age k is a equilibrium constant v e z volume of the column and q is the flow rate. So, t not in to q will give you the retention volume which is four seventy and point four is they epsilon eighty is the volume of the column. So, all in it to do is calculate k the equilibrium constant.

So, it is very strata forward ok.

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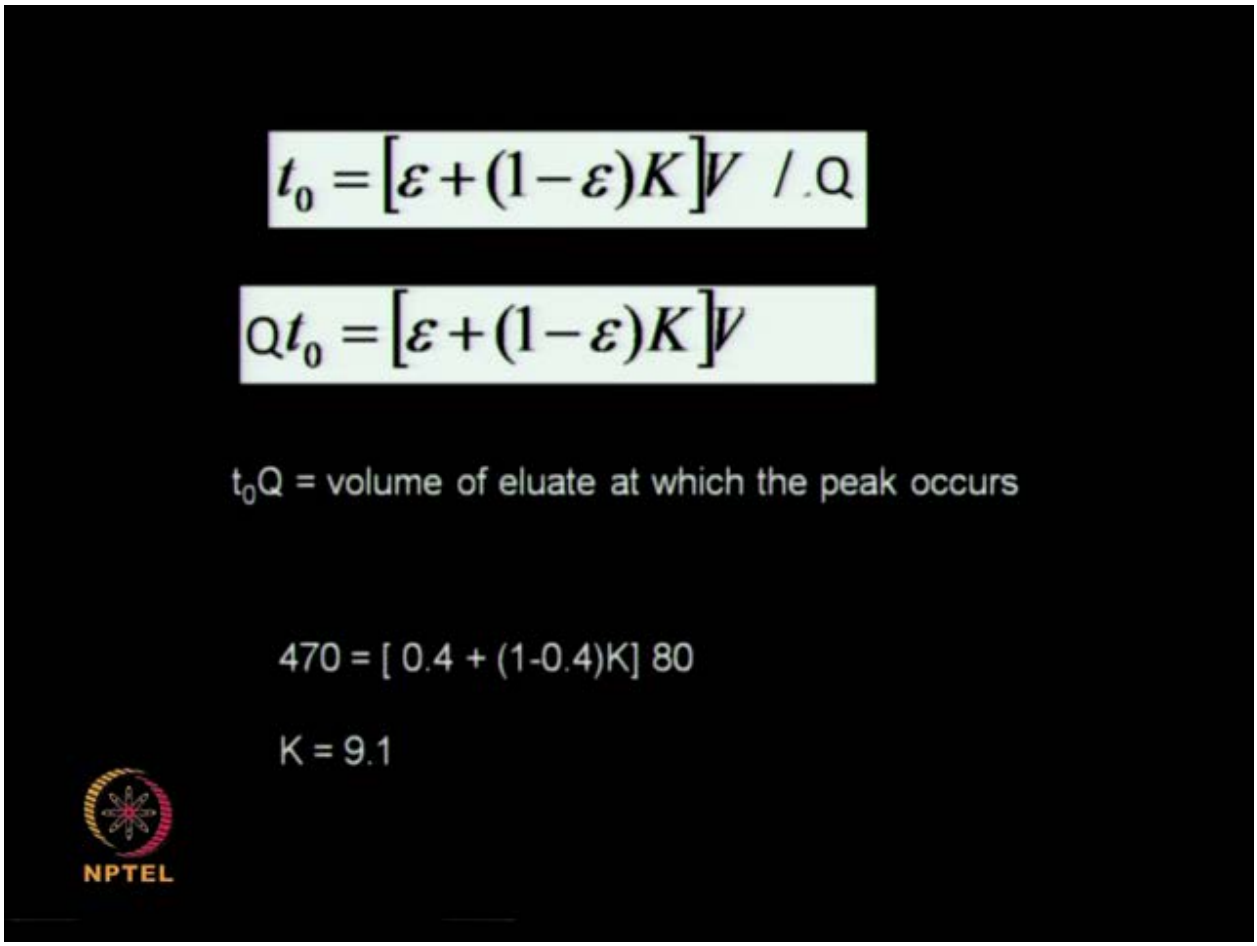

So, same equation. So, if i take the q here q in to t not is equal to epsilon plus one minus epsilon k in to v t not q is the volume of a eluate or volume of a solute which is the peak occurs ok.

So, by substituting these a four seventy epsilon equal to point four one minus epsilon will be one minus point four volume will be eighty liter i get k as nine point one. So, this k is nothing, but the linear absorption isotherm constant which relates the concentration of the solute in the stationary phase as against the concentration of the solute in the mobile phase if you remember q equal to k in to c where q is the concentration of the solute in the stationary phase and c is the concentration or the solute in the mobile phase.

So, very use full data to have. So, you get in a idea about the thermo dynamics of the solute interaction with the stationary phase and the mobile phase by just doing in injection and collecting certain amount of liquid and look add the retention time.

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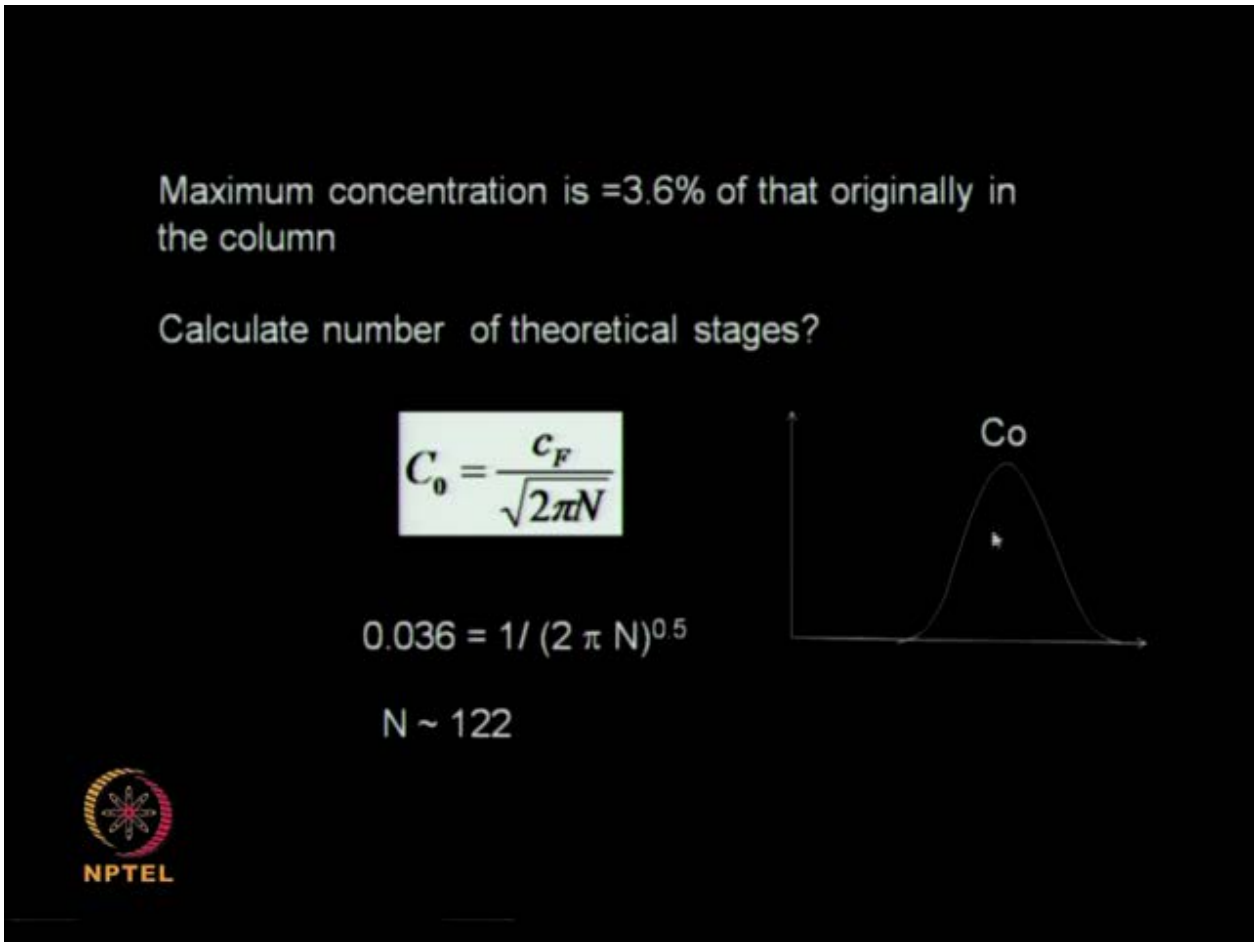

Now, let us go forward let us look at another problem same problem, but an extension now the maximum concentration is three point six percent of that originally in the column ok

So, calculate the number of the theoretical stages last and we look at different ways of calculating theoretical stages based on the retention time and standard deviation based on the retention time and with at half maximum now this is third approach by which we can calculate the number of theoretical stages this is we now the maximum concentration is three point six percent of that originally in the column

Now, you want the calculate the number of the theoretical stages. So, which we cation the use if a remember there is an equation relating c not where c f that is a whatever is there in the original c not is equal to c f divided by squire rote of two phi n. So, c not by c f is point zero three six. So, from there we have to calculate the n what is the number of theoretical state forward n comes how to be one twenty two when interesting. So, we can use this type of approach the calculate the number of theoretical stages

So, you see if the number of theoretical stages or very very large because it comes in the nominator c not will become smaller. So, we will get very small and small p.

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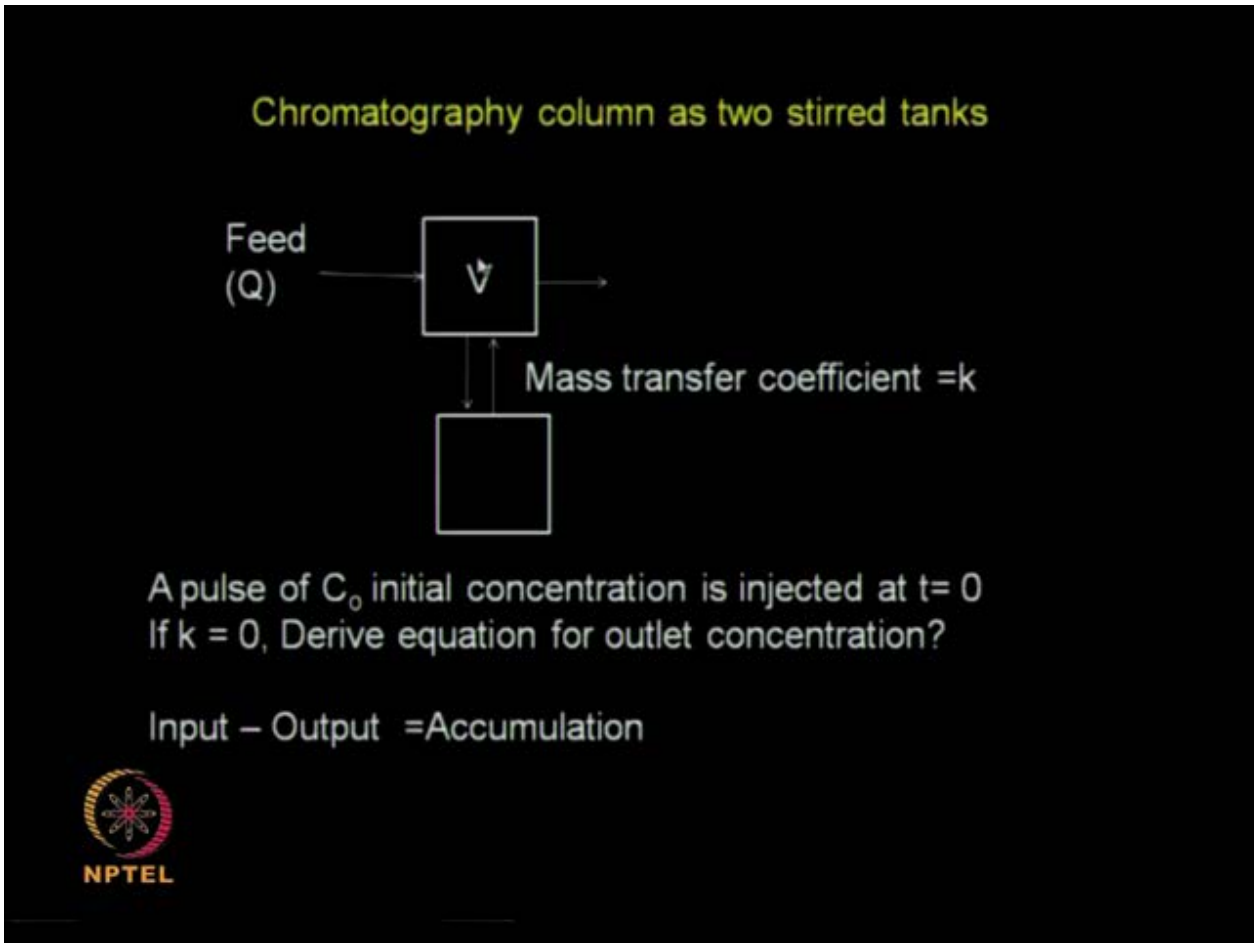

Now, let us look at the chromatography column m like a stirred tank reactor originally you assume a single stirred tank reactor m and try to model the chromatography column

When that is a very very approximate model m then we are extending it to n stage system where n is very very large if when is very very large then the relationship becomes much more uniform or normally distribute red or gaussian distribute red curve now looks at another approach where your trying to model the chromatography column like two stirred tank.

