Principles of downstream techniques in Bioprocess – a short course
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Lecture-17
Chromatography [Continued 1]

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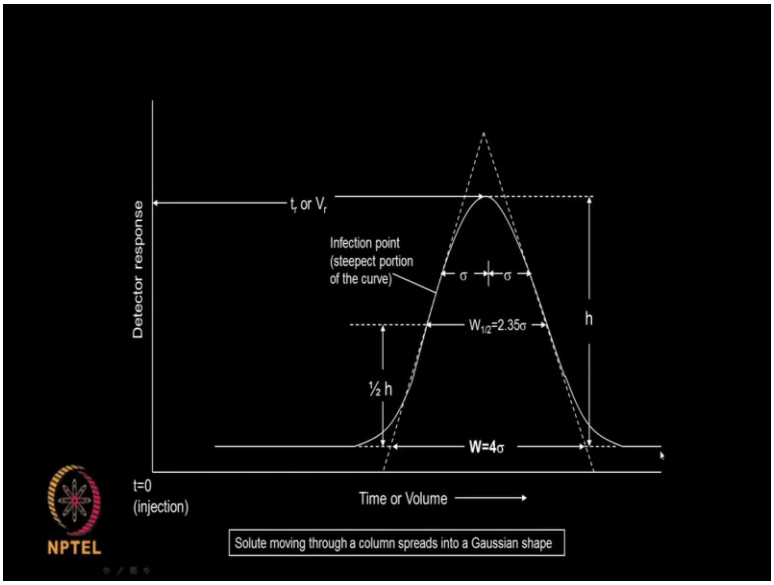

We will continue on the topic of chromatography. So as I mentioned that the peak that is coming out of the column will appear like a normal distribution or a Gaussian distribution and you have the width at the base and this is called the half width at half the maximum. Okay. This is the width at half the maximum. These parameters are used quite a lot, for doing some calculations which we will see that later.

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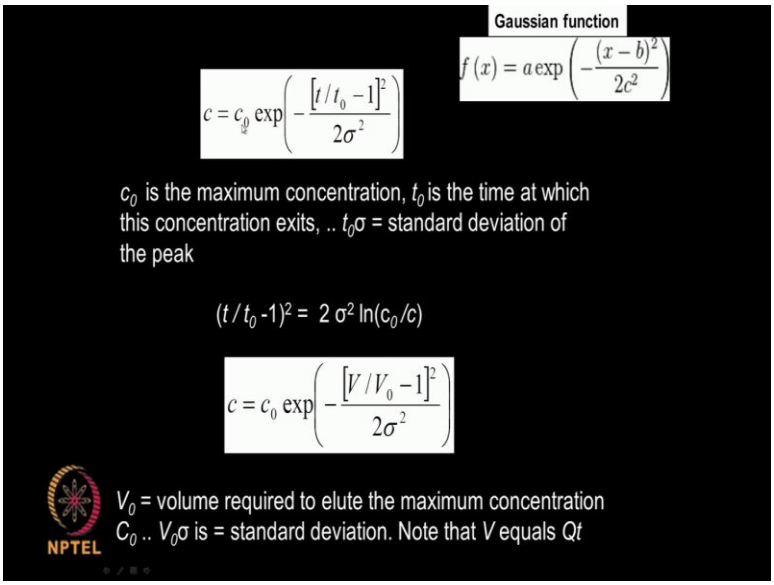

So if you assume a Gaussian distribution, then the as a function of time will appear like this. It will be c0 exponent t/t0 - 1/2 sigma square, t0 is called the retention time, c0 is the maximum concentration, the solute will appear okay and t0 sigma is the standard deviation. You can compare this equation with the normal Gaussian function, as you can see know, x-b the whole square / 2c square okay. So that is how it looks. That is how its analogous.

So we can also have corresponding to t and t0, we can also have V and V0, where V0 is the volume required to be eluted out of the chromatography column, for it to reach the maximum concentration okay. So here V0 sigma will be the standard deviation, whereas in the other case, it is t0 sigma is the standard deviation. So how do you get that? You get that based on comparing the traditional Gaussian function. These equations are very useful as you can see later on. We will use it for many calculations.

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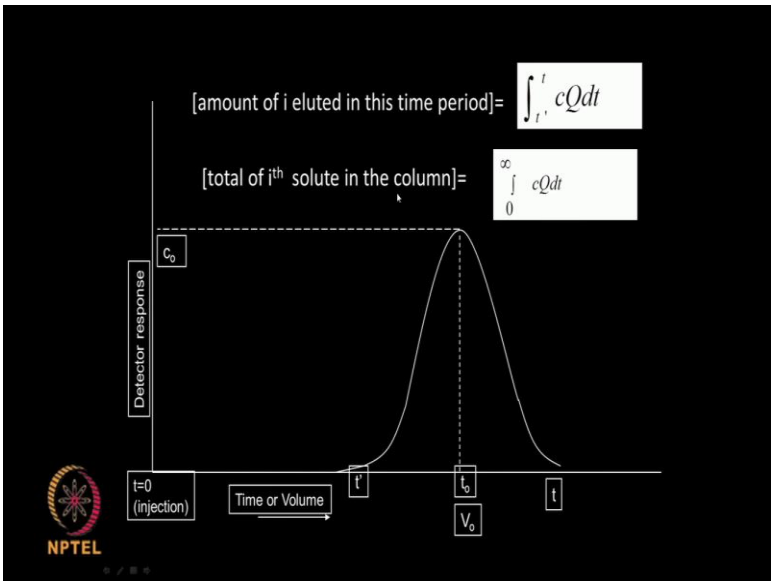

So the total amount of the solute that is coming out of the column is, if I integrate that equation from 0 to infinity but if I collect samples between t' and t, only then the amount of the solute that is eluting out, will be integral of c*Qdt, c is the concentration, Q is the flow rate this time between the two limits of t' and t. Whereas if I am going to collect from 0 to infinity, then the same equation will appear with two different limit, 0 to infinity. So obviously the yield of I will be this/this.

So if I am collecting the sample between t' and t, so I am ignoring whatever is coming out before t' and I am ignoring whatever is coming out after t. So some material will be lost. So the yield will be this / the bottom equation and that is what it is as you can see. And,

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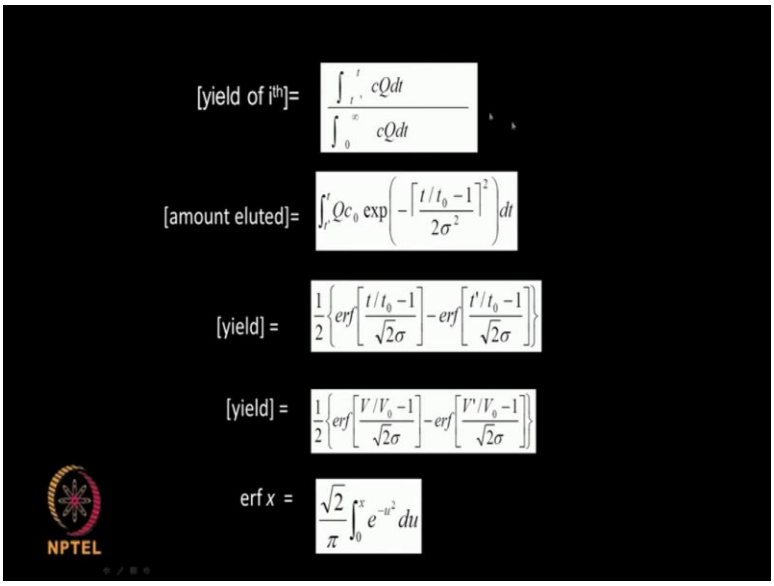

Then we can, the amount eluted out will be given by this, okay because c is given by this relationship as you shown before. Okay. So we can substitute in that and we come up with a new function that is called erf. erf is called error function okay, I will define that more in detail later on. But this is called error function, so yield is given by half of error function of t/t0-1 / square root of 2 * sigma - error function t'/t0 - 1 /square root of 2 sigma okay.

Here you collecting samples between t' and t, okay similarly if we want to use V, then the nature of t, we put it as V. So we get V/V0 and V'/V0. Now what is this error function? The error function is defined like this. Error of x=square root of 2 / pi integral 0 to x e - u square du okay.

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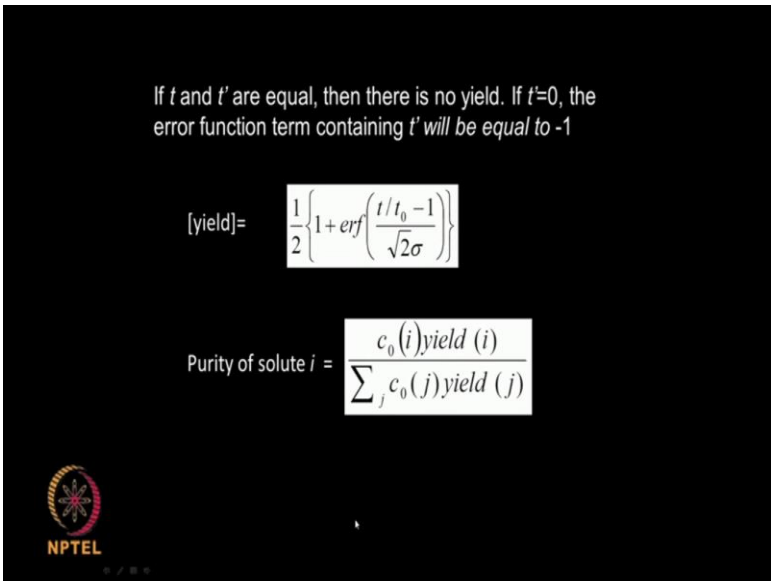

So you can, there is a table available for different values okay of x. What is the corresponding error of x? Okay there is a table available which we can use it okay. I will show you that. So if t and t' are equal okay, then there is no yield okay. That means t and t' are equal if t'=0. If the error function term containing t' will be = – 1, okay. So you will end up with half of 1 plus error function t by t0 - 1 by square root of 2 sigma okay. So I you are collecting the sample from time = 0, right up to time okay then you will get a yield equation of this form.