So, you have a stirred tank of volume v and there is another tank, but they feed is entering this first tank and the product is going out from the first time only, but there is mass transfer or there is a flow of solute in and how'd the first and the second tank they

are connected like this. So, the feed is entering ally the first tank and a product is going out on ally first tank and e volumetric flow rate of the feed this q there is a mass transfer coefficient which relates the amount of solute in and out of the tank one and tank two. So, in the tank two does not have any in or out streams ok

Now, assume this type of system imaging in your giving a pulse of c not initial concentration is injected in time is equal to zero suppose if k is equal to zero; that means, there is now in and hawed can you derive and equation for the concentration of this pulse as a function of time. So, there is no mast and should taking place k is equal to zero

So; that means, this stirred tank is completely cut off from these stirred tank now your injecting yeast pulse at time equal to zero initial concentration of the pulse c not can you find out in equation which relates the concentration living the tank one as a function of time. So, it is very striate forward we have to do in a mass balance input minus output equal to accumulation

So, it because there is a no reaction we always say input minus output is equal to accumulation. So, it because there is a no reaction we always say input minus output is equal to accumulation in there is a reaction then; obviously, there is going to be lose because of reaction now what is input whatever is coming in of the solute in the feed what is output whatever is going out of the solute

Now, there is no interaction between tank one and two. So, we done have to considered that and that is equal to accumulation.

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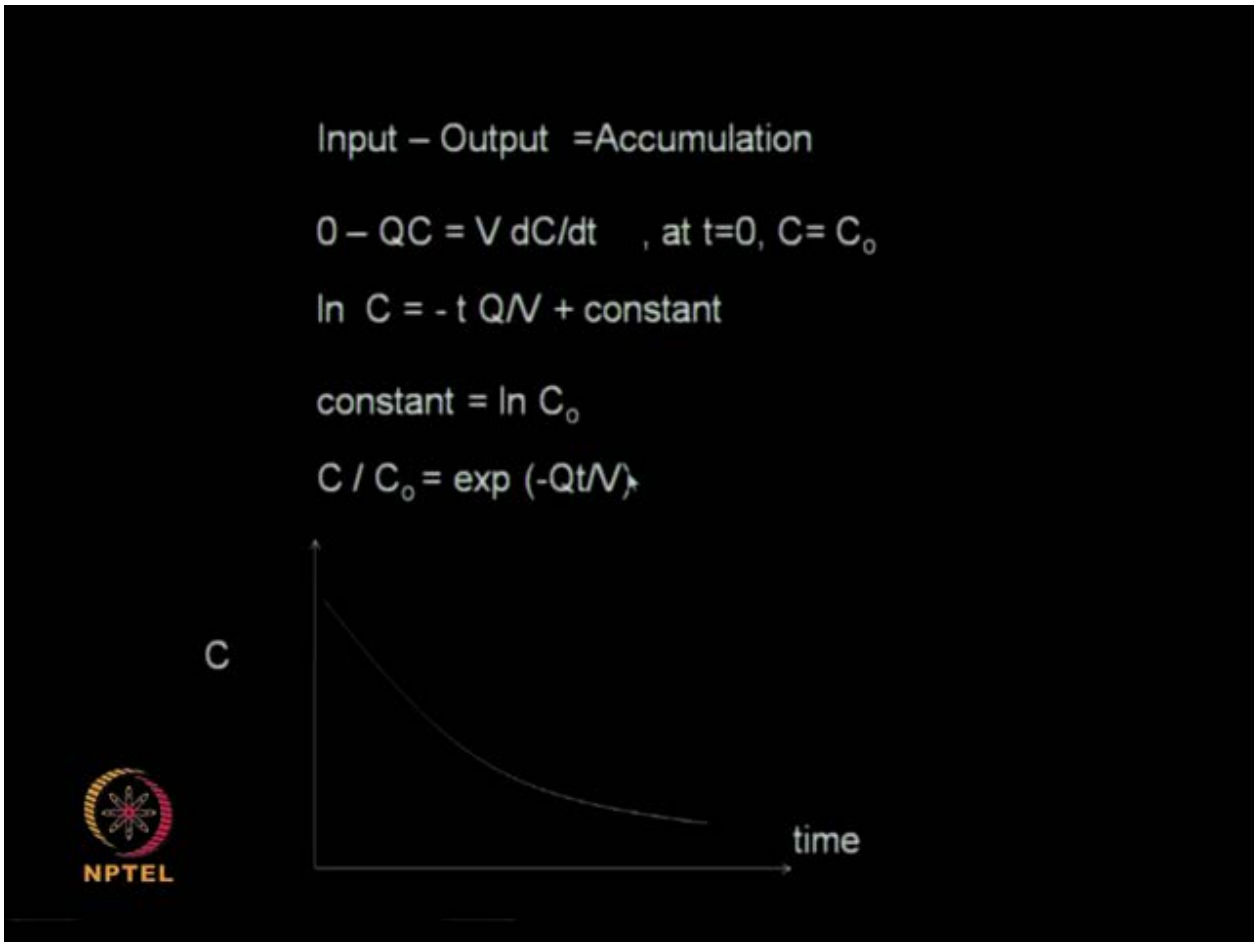

So, input will be is equal to its zero because there is no solute entering we were just injected some solute of concentration c not at time is equal to zero that is all after that there is no solute entering

So, please remember that that is why input is equal to zero, but there is going to be some solute living that tank because we have injected some solute of concentration c not at time is equal to zero so; obviously, the output will be they present at input will be zero. So, output will be q is the volumetric flow rate c is your concentration

Now, this is equal to accumulation what is accumulation v d c by d t. So, it c it is a first add the deferential equation at t equal to zero we are injecting some solute. So, at t equal to zero c c will be equal to c not. So, if you integrate this and substituted these you will end up [vocalize noise] c by c not equal to exponent of minus q in to t divided by v. So, c is the concentration of the solute that is the living the tank c not is the concentration or the solute that was injected time is equal to zero that is equal to exponent of minus q

Q is the volumetric flow rate of the m solve n that is flowing in t is the time divided by v is the volume. So, how will this equation look like it will be exponentially falling like this. So, initially it will start from c not at time equal to zero here and then as time progresses this will keep falling and an exponential fashion ok

Now, this v volume divided by q flow rate resident the is cold residents time of the tank v by q is the residence time of the tank. So, you c that c by c not is equal to exponent minus t by residence time. So, there is a residence time has a very important effect on the way this particular concentration keeps fowling as a function of time ok

So, we had this particular problem where we have two tanks the tank one there is solve n flowing in a all the time solvent is coming out at time equal to zero your injecting a solute of concentration c not and the mass transfer between one and tank two if we neglect; that means, you assume the mass transfer co efficient to be zero ah

We are as to do derive a relationship for the concentration solute living tank one as a function of time and this is the particular relationship it is a first order process because here we have the first order process. So, a solution for the first order process will always look like this. So, its cut e exponent minus q is the flow rate t is the time divided by v is the volume of the tank

So, it is going to fall down exponentially scorch strait forward.

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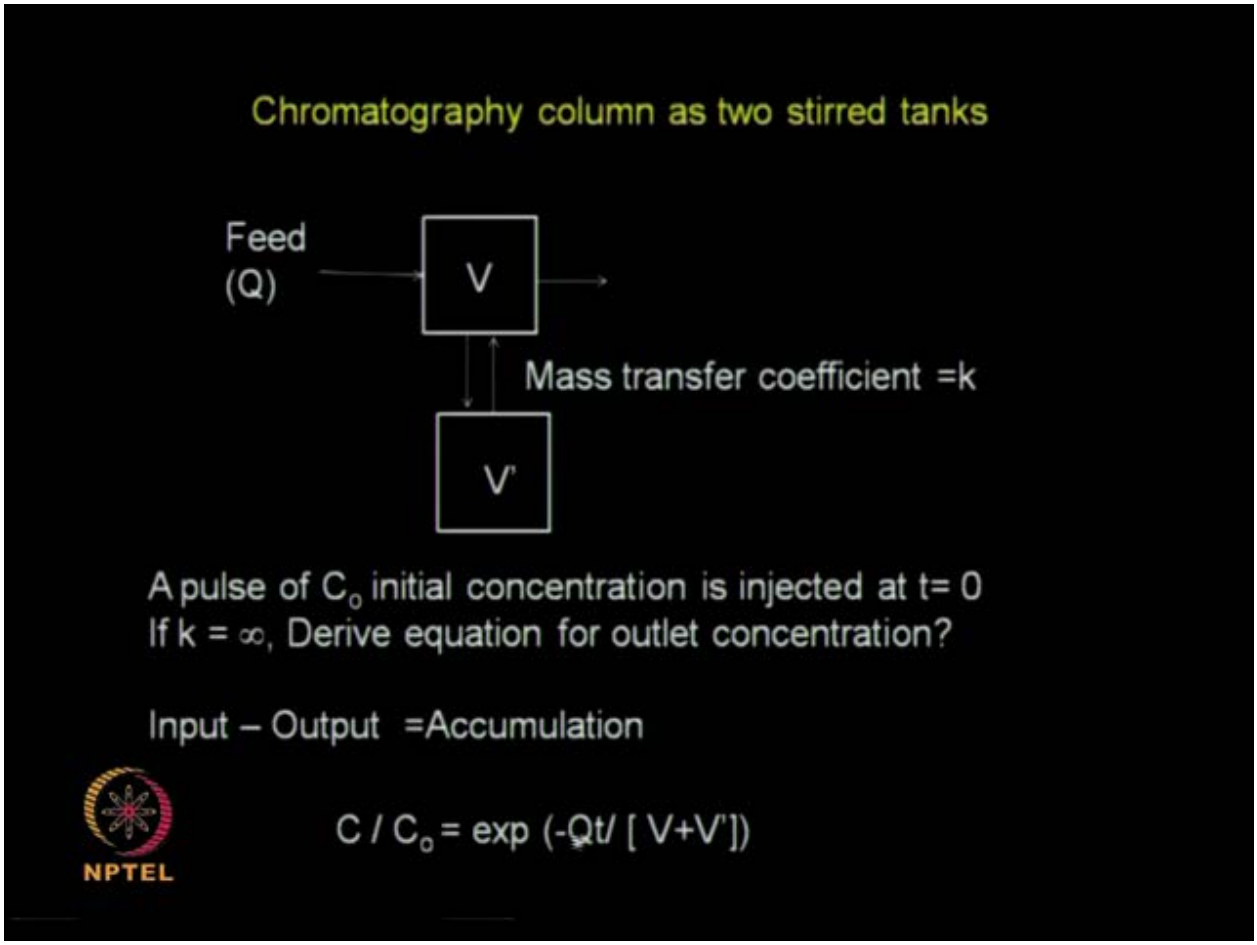

Now, let us look at a slide modification to this problem. So, again we have the same two stirred tank tank v and this tank is v dash now the solvent is flowing at a flow rate q and then solvent is coming on it is flow rate again at q there is in exchange of material because of mass transfer coefficient k ok

And there is now streams entering this second tank or the streams living the second tank now again a pulse of c not is injected at time equal to zero now assume k equal to infinity; that means, there is complete mass transfer from tank one to tank two there is no resistance to mass transfer can you derive in equation for the concentration of the solute a living this tank

First tank as a function of time. So, that problem here is different for the problem before in the privies problem v assume the k equal to zero; that means, tank to is not contributing any think, but in this second problem we are assuming k equal to infinity mass transfer; that means, there is a complete exchange there is no resistance at all fall material to get transferred from stage one to two or tank one to two or tank two to one

Now, again we go back we take input minus output equal to accumulation and again we say when we do that will end up the equation of like this c by c not equal to exponent of minus q t divided by v plus v dash please note in the privies problem we had c by c not equal to exponent of minus q t by v whereas, here because there is absolutely no mass transfer resistance we can assume this hold two tanks in to the one single tank of volume v plus v dash. So, basically this in this problem we are assuming that the the tank has a volume of v plus v dash that all c by c not equal to exponent of minus q t by v plus v dash.