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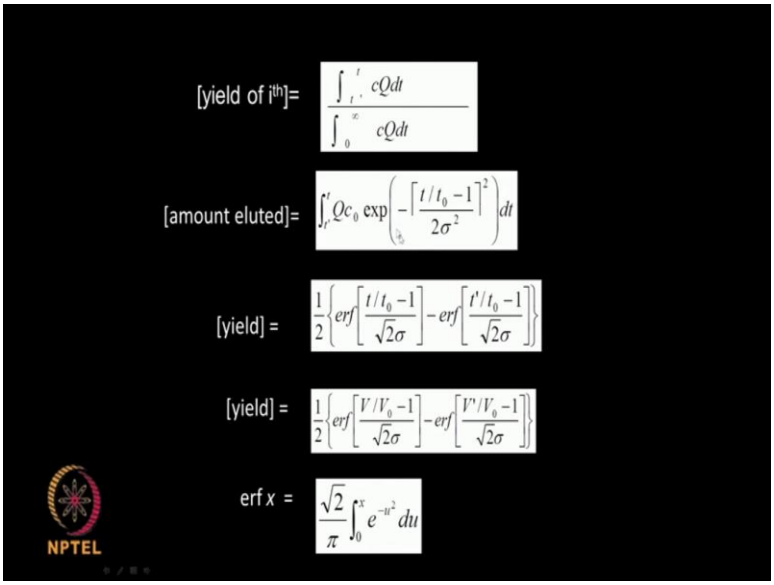

Okay. So when you are collecting time = 0, okay this term will go away actually. This term will go away, that will be -1. You will get half of one plus this term actually. So you are collecting

sample from time = 0 up to time = t. So you will end up with the equation of this form. So if there are many solutes I j k and so on okay many solutes, then of course purity of solute, it will be c0 of I * yield / summation of c0 of j * yield okay. So this could be many solutes available. So you will have with the three solutes you will have three terms in the denominator. That is the purity it is obvious right.

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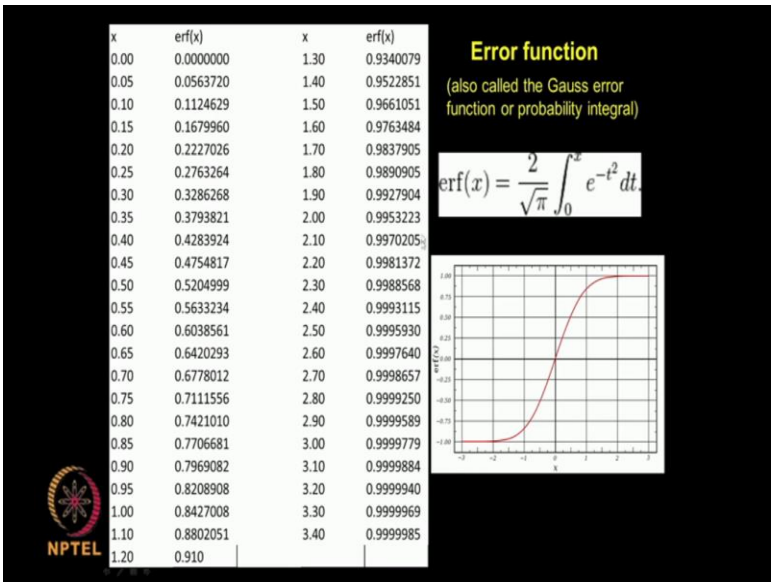

x	erf(x)	x	erf(x)
0.00	0.0000000	1.30	0.9340079
0.05	0.0563720	1.40	0.9522851
0.10	0.1124629	1.50	0.9661051
0.15	0.1679960	1.60	0.9763484
0.20	0.2227026	1.70	0.9837905
0.25	0.2763264	1.80	0.9890905
0.30	0.3286268	1.90	0.9927904
0.35	0.3793821	2.00	0.9953223
0.40	0.4283924	2.10	0.9970205
0.45	0.4754817	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		

So let me define what is error function. Error function, as I said for different values of x, there is a table available which tells you the error of x. okay. So when a error x is 0.00 that means if you integrating between 0 and 0 then the error function will be 0 okay. If you are integrating between 0 and 1, you end up with the error of x as 0.842 because you have 2 / square root of pi. So when it goes to 3.4 and above 3.41 and so on okay because then you will get error function as 1.

So basically error function graph looks like this. So this is the change in x and this is the y axis gives you the error of x. So it is almost going up and then reaching an asymptotic value above 3.4 on that. This is how the error functions like actually. This is also known as probability integral. This is also known as Gauss error function and so on actually.

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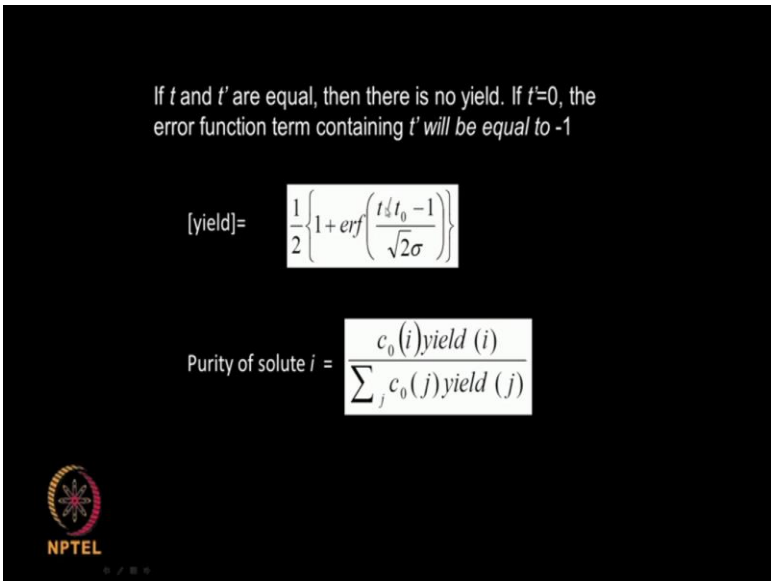

So this table is very important if I want to calculate for example yield. If I am collecting samples from 0 time to time t I use this equation. So I will calculate all these terms and error of x whatever is this, then I go to the table and pick up my corresponding value okay and that is how it works.

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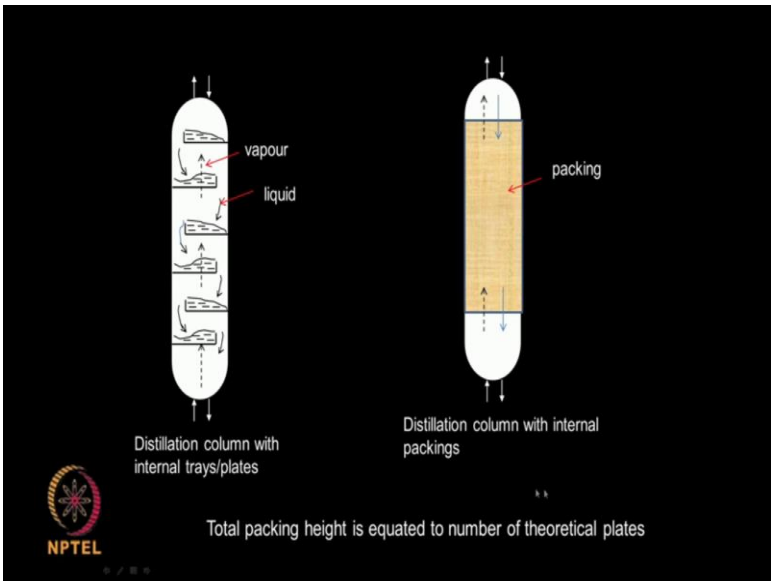

I want to introduce some new terminology theoretical plates. I introduce this terminology in the previous class. It a chromatographic column can be expressed in terms of theoretical plates, which gives a quantitative measure of its efficiency or separating part. How did this theoretical plate come? It came from chemical engineering. So if you look at an original distillation column

in chemical engineering, distillation is used for separating liquids with different boiling points or liquid, which have the different vapor pressure right.

So originally the distillation columns, tall columns, the trays inside or plates inside. So the vapor will flow upwards and the liquid will flow downwards. So it will come from one tray to down another tray to down another tray and so on. And at each tray there is a vapor liquid contact equilibrium mixing and all takes place okay. So more number of trays you have more efficient was the distillation column in separating two liquids. Okay.

So more number of trays it can separate out two liquids in closer boiling point that is how it happened. So they believe that when you have many plates you will have better distillation efficiency. When they move into packed column that means, you have packing inside, you do not have trays inside. If they want to compare this with the original tray distillation column or the plate distillation column, they wanted to have some relationship of converting the packing height to number of trays so that it becomes easy for one to compare.

Okay so they there some equations were developed, which related the packing height to number of trays. So the same thing is used in column chromatography also because you have a packing in the as a stationary phase. So can I call a the certain length of the stationary phase packing equivalent to certain number of trays, then it becomes easy for you to compare different chromatographic columns. We can say this column A has 100 plates and column B has 110 plates, then column B is more efficient than column A.

Because it has got more number of theoretical plates, so that is how the concept of theoretical plates were extended from distillation column, which had plates or trays to pack distillation column and then to chromatographic columns as well okay.

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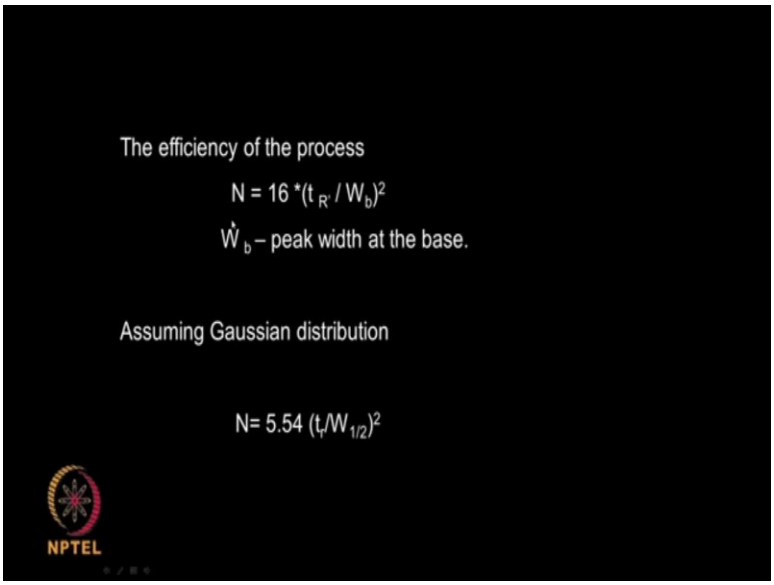

So the efficiency of the chromatographic process is measured by the number of theoretical plates, it is not measured by the column length but because column length will alone is not enough to tell the efficiency. There are three type of packing, so many other factors come into deciding the efficiency. So if you convert this into N number of theoretical plates, then it is easy for one to compare 2 different columns and we can also say whether this column is good or not very good and so on.