So, in this second problem because v plus v dash is grater then v v can [vocalize noise] c that the residence time of this system is much larger than the residence time of the first system.

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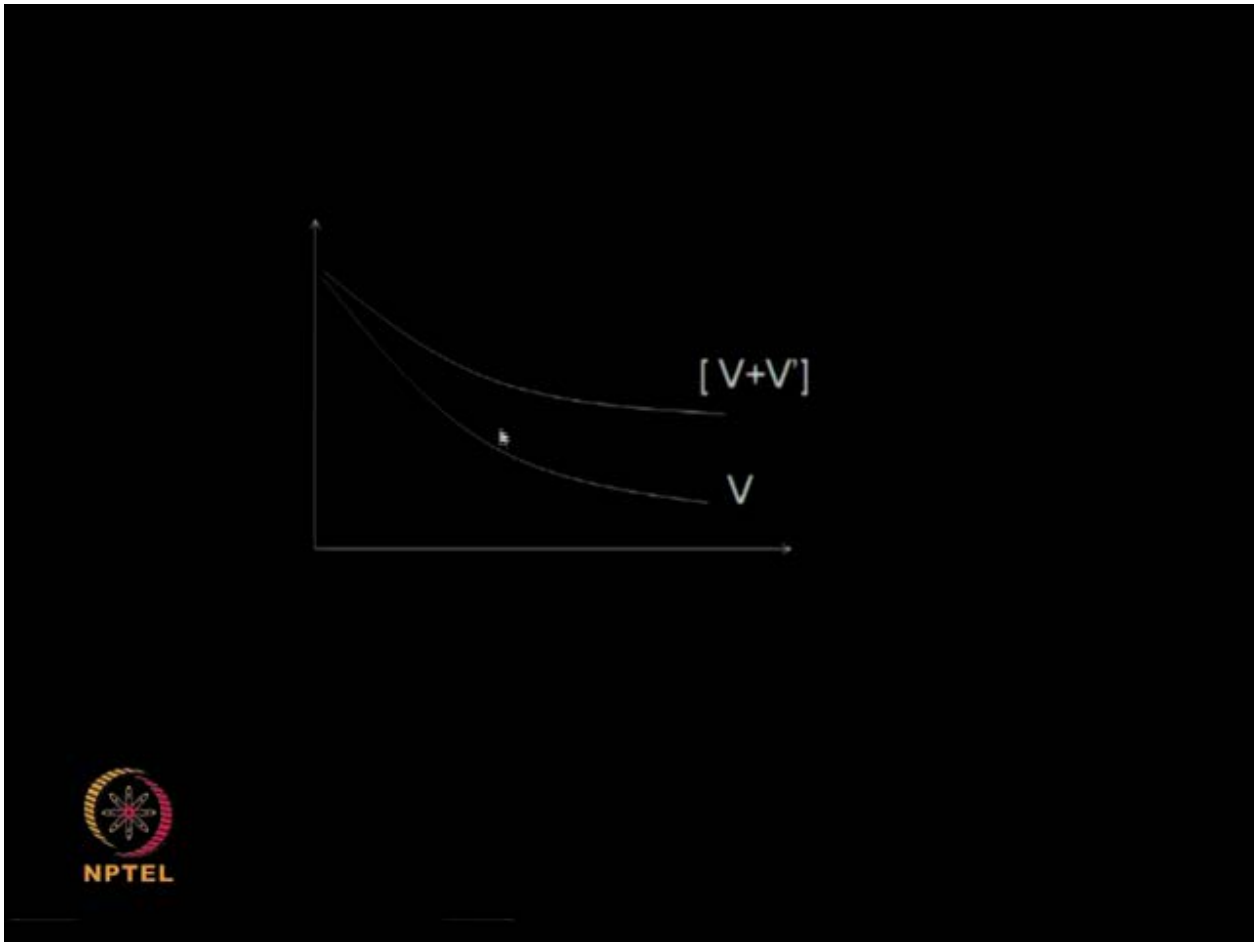

So, if we look at both the problem is together in the first case when k equal to zero the concentration of the solute living the tank will be like this, because the second tank is not contributing anything where as in the second case when the mass transfer is coefficient is infinity we are clubbing or combining both the tank. So, it is like a combine and volume of v plus v v dash. So, the concentration of the solute as a function of time living the system will be like this ok.

So, in reality if we have some value for k; that means, there is some must and should coefficient value which may be between zero to infinity then the performance of such a system will live between these two extremes. So, we have two extremes extreme one is when the must and should coefficient in the zero; that means, tank to is not contributing at all in the second case extreme we assume in a must and should coefficient in infinity that is it is almost like a tell scoping or combining both the tanks the v dash to gather.

So, it is like one single tank of volume v plus v dash. So, that solve the performance will look like in case to this is how the performance will look like in case one. So, in real in a real stick system where k has a value night her zero or infinity, but something more reasonable then the performance will fall within these two extremes. So, will we are looking a different types of assumptions for the chromatography column.

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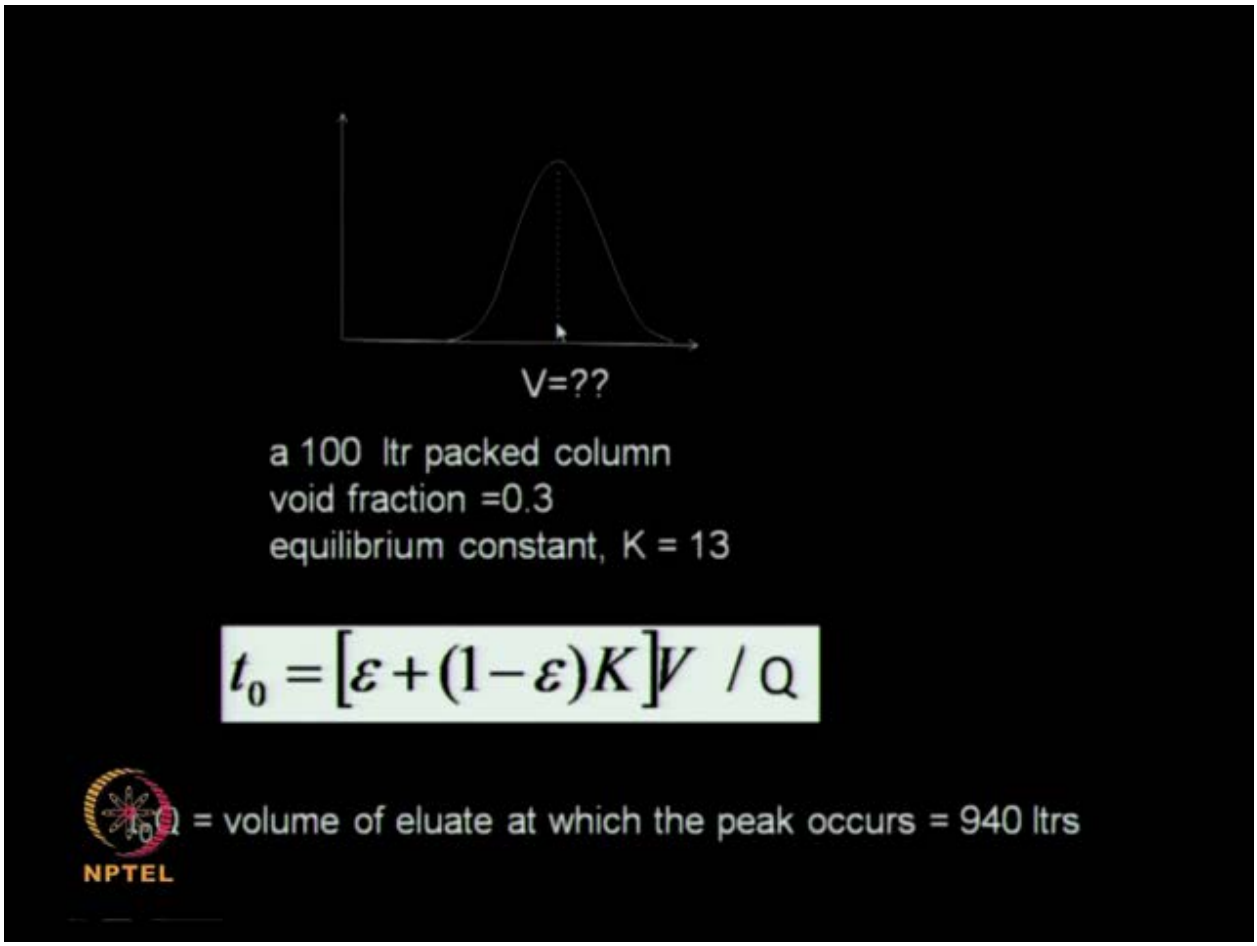

Now, let us go for their imagine I have a hundred liter packed column with god a void fraction of point three equilibrium constant is k is equal to thirteen what is the volume at which they elusion takes place ok.

So, what is a elusion volume. So, how long should I keep collecting until the maximum of the peak appears. So, again me use one of those equations there equations where you have t not equal to epsilon plus one minus epsilon k v by q. So, when I take q together with t not we will get this elusion volume write this is elusion time when I multiply this with q that is the flow rate I get q to t not that is the elusion volume now i no epsilon which point three I know k which is thirteen I know v which is hundred liters by substituted and calculate the elusion volume very stride forward that is equal to nine hundred and forty liters.

So, it means that i have to collect nine hundred and forty liters of the solution and then [vocalize noise] the peak will appear here ok.