So the number of theoretical plates for a chromatography column can be estimated by this formula equal to 16 / tr that is the retention time / the width at the base of the B square okay. If you assume it as an Gaussian distribution, then the number of theoretical plates also again 5.54 * tr, that is the retention time / the width half peak height square. So we can use this formula or this formula, if I know the width at the base. I can use this, if I know the width at half peak height, I can use this formula.

So I have to, all I have to do is, I inject the sample into my column chromatography and then see when the return gets eluted out. That is the retention time and I determine the width at half the height or width at the base of the peak and then I use one of the formula to calculate n. So I can, if I have two columns, I can do the same injection of the solute in both the columns and I can estimate from the tr and Wb or w half the number of theoretical plates. So I can say this column

is more efficient, that is this column has more number of theoretical plates, than the other. Okay this is how the theoretical plate concept was extended to chromatography as well.

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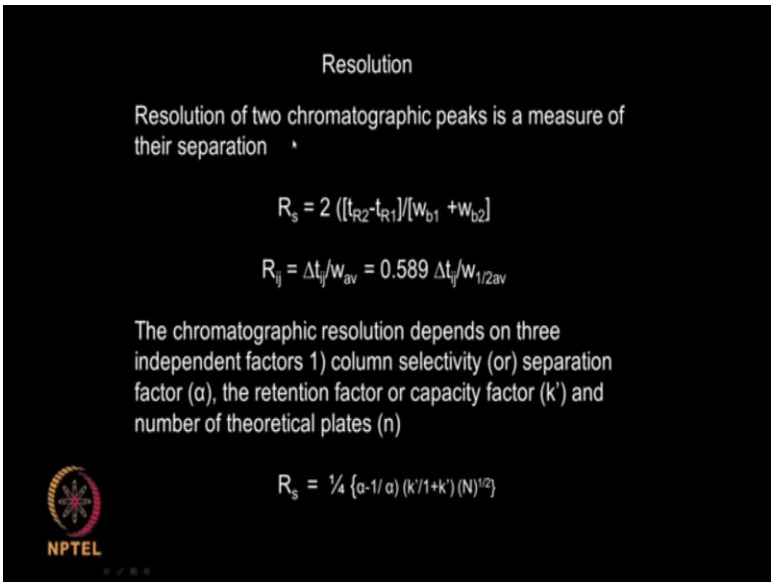

There is something called resolution. Resolution is a measure of how two chromatography peaks are separated. Are they good or are they bad. Basically resolution is determined by this, 2 into the retention time for peak 2 - retention time for peak 1 / width of base 1 peak 1 + width at base for the peak 2 or we can say delta t, that is the difference in the retention time / w average or if it is a, if you are measuring the width at half the height, then it will be 0.589 delta t / W average okay. So the resolution depends on column selectivity separation factor, retention factor, capacity factor and number of theoretical plates and so on actually okay.

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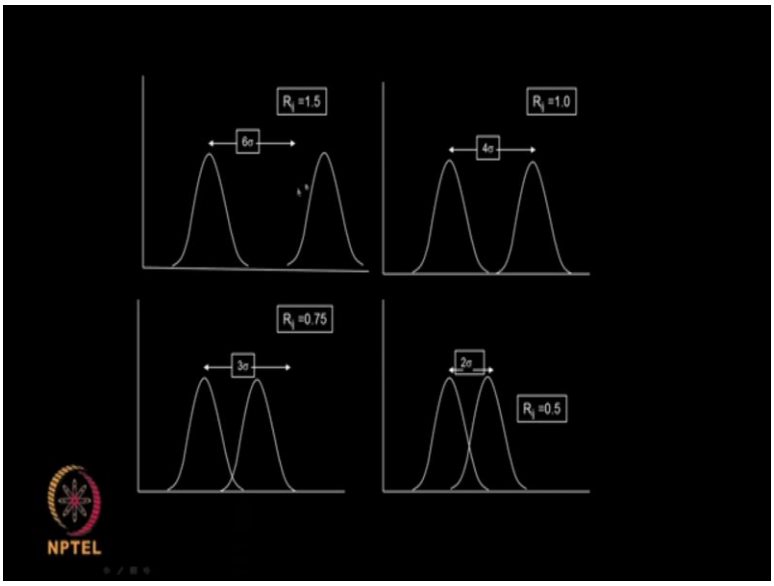

So resolution, this is how it looks like. Okay these peaks are very well dissolved, okay. So you can have this is almost at a distance of six sigma resolution may come out to be 1.5. So resolution 1 means they are just touching each other at the base and as the resolution keeps going down this starts slowly overlapping. So when poor resolution almost 0.5, we can see large overlap between these two peaks okay.

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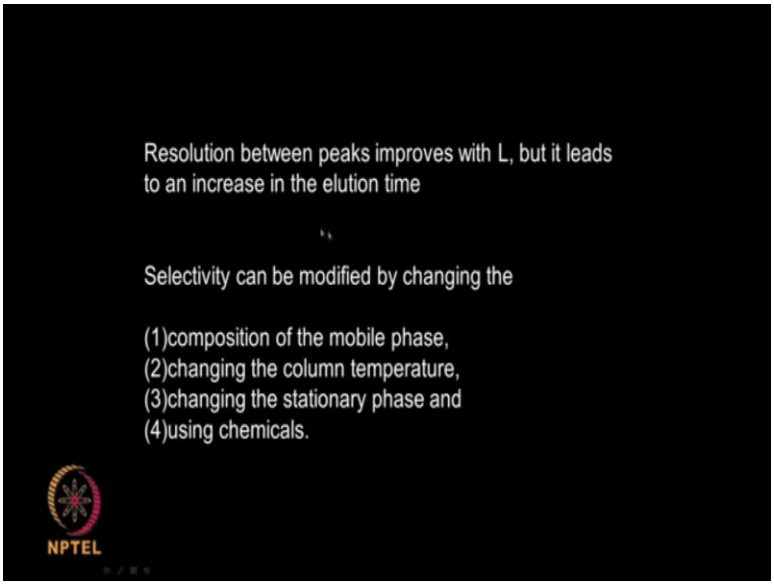

So the higher the resolution further away are the two peaks okay, the resolution between peaks improves with the length of the column but the problem is, if we increase the length, the illusion time also increases. The selectivity can be modified by modifying the composition of the mobile

phase, changing the column temperature, changing the stationary phase, using some chemicals and so on actually.

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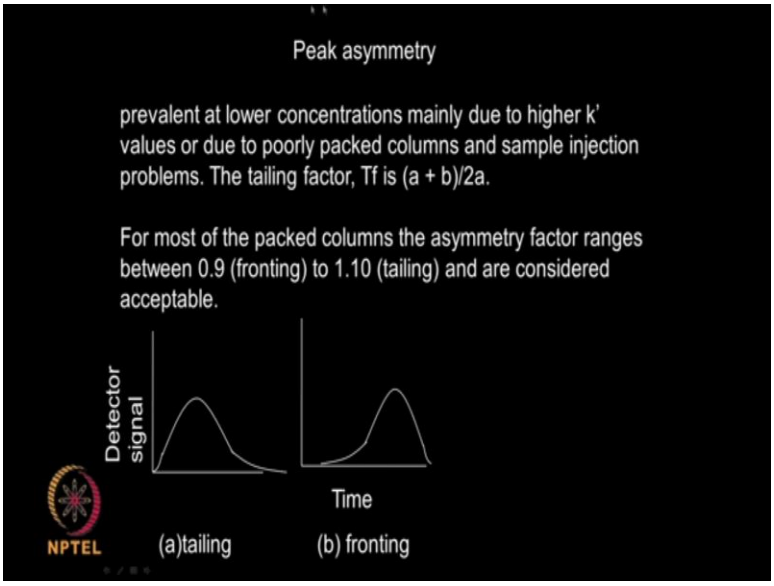

There is something called peak asymmetry because it almost we say Gaussian peak and so on. But peaks are never Gaussian, you may have a tailing, that is slowly its decaying or it is called the fronting. So both are possible actually.

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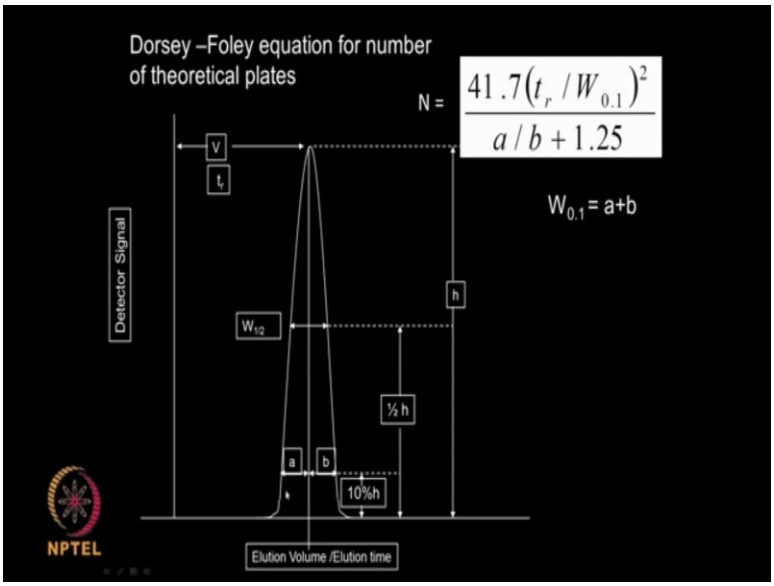

So the tailing factor is defined by a + b / 2a. What is the front portion of this is called a and the back portion is called b okay. So the tailing factor tf is a + b / 2a. Generally this factor will vary

between 0.9 to 1.10, you will never get exactly 1 but it will vary between 0.9 to 1.10. Okay so you can have some tailing or some fronting okay a. a is an indication of the fronting and b is the indication of the tailing. So if we have that, then we can get an equation for the number of theoretical plates 41.7 tr / W0.1 its not w half or w base its point 1, that means the width at 10 % of height okay / a / b + 1.25 okay.

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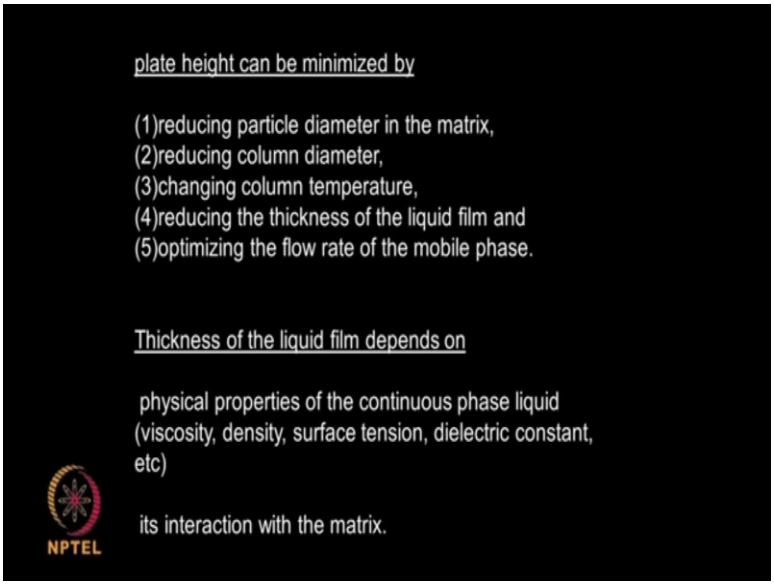

So there are different ways, by which one can calculate the number of theoretical plates using width at the base or width at the 10 % of the height or width at half the height and so on. So there is something called plate height. So if you have number of plates, then you have plate height. If you multiply together, that will give you an idea of the length of the packed portion. So plate height can be minimized by reducing the particle dia.

Okay smaller the particle better is separating efficiency reducing column dia, changing column temperature reducing thickness of the liquid film that is forming on the packing optimizing the flow rate of the mobile phase. So all these can reduce the plate height, I said thickness of the liquid film. So that depends of the physical properties of the continuous phase, like viscosity, density, surface tension, dielectric constant interaction with the matrix and so on actually. So all these are issues which can affect the plate height.

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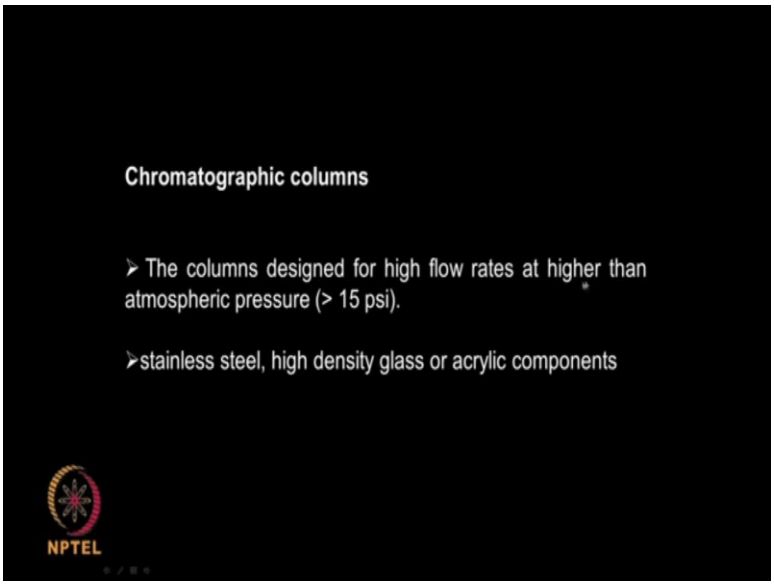

The columns designed for high flow rates are higher than the atmospheric pressure. These columns are stainless steel or high density glass or acrylic components. These are the chromatographic columns are made actually.

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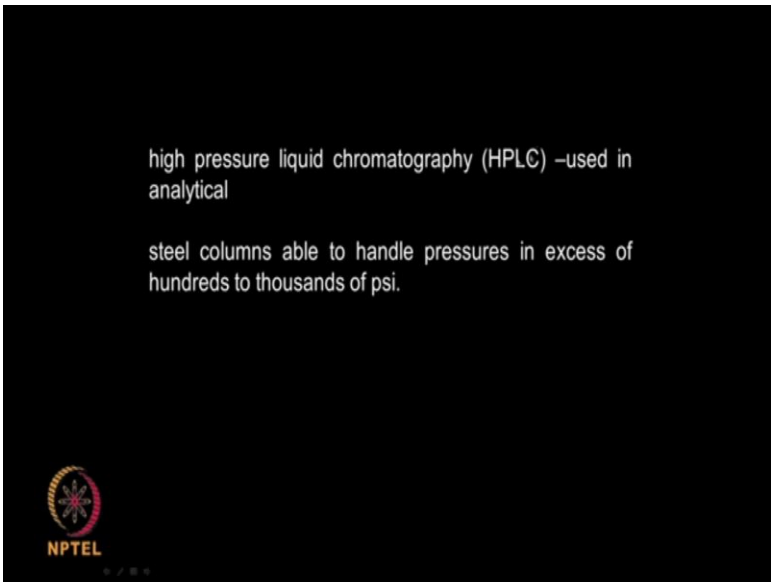

You all HPLC that is the high pressure, high performance liquid chromatography. Generally they are used for analytical purposes, they are not generally used for separations in industrial scale because they are very small diameter. So sample injection is very limited in this actually.

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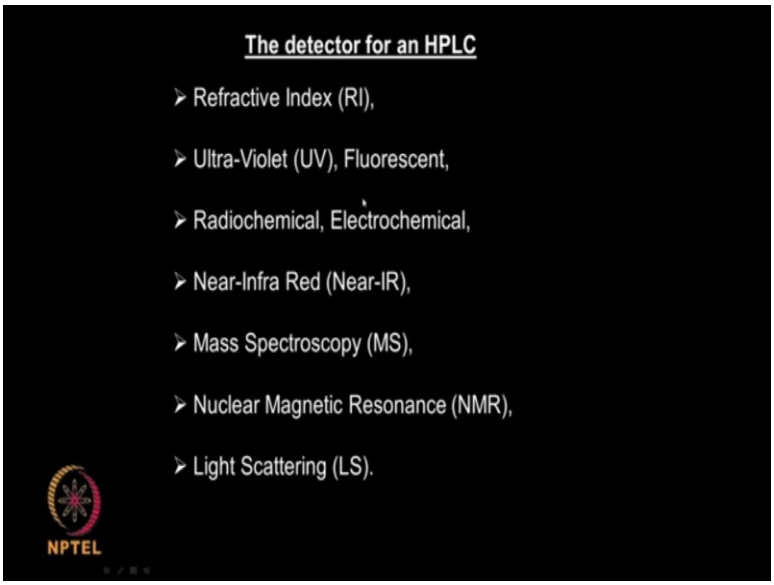

So what are the detectors? The HPLC detectors could be Refractive Index Detector, Ultraviolet Fluorescent Detectors, Radiochemical, Electrochemical, Near-Infra Red Mass Spectrometer, Nuclear Magnetic Resonance Light Scattering. So large number of detectors can be used. We will not spend time on those because we have shortage of time.

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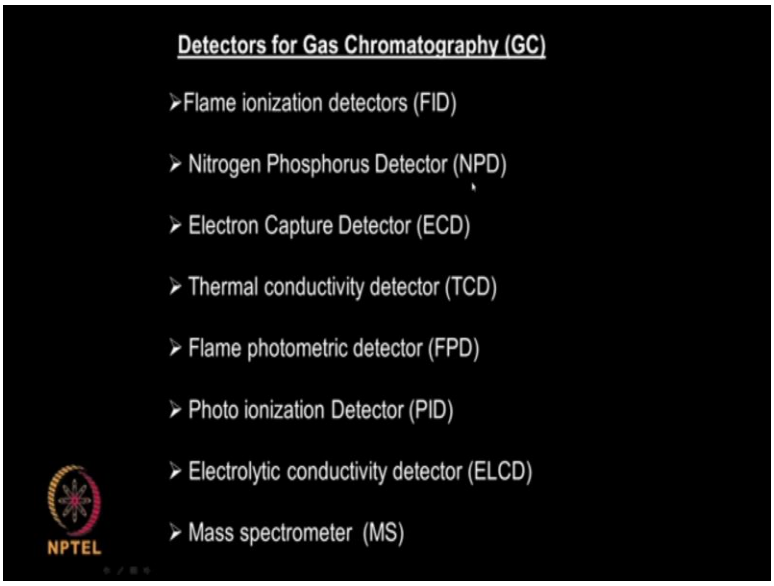

Similarly detectors for Gas Chromatography, We Can Have Flame Ionization Detector, Nitrogen, Phosphorous Detector, Electron Capture Detector, Thermal Conductivity Detector, Flame Photometric Detector, Photo Ionization Detector, Electrolytic Conductivity Detector, Mass Spectrometer and so on. So large number of detectors depending upon type of compounds you

are separating whether it is ionizable non ionizable, whether it is ui visible, all those things actually.

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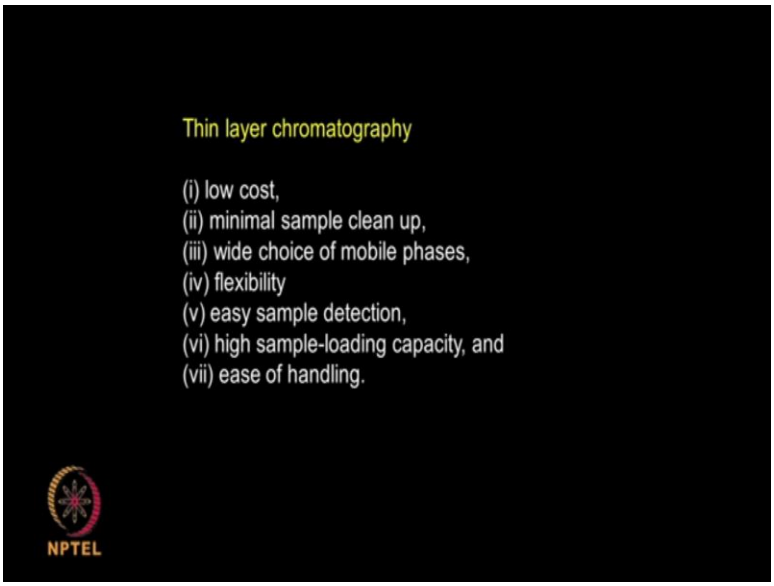

Okay there is something called Thin Layer Chromatography. This is we do it on a piece of glass plate which I coated by silica. So it is very cheap, minimal sample clean up, wide choice of mobile phases, flexible, easy sample detection, high sample detection, ease of loading. This is widely used in chemistry, synthetic labs for a very very long time, which it can be used for small molecules. It can be used for larger biopolymers as well actually.