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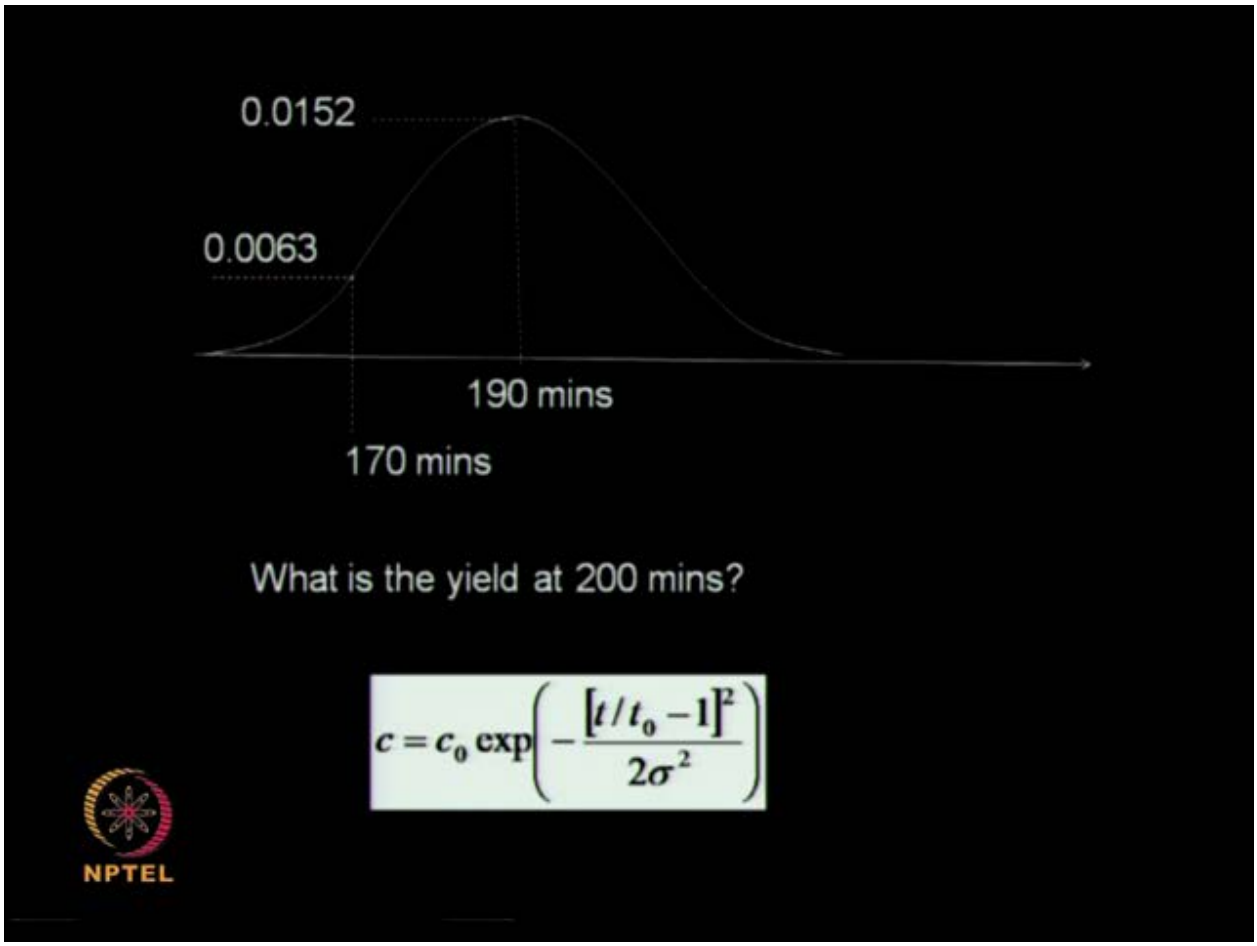

Now, let us look at another problem where we are going to unique use of concept like yield. So, very long time back i introduced something cold yield where if you collect a component between certain times say time equal to t dash and time equal to t.

Then we want we can find out what will be the yield of that particular [vocalize noise] solute which we have collected. So, that is what we want the calculate here in this particular problem. So, you have a chromatographic peak like this at hundred and seventieth minute the concentration is point zero zero six three units at hundred and ninetieth minute that is where the maximum occurs that is that is the retention time we have a concentration at point zero one five two units what is the yield the two hundredth minute.

That is on the other said after this suppose if I collect. So, much of comp on component that is beyond hundred and ninety minute up to two hundred minute what is yield. So, what equation to do I use you are the go back now you no c is equal to c not exponent minus t by t not minus one hold squire divided by two sigma squire write. So, here I now t not is one ninety and c not is this from there i should be able to calculate this particular

term cold this sigma then I can use this for calculating the yield at two hundredth minute ok.

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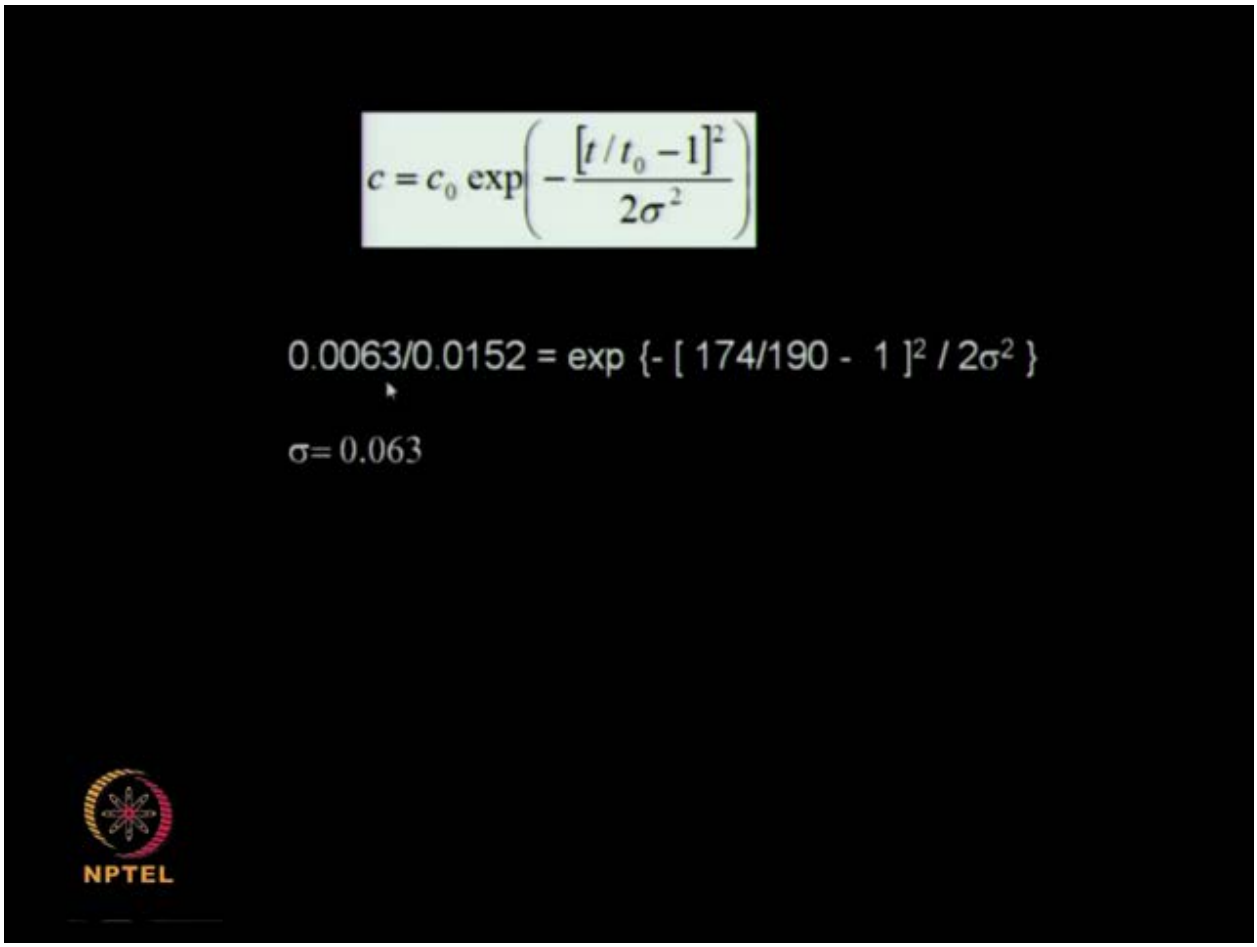

So, i come back again same equation c is given as if we go back.

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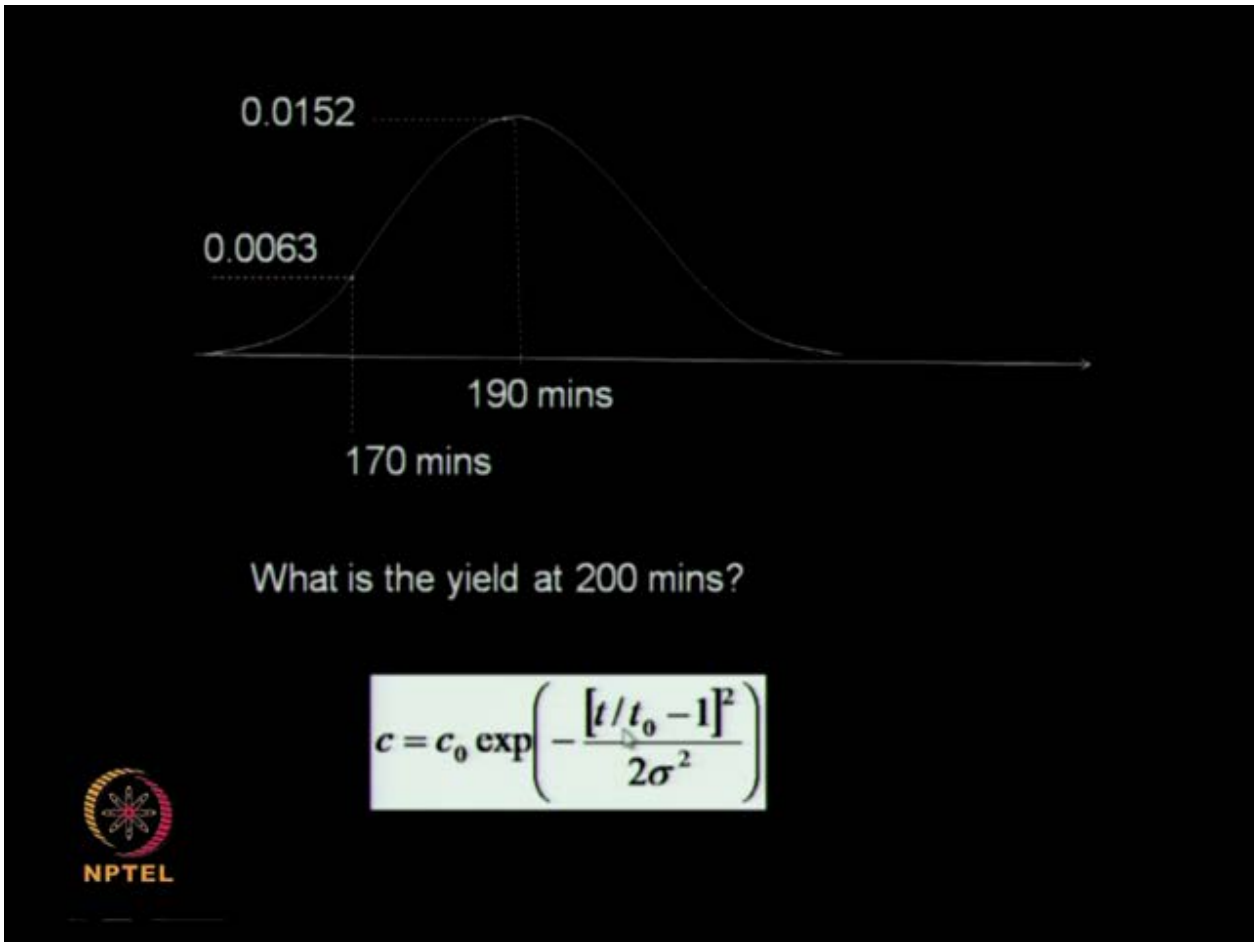

At hundred and seventieth minute c is point zero zero six three c not is point zero one five two write. So, c not is point zero one five two as c is poin zero zero six three t is one seventy minutes t not is one ninety minutes. So, I can substituted all these terms and I [vocalize noise].