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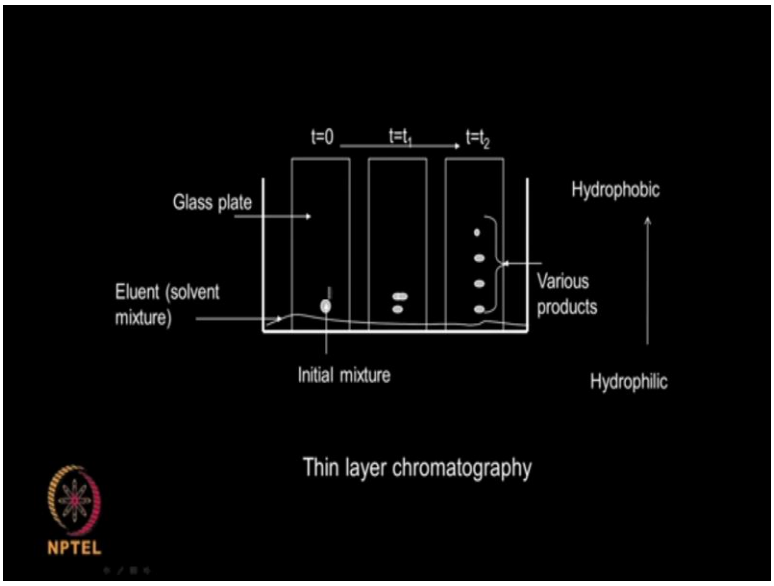

So basically what you do? We have a glass plate, we coat it with the silica and then you put an initial mixture of your the mixture and then you have a solvent here, could be a mixture of solvent. As time progresses the spot keeps moving up, as you know silica is extremely hydrophilic. So it does not move, whereas hydrophobic material keeps moving. So as time progresses and progresses, you are going to have many spots depending upon the optimization of the sol mixture of solvents.

You will get clean and beautiful spots. So the top could be hydrophobic and the bottom most is the most hydrophilic. It is a very good technique and this is called TLC or thin layer chromatography.

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Alumina is a strongest adsorbent followed by charcoal and so on… silica gel is the least adsorbing in the group okay.

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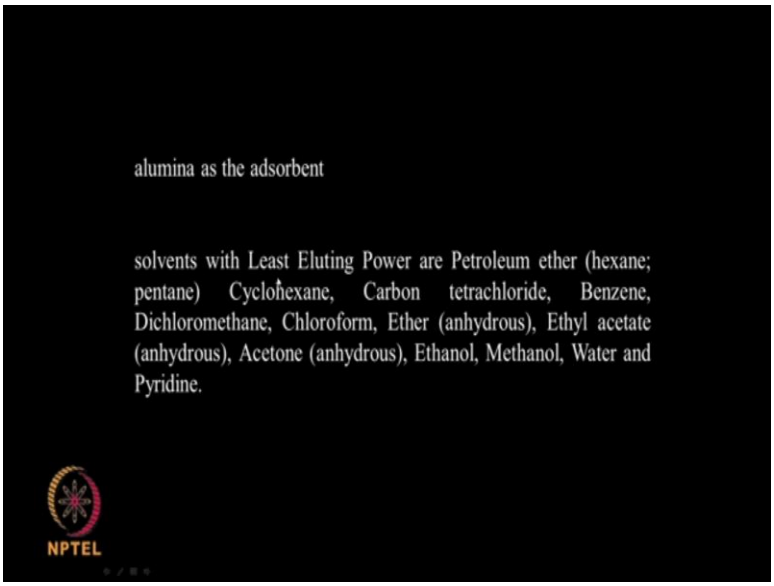

Alumina as the adsorbent, then you use different solvents for these solute mixture to travel upwards right. So you can have Petroleum Ether And They Have The Least Eluting Power And They Have Cyclohexane, Carbon Tetrachloride, Benzene, Dichloromethane, Chloroform Ether, Ethyl Acetate, Acetone, Ethanol, Methanol, Water And Pyridine. So if alumina is the adsorbent, then least eluting power solvents are given in that order.

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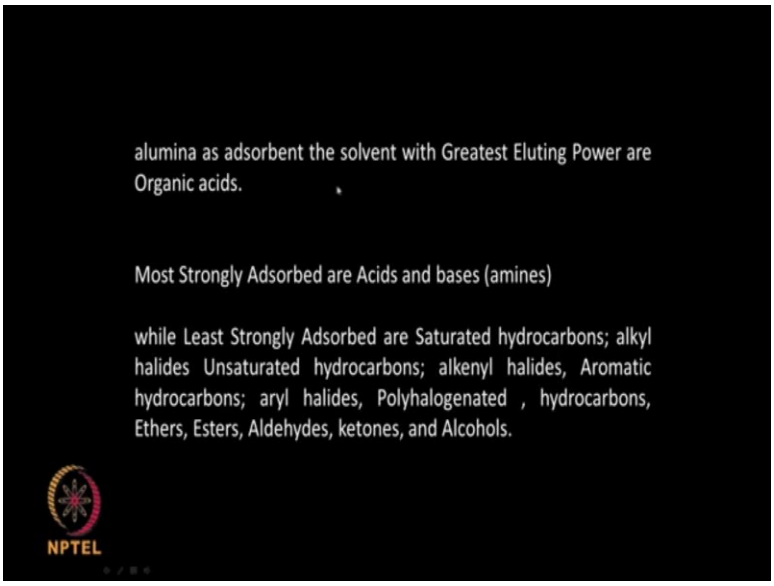

The great if alumina is adsorbent, the greatest eluting power organic acid more strongly adsorbed are acids and bases and amine. Where least strongly adsorbed are saturated hydrocarbons, alkyl halides, unsaturated hydrocarbons. So they will travel much faster in that order you know. Saturated hydrocarbons are extremely hydrophobic right. So they will travel faster and reach the top first and so on… As you come down to ethers esters, aldehydes, ketones and finally alcohols.

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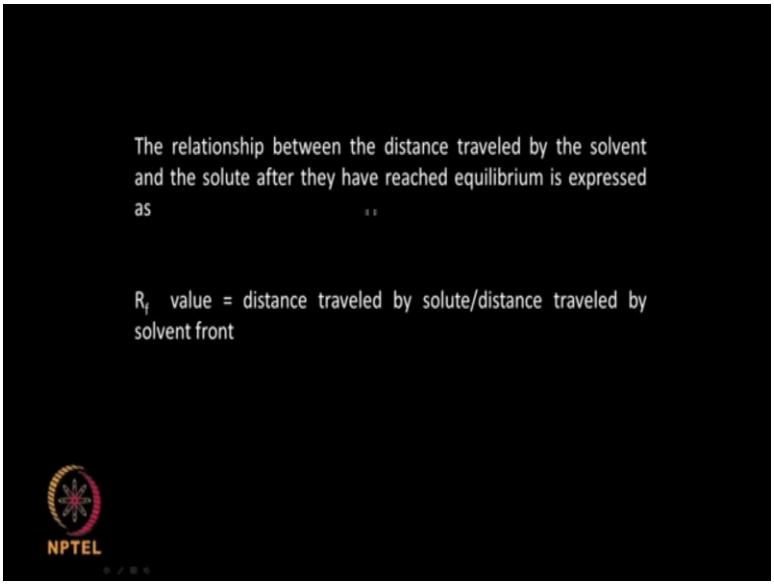

The relationship with the distance traveled by the solvent and the solute after they have reached equilibrium is expressed as Rf value. That is distance traveled by the solute / the distance traveled by the solvent front. Okay. So that is called the Rf value. So each component will have the Rf values and that is very characteristic of the component.

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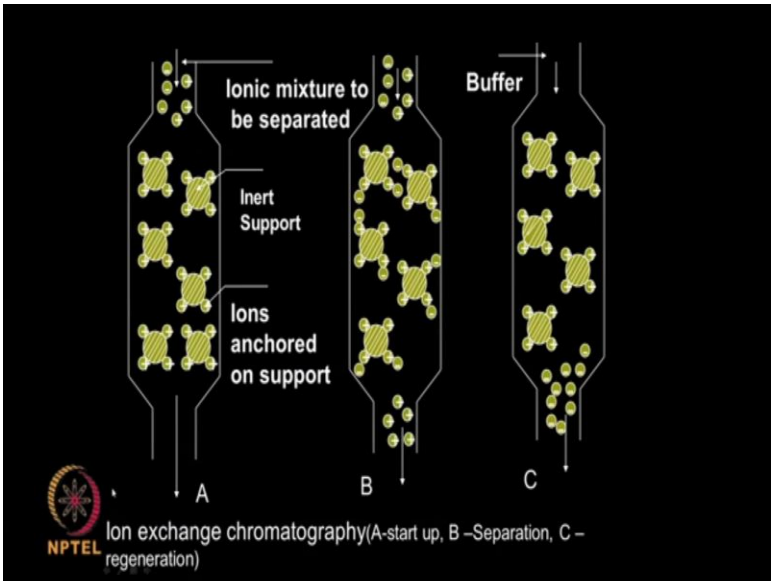

Now let us look at something called ion exchange chromatography. Okay. So basically if you have a ionic mixture, like you have a positive and negative okay, so we have a packed column okay which has ei either positive ion anchored on the support or it could be negative ions and sometimes it can have mixtures also. So what happens when you pass ionic mixture, okay, so the negative ions will get captured on the ions which are anchored.

So the positive ions will flow nicely okay, so later on I can use a buffer to elute out these negative ions. So where do you use this? We can use this suppose I have a protein and salt solution I want to remove the salt because salts are charged. So I can use a ion exchange chromatography. So I can use both cationic and anionic anchored to the support. So the sodium chloride can get captured and my protein can move down. That is the advantage of ion exchange chromatography.

So we can have both ions present anchored to the support cationic and anionic, both together. So how do we recover? We can pass a buffer okay, so that they get removed or we can change the pH of the solution, so that the whatever salts are captured, can be removed. This is ion exchange chromatography.

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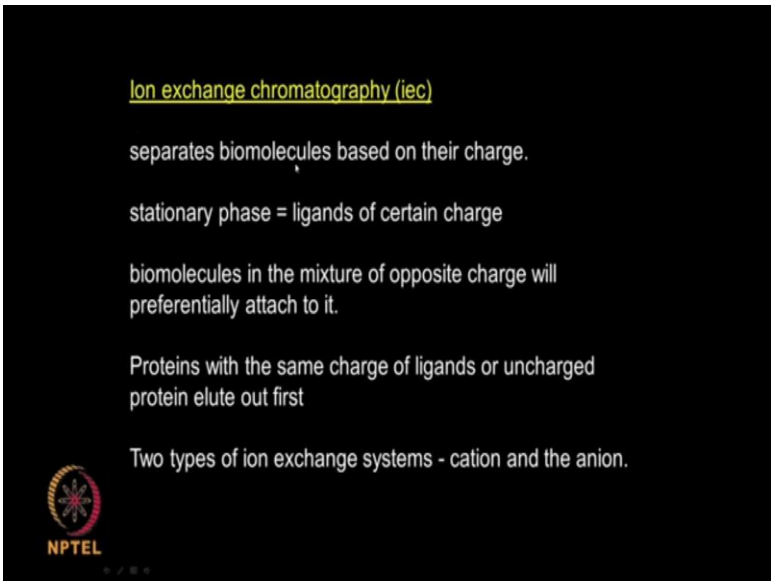

It separates biomolecules based on the charge, stationary phase ligands of certain charge. So it is all based on charge biomolecules in the mixture of opposite charge will attach to it, whereas with the same charge. It will pass through obviously right. So we can have cation and anion.

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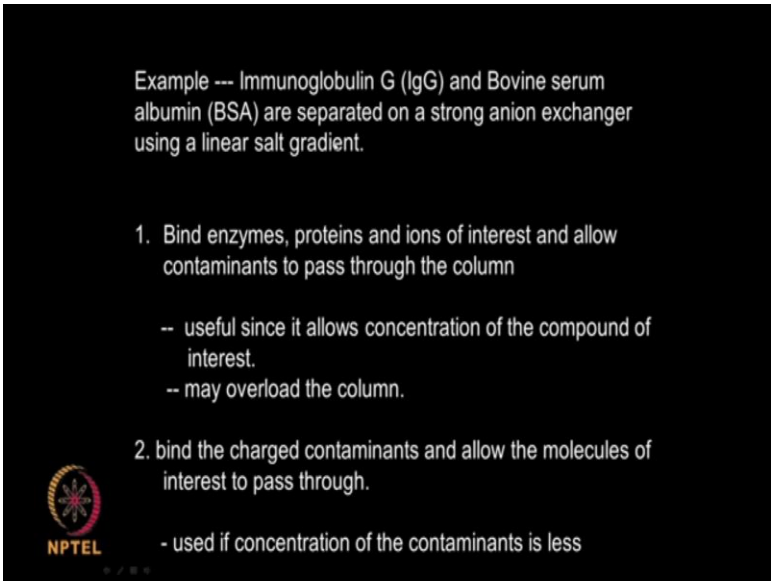

So immunoglobulin g and bovine serum albumin are separated on a strong anion exchanger using a linear salt gradient. Okay so bind enzymes, proteins and ions of interest and allow contaminants to pass through okay. So that is later on bind the charge contaminants other approach, binds the charge contamination, allow the molecules of interest to pass through depending upon the concentration of the impurity. We can do both. If the impurity concentration

is very little, we can try to capture them and allow your product to pass through and vice versa actually.

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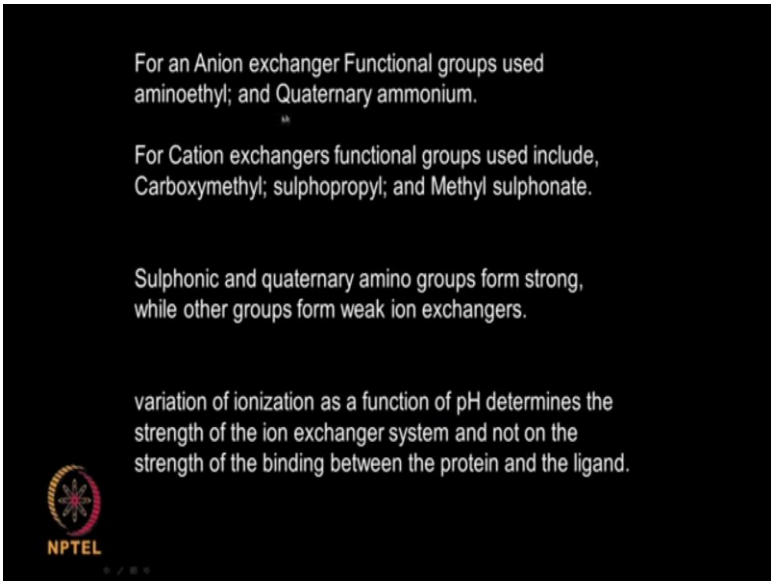

For an anion exchange functional groups used are amino ethyl at quaternary ammonium, whereas for cation exchange functional groups used are carboxymethyl, sulphopropyl and methyl sulp sulphonate okay. Sulphonic and quaternary amino groups form strong, while other groups form weak ion exchangers. So variation of ionization as a function of pH determines the strength of the ion exchange system. So when I change the pH obviously the ionization changes. So that strength also change okay. The binding also changes actually.

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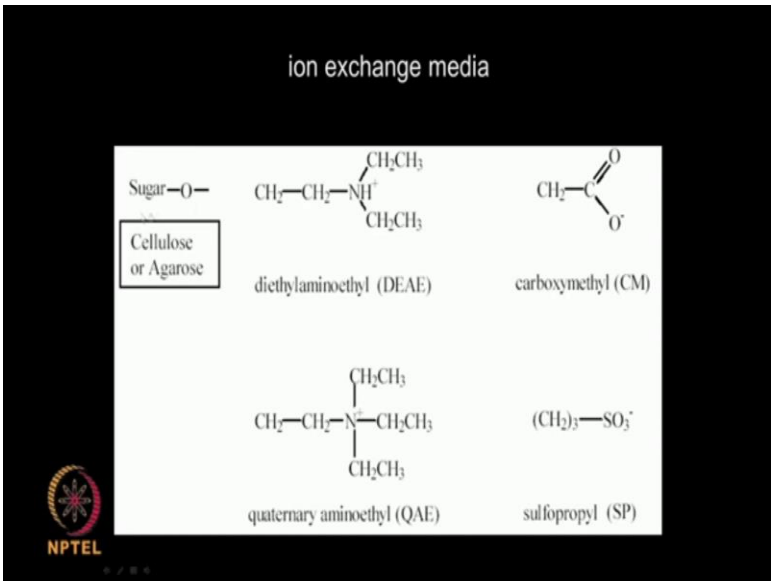

So these are ion exchange media like I have a cellulose or agarose, okay. Then I can have a diethyl aminoethyl. So will get lot of positive charge or quaternary amo amoniaethyl or if I want to have a negative carboxymethyl group, so they have a negative charge or sulfopropyl group this also has a negative charge okay.

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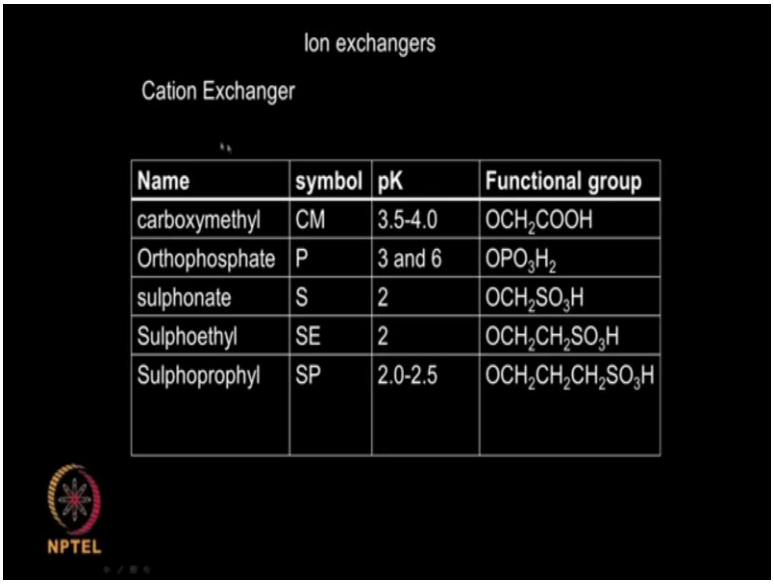

Name	symbol	pK	Functional group
carboxymethyl	CM	3.5-4.0	OCH_2COOH
Orthophosphate	P	3 and 6	OPO_3H_2
sulphonate	S	2	OCH_2SO_3H
Sulphoethyl	SE	2	$OCH_2CH_2SO_3H$
Sulphoprophyl	SP	2.0-2.5	$OCH_2CH_2CH_2SO_3H$

So cation exchangers will have carboxymethyl, as you can see carboxymethyl cation exchange or orthophosphate, sulphonate, sulphoethyl, sulphoprophyl and so on… So these are the functional groups there okay, here it gives you see the pK values also here very acidic okay.

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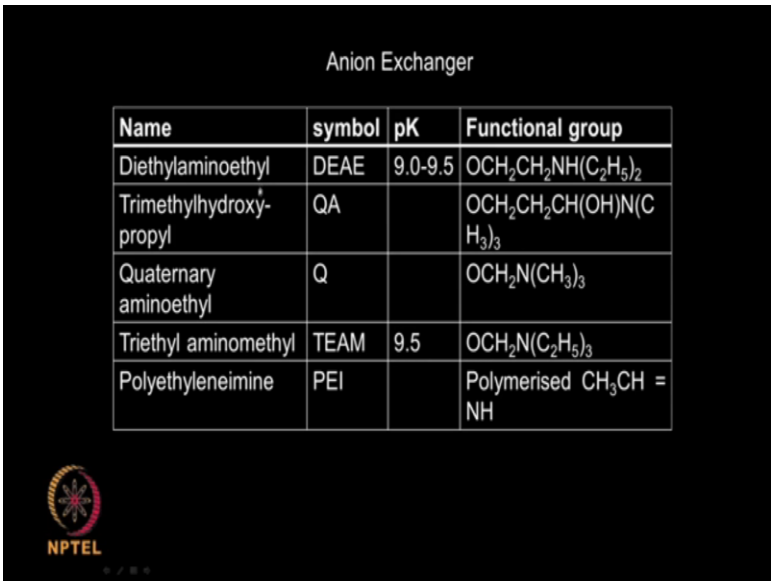

Now let us look at the anion exchange. We have diethylaminoethyl okay. So it will have a + all these have a trimethylhydroxy-propyl, quaternary aminoethyl triethyl, aminoethyl. So pK values will be in the basic region 9 9.5 polyethe ethyleneimine amin and so on actually. So these are anion exchange whereas cation exchange is as I said its carboxymethyl orth sulphonate, sulphoethyl, sulphoprophyl and so on actually.