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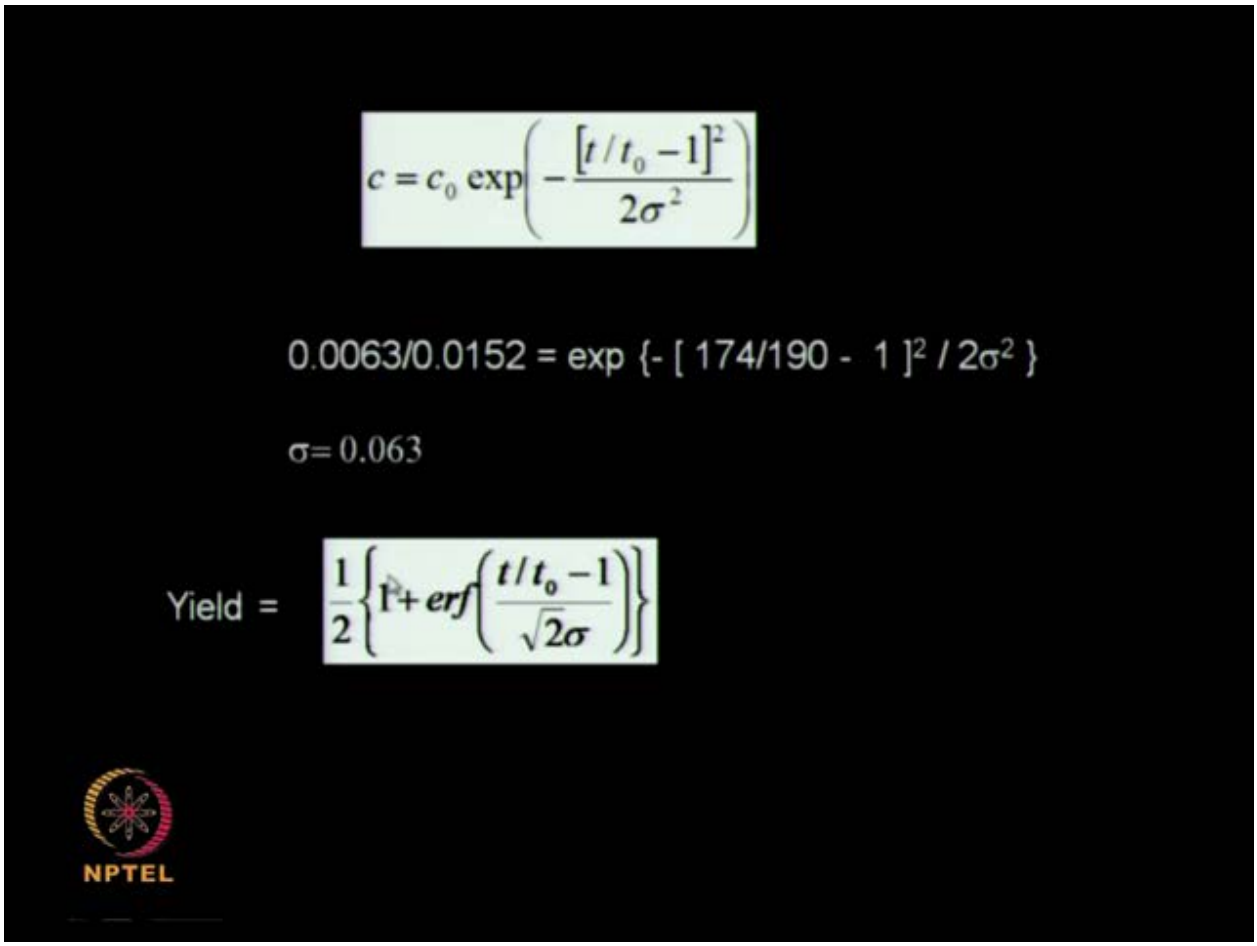

Calculate the sigma a. So, I substituted c is point zero zero six three c not is point zero one five point.

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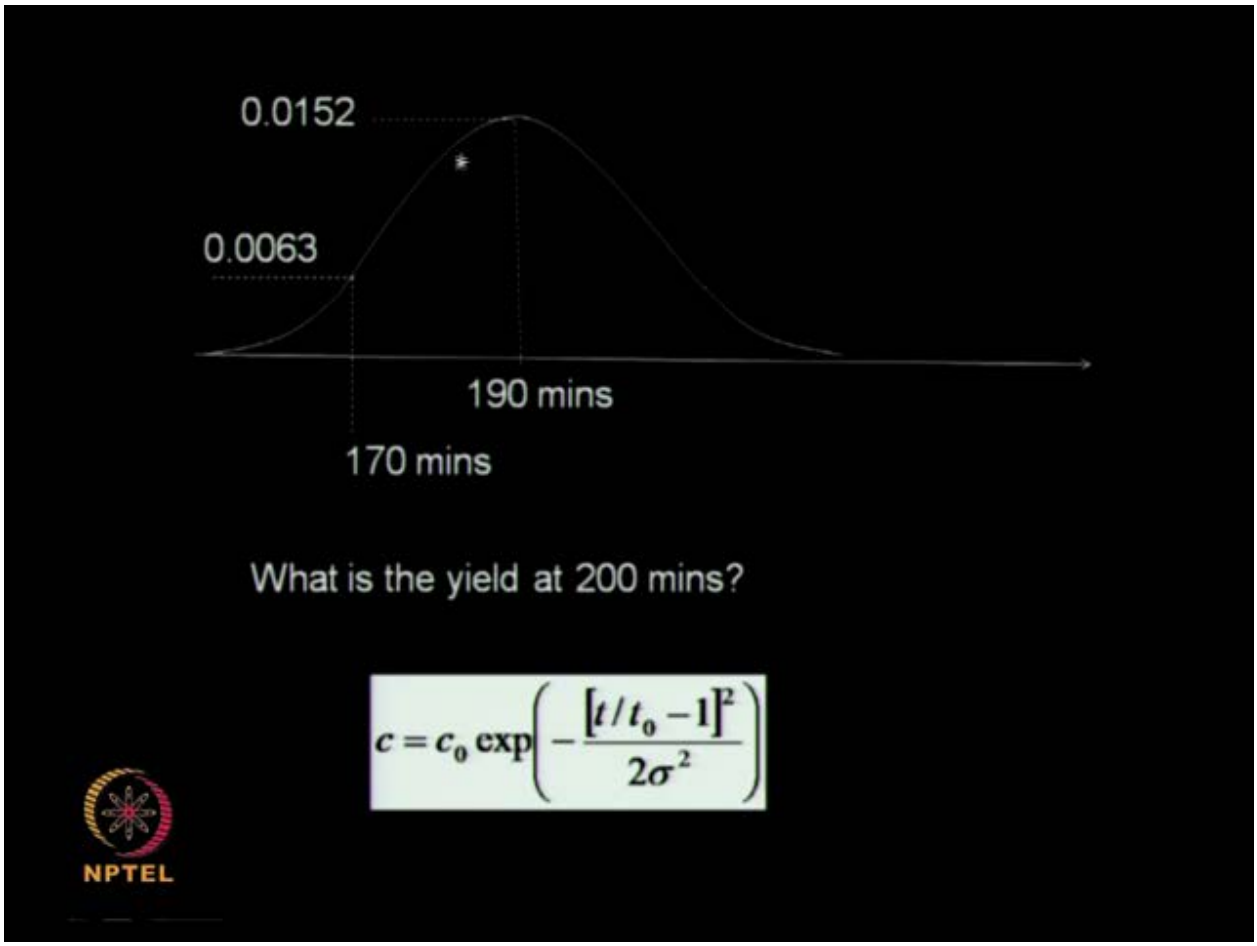

The yield if you are collecting up to this it is point five and whatever is collected behind is behind point five because we assume this particular curve to be uniformly distributed. So, if I collect up to one ninety minutes the yield will be fifty percent or point five and if I collect behind one ninety minutes it will be point five plus other term.

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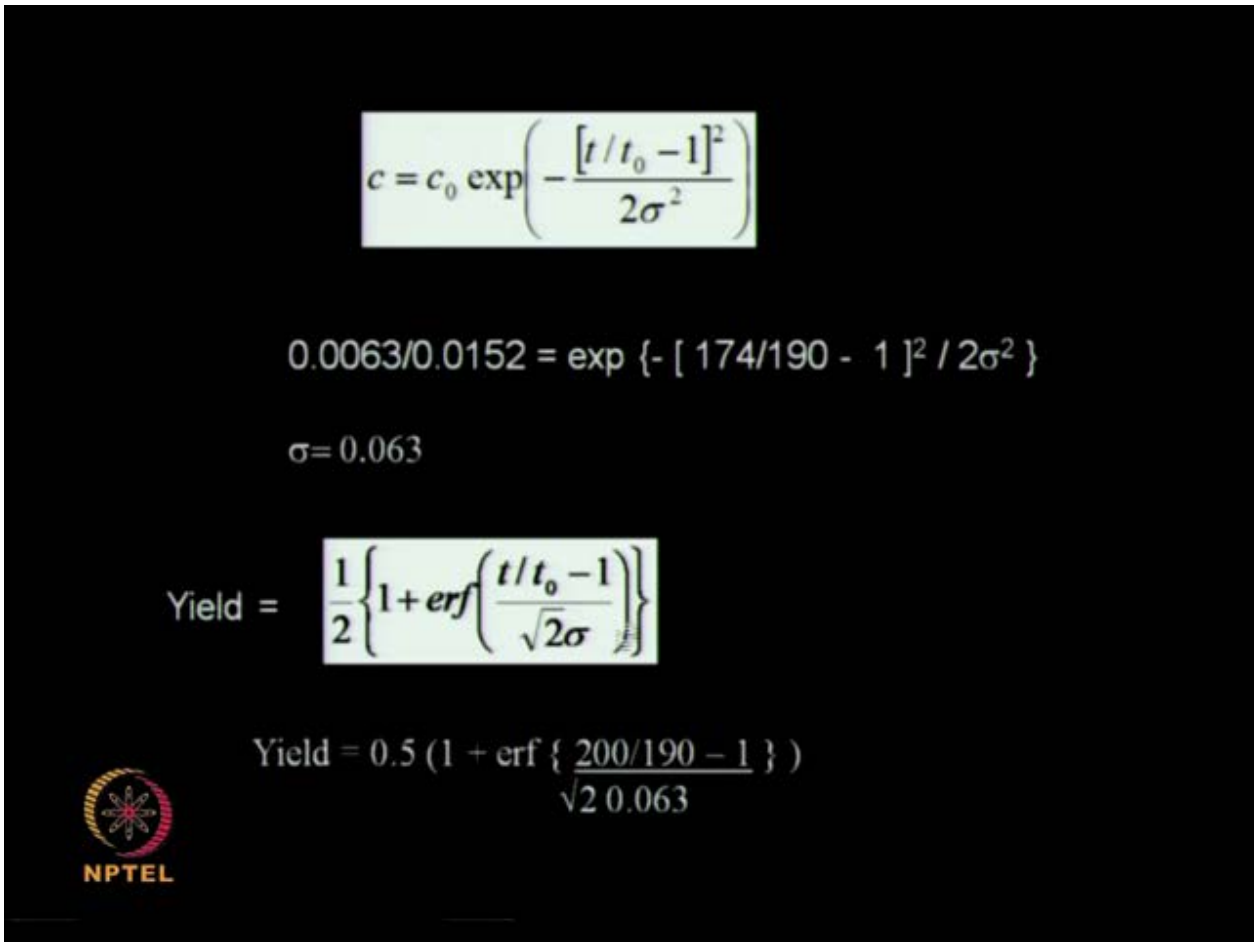

So, the equation for the yield is given by half in to one plus error function of t by t naught minus one divided by square root of two sigma now I know sigma t is two hundred minutes t naught is one ninety minutes. So, I can substitute and then if I know how to calculate error function I will be able to calculate yield. So, it is very straight forward. So, here t naught is one ninety t is two hundred.

Because I want to calculate the yield at two hundred after two hundred minute sigma is given here point zero six three. So, I just substitute I just have to calculate the error function that will give me the yield now let us look at how to calculate error function how to calculate error function straight forward.

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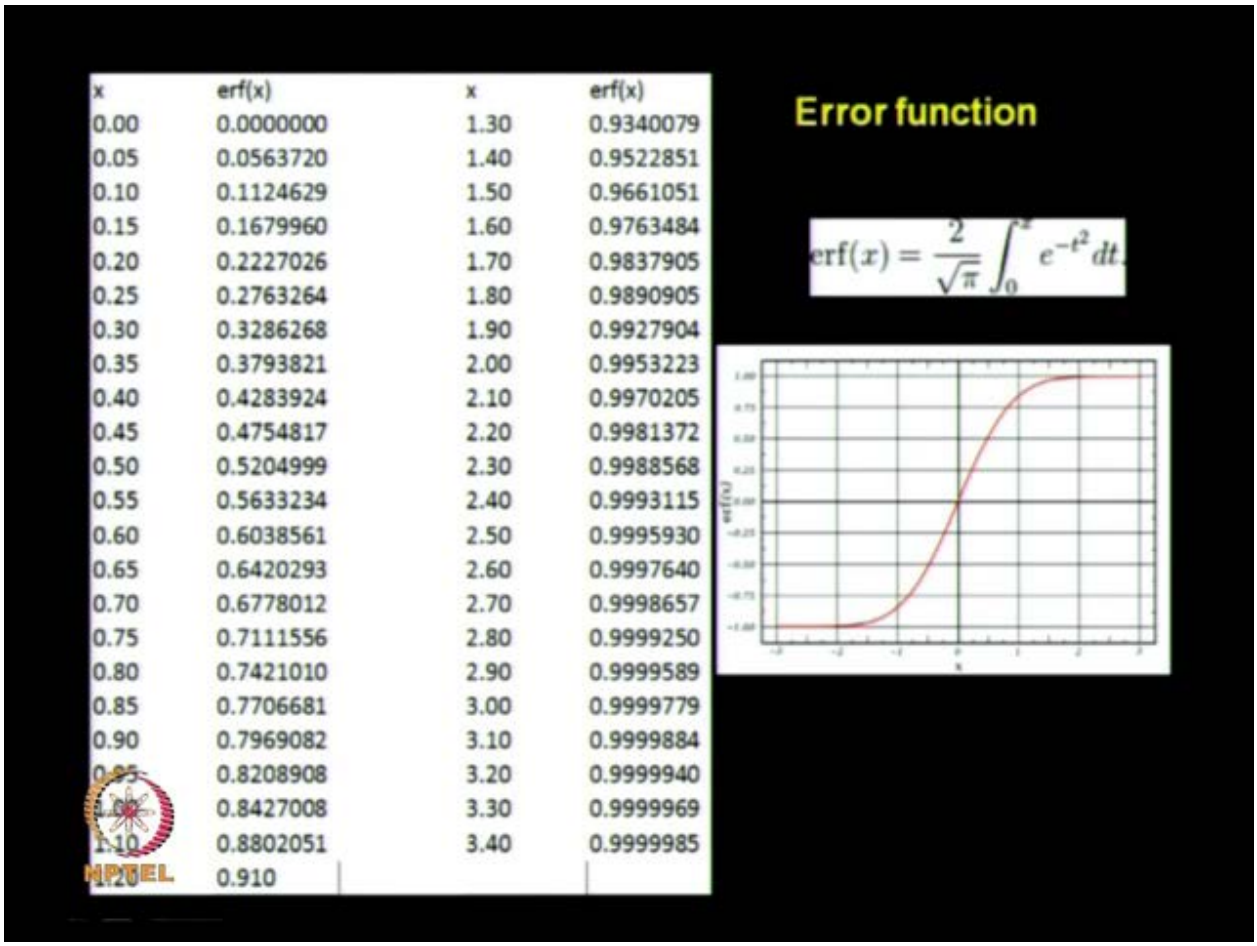

x	erf(x)	x	erf(x)
0.00	0.0000000	1.30	0.9340079
0.05	0.0563720	1.40	0.9522851
0.10	0.1124629	1.50	0.9661051
0.15	0.1679960	1.60	0.9763484
0.20	0.2227026	1.70	0.9837905
0.25	0.2763264	1.80	0.9890905
0.30	0.3286268	1.90	0.9927904
0.35	0.3793821	2.00	0.9953223
0.40	0.4283924	2.10	0.9970205
0.45	0.4754817	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
[illegible]	0.8208908	3.20	0.9999940
[illegible]	0.8427008	3.30	0.9999969
1.10	0.8802051	3.40	0.9999985
1.20	0.910		

Error function is a number which can be calculated using certain numerical technique error function is defined like this error function of x is equal to two by square root of phi zero to x e per minus t square d t and error functions look like this. So, x varies like this error function looks like this.

So, it is very very low that is minus one and it rises sharply it is like a sigma model curve and then after some value of x it reaches and remains constant. So, the error function that is the left hand side varies between minus one to plus one like this when we move the x that is whatever a term inside the parenthesis when you move from a negative value to the positive value ok.

So, if we consider only positive range for x; that means, x varying from zero one two three and. So, on. So, error function will error function of this particular x will start from zero it will rise and then it will saturate at the value of one. So, the error function x is given by two divided by square root of phi integral zero to x e power minus t square d t. So, for different values of x there is a table which gives the corresponding value of the y that is the error function of x.

In fact, this is what it is. So, for different values of x the erf x that is what ever is there on left hand side is given in the form of a table. So, we can get erf x when i know x. So, when x is equal to zero error function x is equal to zero as well as the x goes up this also rises sharply and then it flattens out. So, you see after sometime it is practically one because there is a saturation it then does not cross one. So, it reaches the saturation value of one.

So, initially it start from zero and then it raises sharply then after that you can see that it is faltering out and it will never cross one. So, after about one point seven or one point eight we can practically assume. So, if the x is above one point eight or one point nine and. So, on we can practically assume error function of that is almost equal to one. So, that this particular table we are going to use in our problem to calculate yield ok.

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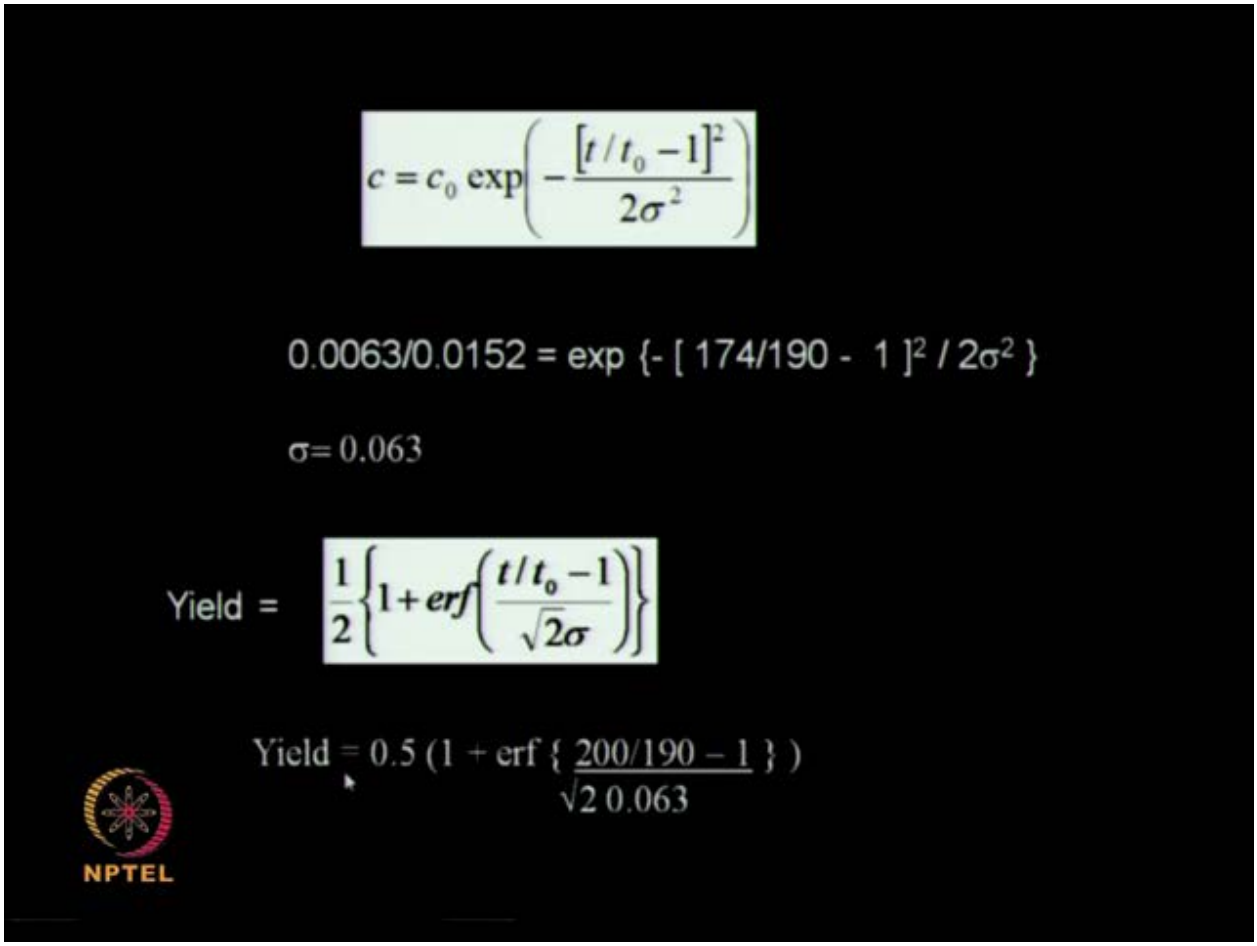

So, we take this equation again and we know t which is two hundred and we know t naught this hundred and ninety we know sigma which is point six three. So, we substitute

here. So, we will get some value for the x once I know the x I can use the table and they calculate error x which is substitute here and then yield will be equal to point five multiplied by one plus that particular value.

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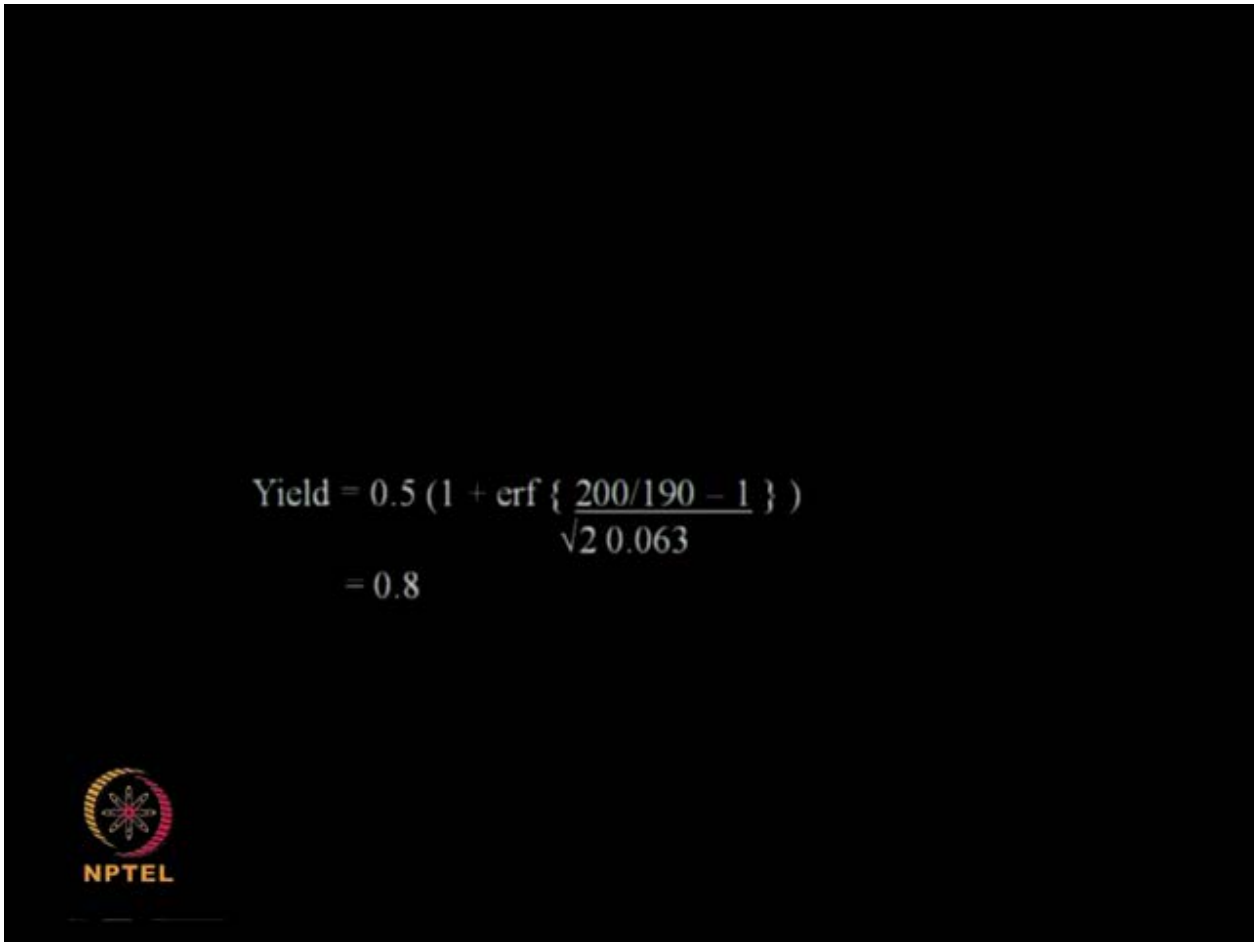

So, when I do that I end of as yield equal to eighty percent. So, the yield up to the hundred and ninetieth mint that is where the retention time of the maximum happens the yield will be point five and when i go beyond the hundred and ninetieth minute the yield will be much more then point five which is given by this extra tern here.