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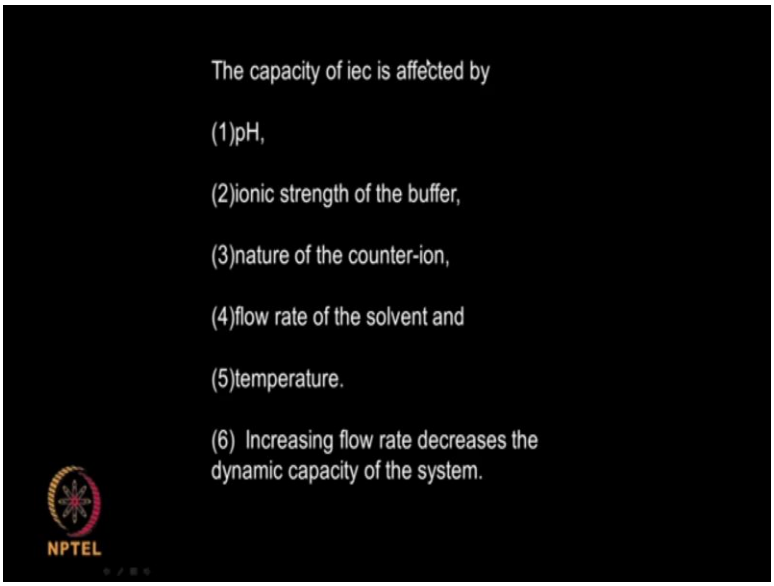

A capacity of ion exchange chromatography depend on pH, ionic strength of the buffer, nature of the counter ion, flow rate of the solvent, temperature for increasing flow rate, decreases the dynamic capacity of the system obviously right because the residence time decreases.

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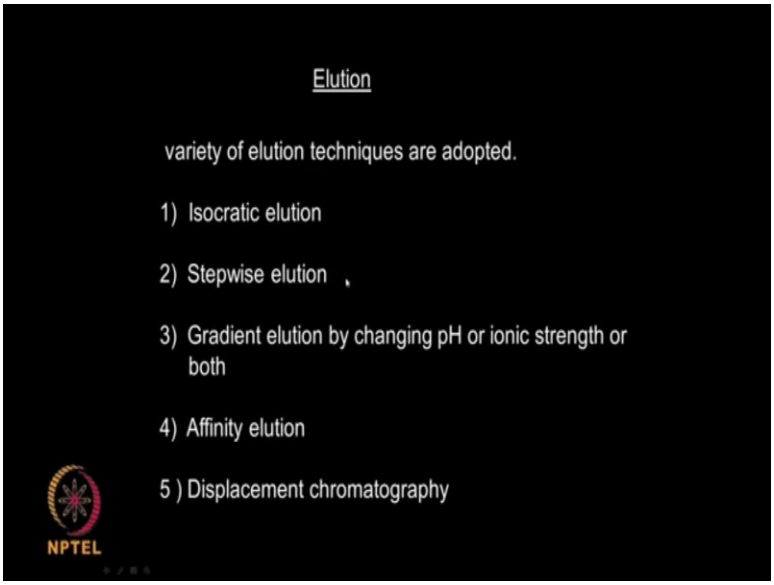

So how do you elute out? We can have different techniques for eluting out. We can have isocratic elution that means concentration salt of certain concentration stepwise elution. I will change the concentration from one value to another gradient elution continuously changing the pH or ionic strength affinity elution. I can have a affinity ligand passing through which will capture whatever has been bound. Displacement chromatography that means I can displace whatever has been don done using some other solvent or some other material.

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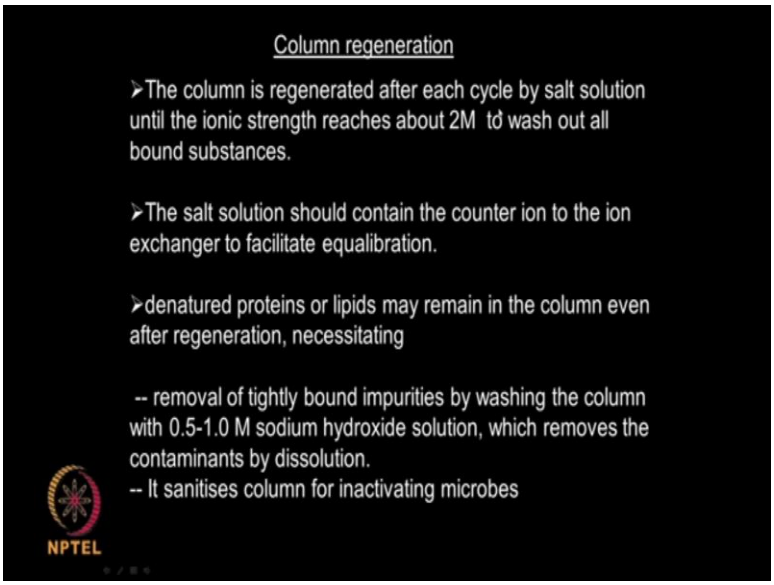

So this is how you regenerate your column. You have to regenerate after each cycle, you can use the salt solution. Until the ionic strength reaches about 2 molar, the salt solution should contain the counter ion to the ion exchanger, so that there is equilibration takes place. There could be denatured proteins or lipids which may remain in the column even after regenerating okay. So we need to wash it then with sodium hydroxide solution, so that the contaminants get roved actually.

So it is quite strong sodium hydroxide because some denatured proteins or lipids may be still stuck okay. And sometimes if there are microbes present, we need to sanitize of course the sodium hydroxide also helps in the sanitization.

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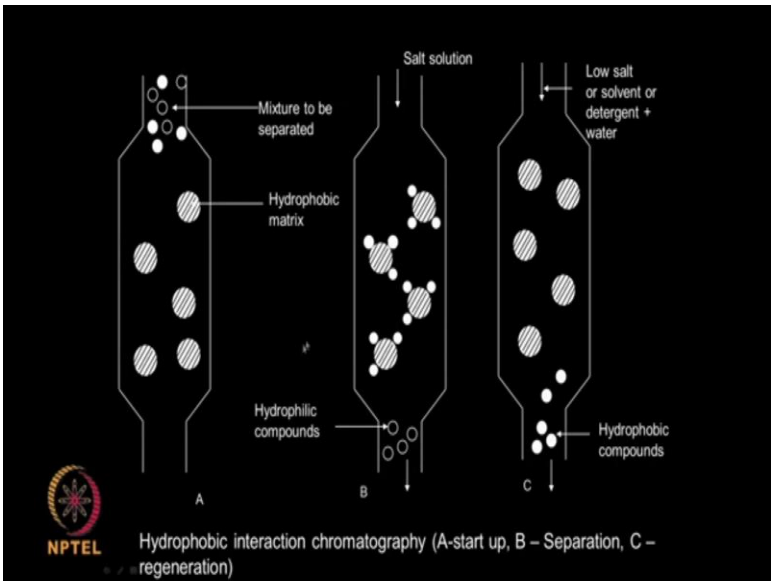

Next one is hydrophobic interaction chromatography. We looked at ion exchange. So in this hydrophobic interaction, we are separating proteins based on their hydrophobic nature of the surface. So I will have matrices which are hydrophobic. Okay. So what will happen proteins which are hydrophobic will get bound and proteins which are hydrophilic will get flushed out okay.

Then I can have a change in the salt concentration or I can use a detergent or a solvent. So whatever has been bound the hydrophobic ones can be collected okay. So this is how the hydrophobic interaction chromatography works. So basically its separation based on the differences in the hydrophobic nature of the proteins okay.

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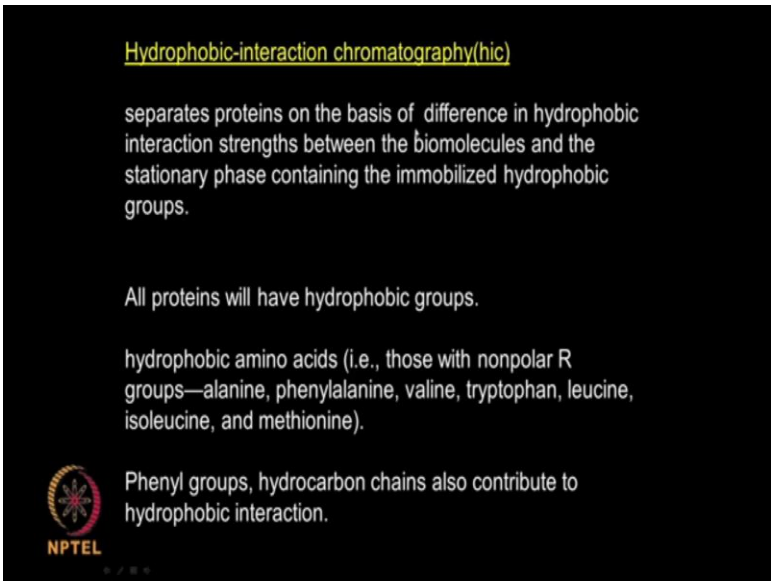

These are differences in the hydrophobic interaction, all proteins will have hydrophobic groups. There could be hydrophobic amino acids okay basically those with the with non polar groups r group like alanine, phenylalanine, valine, tryptophan, leucine, isoaleucine, methionine. Phenyl groups, hydrocarbon chains, all theses contribute to hydrophobic nature and depending upon how much of these are exposed to hydrophobicity of a protein varies. If they are buried inside, it will look more hydrophilic. Okay. So all these groups contribute to the hydrophobic nature of the protein surface.