So, f x is equal to t by t naught minus one divide by square root of two sigma. So, you calculate the x and then from the table you calculate the f x and then substitute here and this side point five multiplied by one plus something.

So, the yield is point; that means, eighty percent. So, if I collect the sample up to two hundred minute my i will be collecting eighty percent of the sample this is not telling anything about purity of the sample, but it is telling about the yield.

So, if I collect the hundred and nineteenth minute I am collecting fifty percent of the sample if an collecting the two hundred and minute i am collecting eighty percent of the solute or metabolite which I am trying to recover and eighty percent going upping time the collecting more up particular solute or protean buy as a said again taking about the purity if there is another protein that is presents after cretin time that protein may come out as an impurity.

So, generally what happened if i yield increases may purity goes down and if my yield decreases my purity goes up.

So, you need to strike a balance see five I am way interested very pure components compounds or interested more in collecting as much of the product as much as possible. So, if a I am resort to sum other type of purification step beyond and this particular stage i am an interested in collecting the all the materials.

So, I would like to have very high yield low purity and then i am a results of another type of chromatography and to improve the purity I am interested in the parity of the compound; that means, like a form acytile protein or something then I will see to that there is no other routine a coming during my collections in process I may lose lot of this particular design product in the. So, yield may go down considerably you need to decide considerably they want to have all the protein or as much of the protein collected with pore purity or do you want to collect very pure product; that means, the problem will be the pure yield.

So, if you want to do that that product calculation we can use the these equation. So, the main equation that comes here is yield is equal to point five multiplied by one plus.

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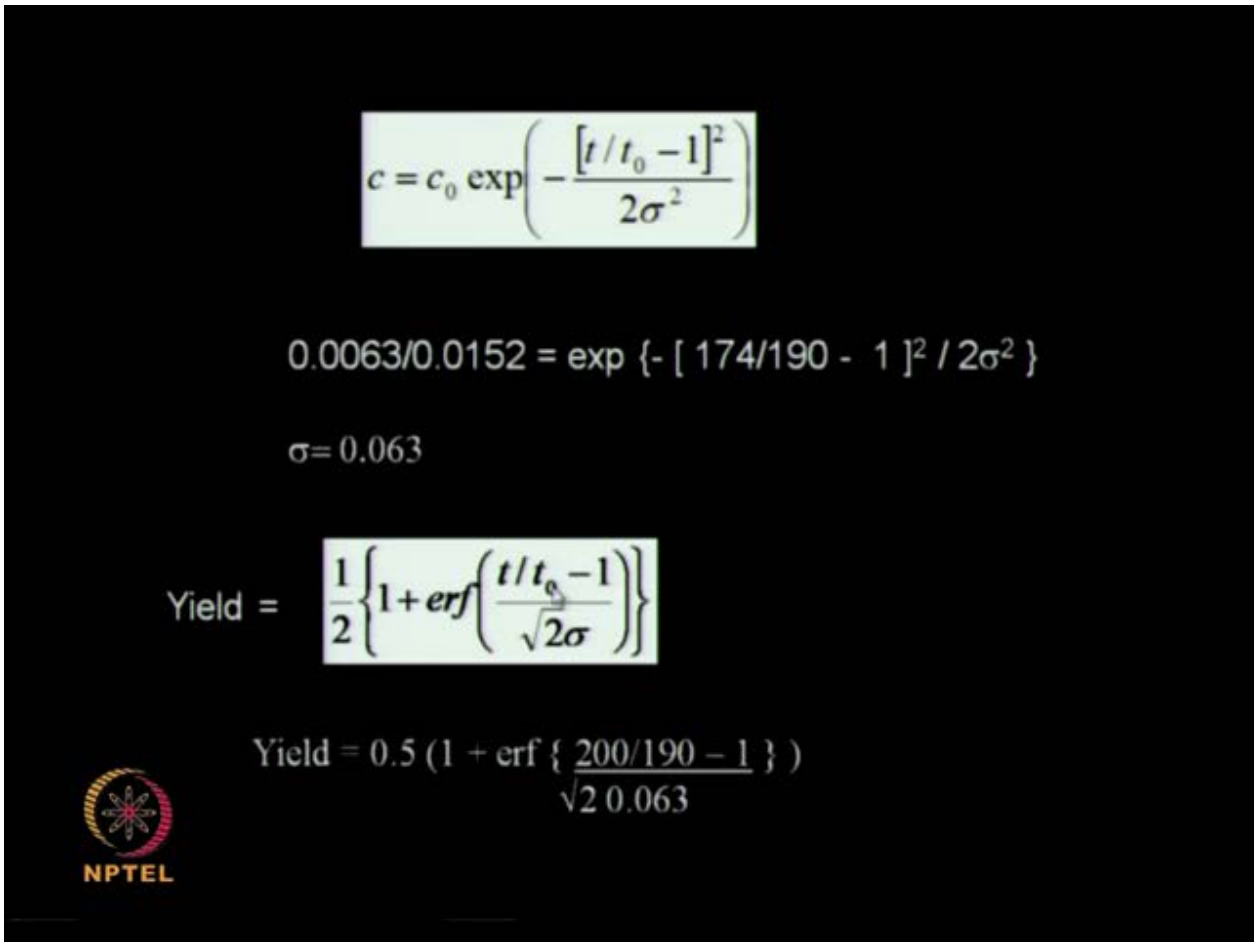

Error f t divided by t not minus one divide by square rout of two into sigma. So, t not is your retention time and t is the time to be collecting your sample sigma you calculate from previous data which is available sigma t not standard deviation gives and distribution let us go forward and see.

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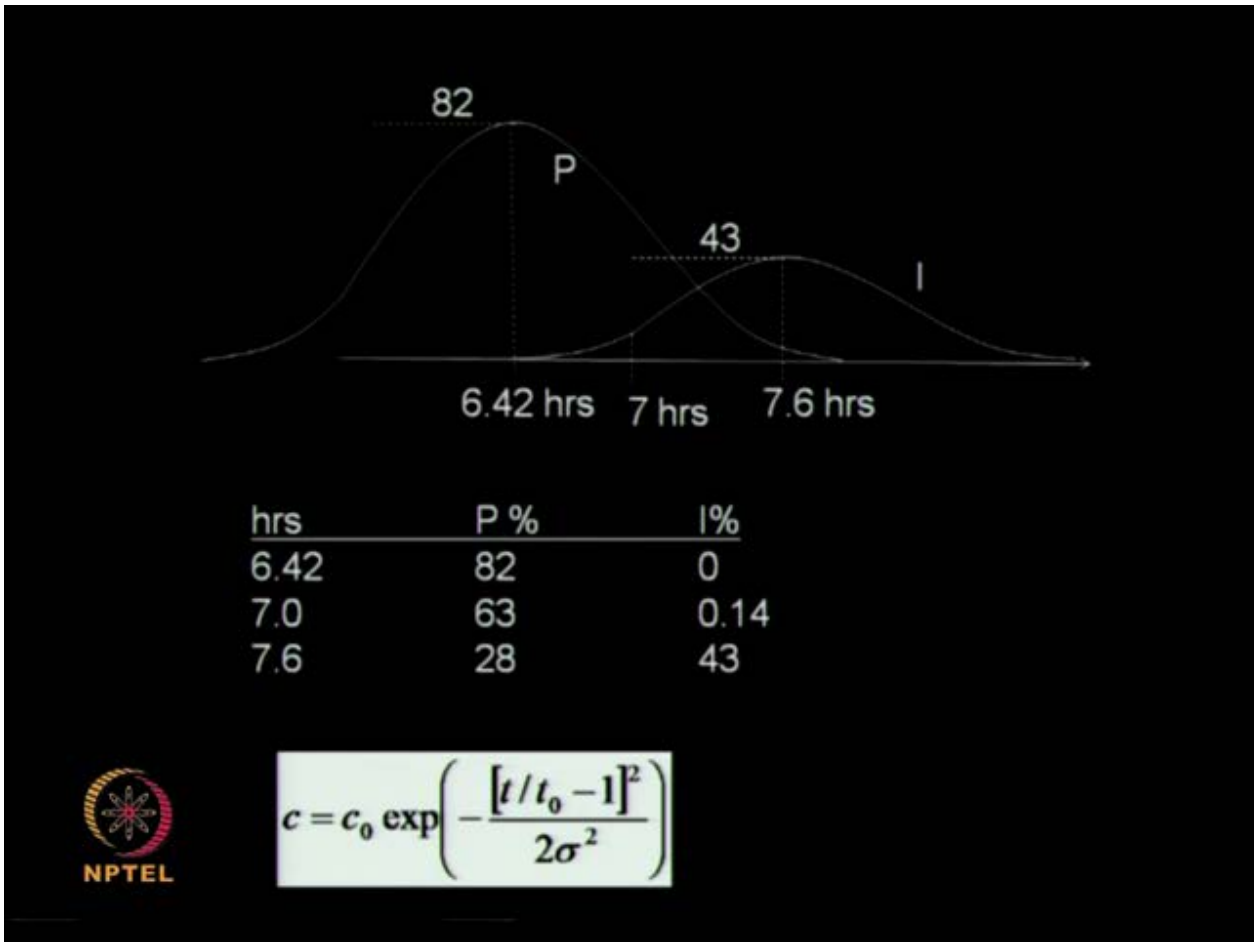

How to analyze this type of problems much more stringently now we are trying to make this problem more complicated now we have two different protein that is eluting out of a chromatography. So, this is protein one this is protein two we call it i impurity.

So, the first protein has elution time of six point four two hours the second protein has elution time of seven point six hours six point two hours protein one is eighty two percent and there is no protein two or impurity. So, it is zero and seventh hour protein one is sixty three percent protein two is point one four percent at seven point sixth hour protein two that is impurity forty three percent protein one is twenty eight percent

Typical example now we can make use of these equation rights by making of this equation what can we calculate we can calculate sigma for protein. We can calculate sigma for impurity correct you can use these equation we can calculate sigma for the protein because you known that t knot for the protein which is six point four two hours ok.

We known that t knot for which is impurity seven point six hours and by using those these two t knots we calculate correspondence sigma for protein as well as for the impurity that is what going to do now.

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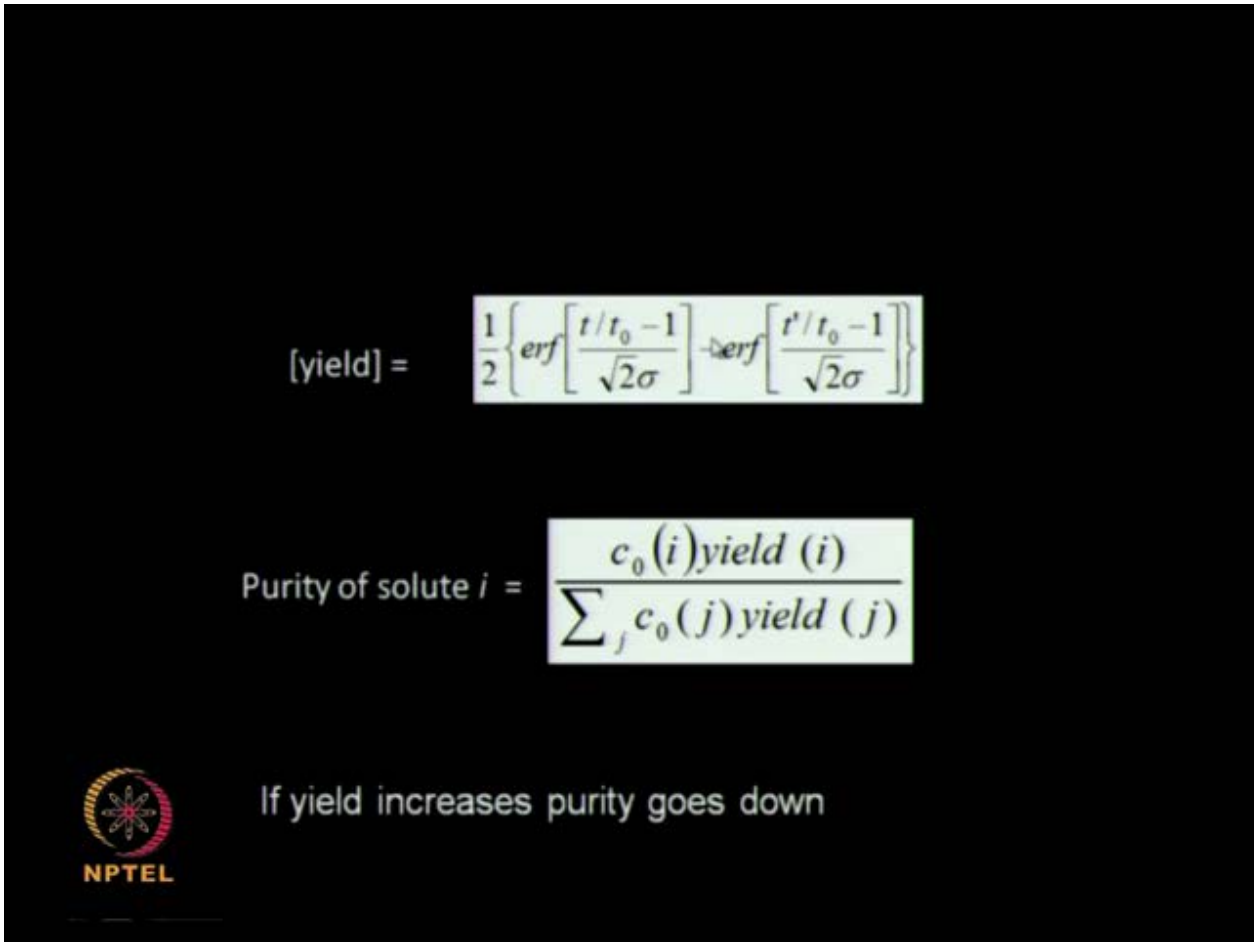

Now, we want to calculate the yield. So, if i am collecting sample between time t dash to t where t knot is the retention time of the component than yield is given by half into error function t t knot minus one divided by square route of two sigma minus error function t dash by t knot minus one divided by square route of two sigma ok.

Now, the purity of solute as a mention now you have a many components in the previous example you have a two components it is given by a concentration into yield of component one divided by concentration of yield one plus concentration of yield two. So, the purity for the solute in any collection part will be how much of concentration and yield of component one and component two a up sense.

So, the n components; obviously, in the denominator we are going to have a summation of all these components. So, if yield increases purity will go down as i said and if yield decreases purity will go up. So, if you have very pure material your yield is going to be sacrifice.

So, we can in the previous problem you can calculate sigma for the protein we can calculate sigma for the impurity and then we can use this equation to calculate the yield and we can use this equation to calculate the yield of the protein we can use this equation to calculate the yield of the impurity if i collecting sample between t dash and t. So, once i know the yield of the protein and once i know the yield of the impurity i can use this particular equation and then calculate what will the purity of the protein in the sample have a collected what is the purity of the impurity that is present in the sample.

So, it is very interesting problem these the realistic situation made be we have two proteins, but in reality there may be many many proteins especially if you have talking about human sample and than i am trying to purify one particular protein from mixtures of protein than you may be have ten thirteen twenty different proteins and separation of those proteins going to be a challenge.

So, the purity always going to be a real challenge. So, here the factors which governed the protein purification depends upon interaction between the protein and the stationary phase and what are the factors which we observed something called that t knot that is the retention time and we observed something called the sigma where t knot sigma is the standard deviation of that particular curve are the concentration leaving column.

So, is very very specific to one particular system or one particular solute. So, t knot will be different for protein one t knot will different for protein two. So, sigma will be different for protein one and sigma will be different for protein two. So, by looking at various time at which these two waves travel out of the column.

We can calculate the sigma and once we know the sigma protein and the impurity we can calculate the yield of the protein than we calculate the impurity. So, we use lot of equation very simple equations most important point is looking at the purities looking at the yields and we also. So, introduce n u trilogy called error function x where error function is integral which has certain limits and this error function x varies between zero to one after words there is no increase.

So, it is like a saturation type of curve. So, there are tables available which gives value of the error function x for a given x. So, if x i can calculate immediately error x x than i can substitute here. So, if I know value of x i can calculate x substitute here i can calculate the yield ok.

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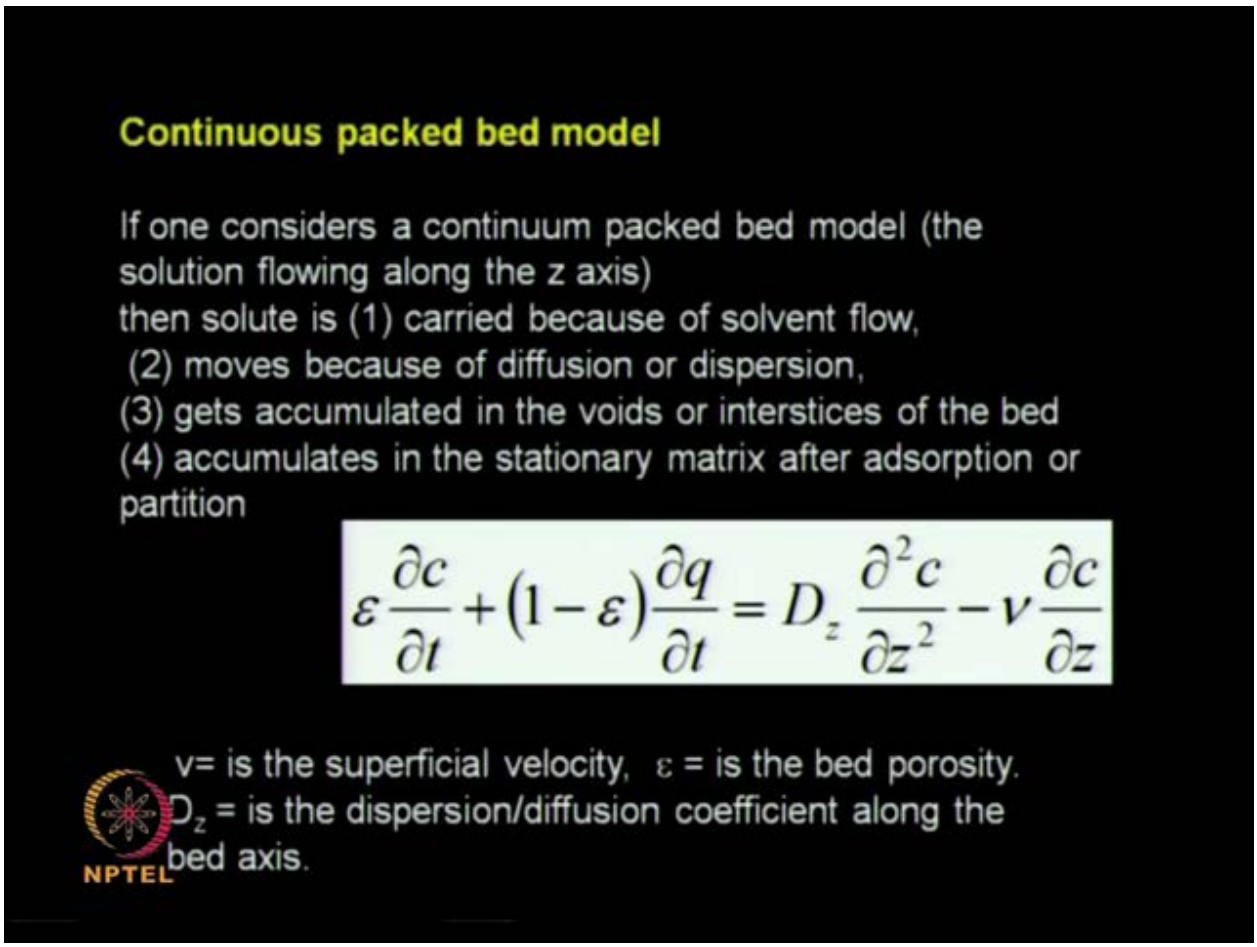

now we are looked at as many things we are looked at discrete stage modeling of a chromatography column; that means, multiple stages

We are looked at a single stage modeling we are looked at two stages connected with moss transpose and. So, on now let us look at another approach that is will continuous packed bed model. So, we assume there is continuous packed bed and the solution is flowing along the x is z axis; that means, flowing is z axis from left to right the solute is carried because of solvent flow number one solute move because diffusion or dispersion solute get accumulated a voids or interstices of the bed the voids

So, there are solute solution intrampt in the void. So, some solute is there. So, solute is accumulate in stationary matrix that is the stationary phase because of adsorbtion or partition or interactionbetween the stationary and the mobile phase. So, many things are happening. So, this is a continuous packed bed model the solute is moving from left to right along the z axis and. So, the solute is carried because of solvent flow solute is carried because of diffusion along the axis of tubular bed or dispersion solute get void or interstices of the bed.

And the solute is also in the present adsorbed on to the stationary matrix. So, all this things happen. So, if all this things happen we can still write some partial different equations which describe this particular type of phenomena and this is out given looks very complicated, but each term has certain particular significance.

Now, this term where we said dis square by d z square into capital d is arising because of diffusion and dispersion of the solute along the axis this term v into d c by d z is arising because of the moment of the solvent v is the superficial velocity solvent c is the concentration of the solute z is the bed.

So, concentration the solute along the packed bed along the packed bed. So, this term arises because of the diffusion of the solute in the column this term arises because of the moment of the solvent and this left hand side we have all the accumulation term v is the concentration of solute in the mobile phase. So, absalom is the widage bed porosity and one minus absalom is the amount of stationary phase d q by d t where d is concentration of the solute which is bound to the stationary material.

So, this left hand side all related accumulation right hand side has one term related to the diffusion or the dispersion of solute along the z axis other one becomes of the moment of the solvent in the this is how we end of with a partially different partition we have z that is the axis of the column. So, there is going to the concentration various along that this is of the column there is going to be concentration as the function of time well.

That is why we have t coming here now see is the concentration of the solute in the mobile phase q is the concentration of the solute that is in the stationary phase that is adsorbed in the stationary phase. So, if you have simple relationship between c and q is a linier fashion than we can combine them together nicely those any problem we can did in

the previous case for example, q can be equal to k c for something like that now let us continue this problem this particular packed bed model in the next class.