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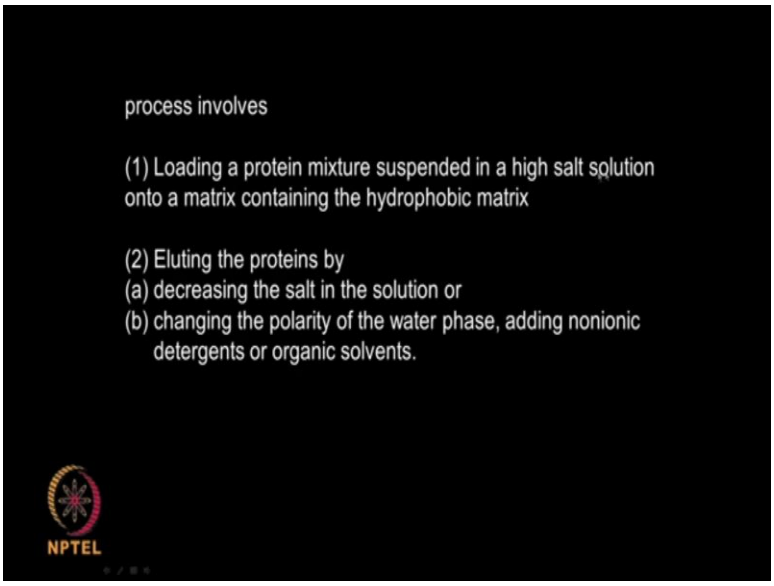

So the process involves loading a protein mixture suspend in a high salt solution. Why are you doing high salt, so that there is some sort of a salting out. Okay. Eluting the proteins by decreasing the salt in the solution or changing the polarity of the water phase by adding nonionic detergent, like adding some sort of detergent or surfactant okay which will try to flush it out or you can even add organic solvents here actually. That is how you elute out okay. So first is loading then is eluting out of protein.

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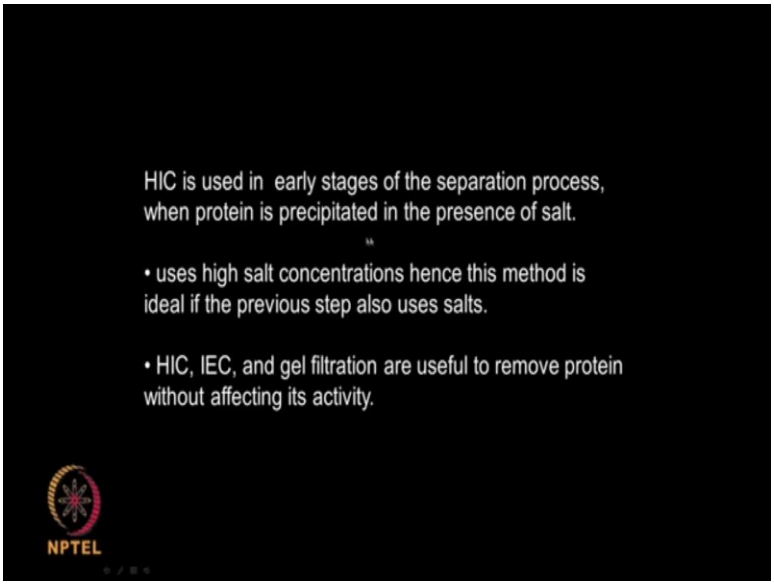

It is used in early stage of separation process, when protein is precipitated in presence of salt. So high is salt concentration is ideal because you are achieving a salting out of effect and the proteins will have better interaction with the hydrophobic stationary phase. So hydrophobic

interaction, ion exchange chromatography, gel filtration are useful to remove protein without affecting the activity. So basically will get the activity in the protein back will talk about gel filtration later but these three are very good because the activity of the protein is not lost.

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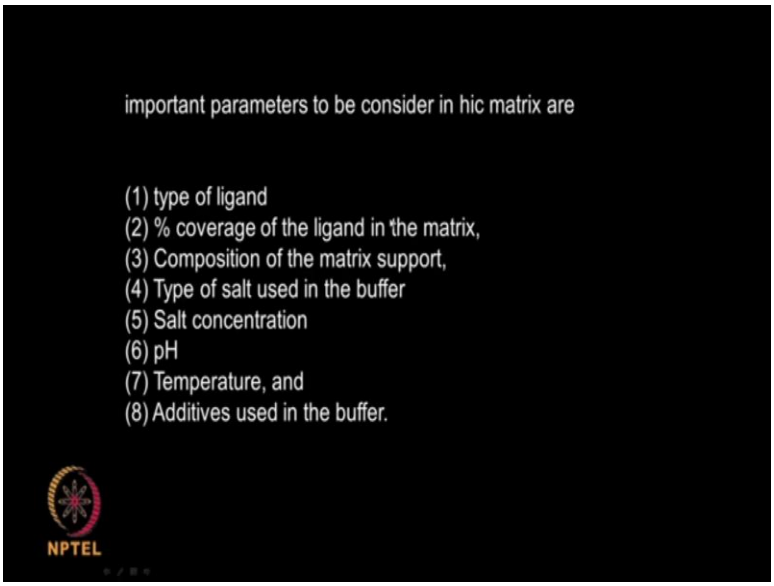

So important parameters I need to consider the type of ligand, percentage coverage of the ligand in the matrix, composition of the matrix, type of salt used, salt concentration, pH, temperatures, any additives that are being used and so on actually.

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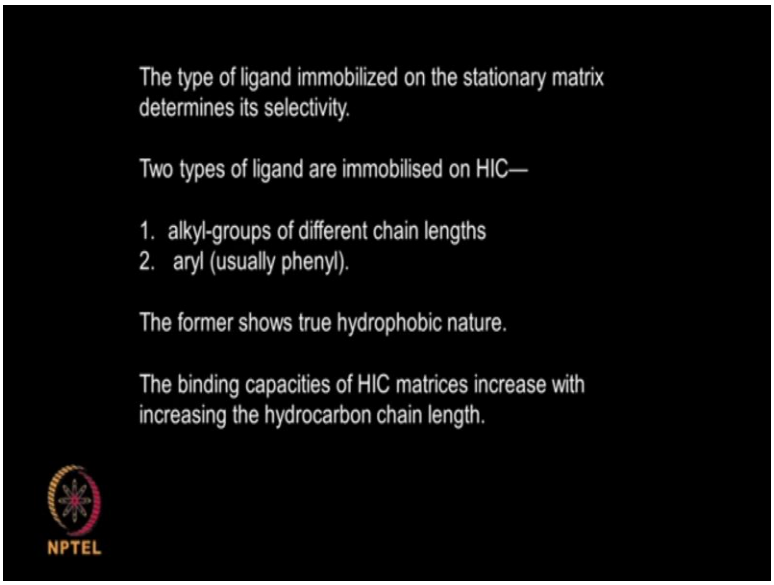

Okay so we can also have different types of ligands attached on the chromatographic support. It could be alkyl groups of different chain length, like I can have a c8 group attached or a c18 group. So it will become very hydrophobic or I can attach a phenyl group which is also hydrophobic. So the former is very very hydrophobic, the binding capacity of HIC matrices increases with the increase in the hydrocarbon chain, like I said c8 versus c18. c18 will be very hydrophobic whereas c8 will be less hydrophobic.

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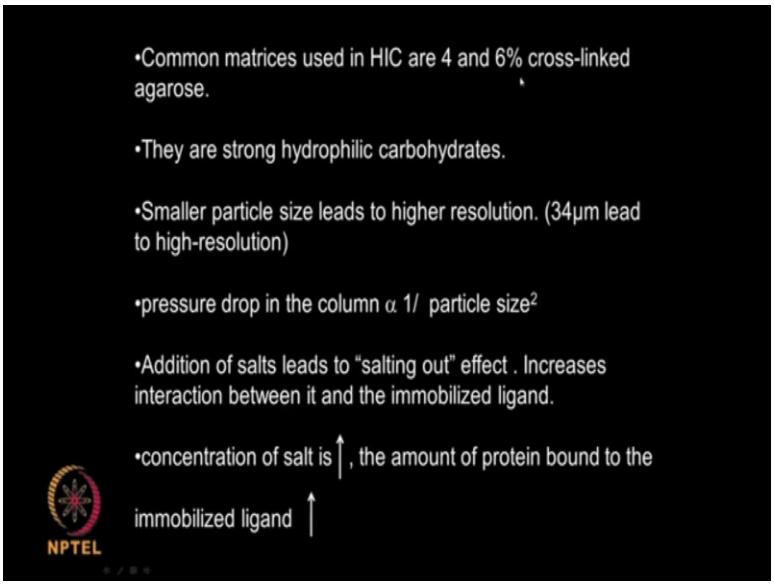

Common matrices used in HIC are 4 to 6 % cross-linked agarose. They are strong hydrophilic carbohydrates. Smaller particle size leads to higher resolution you know but then pressure drop also increases because pressure drop is inversely proportional to 1 by particle size square. So that problem we need to… Addition of salt leads to salting out. So increases the interaction between the protein and the immobilized ligand. So I can have a very high solvent.

So concentration of salt if it is increased the amount of protein bound to the immobilized ligand also increases the salting out. So later on if I want to recover whatever has been bound I will have a low salt so that the bound protein will come back into the continuous phase .

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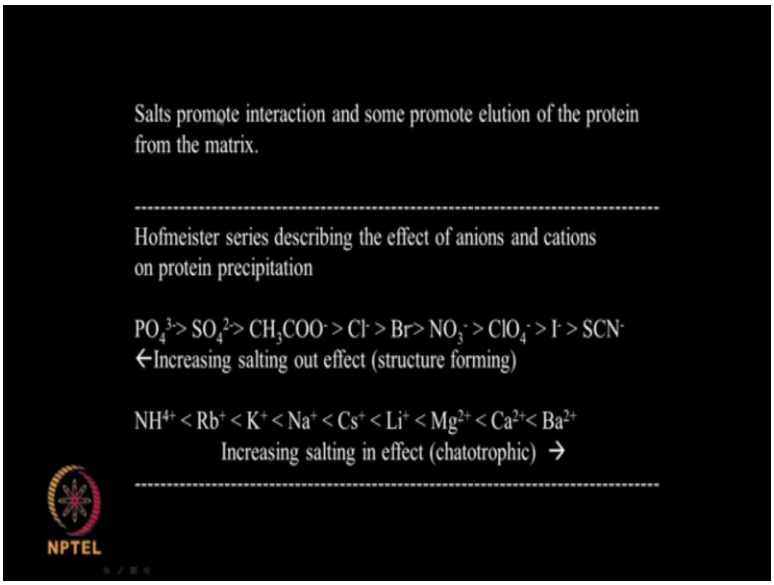

So salts promote the interaction and some promote the elution of the protein. So here the salting out effect which we talked about in the early class will come in, in the Hofmeister series. So I can have different anions cations and salt okay the increasing salting out effect and this is increasing salting effect okay. So I will use consider these salts if I am looking at early initial stage of HIC and I will use this type of salts, if I want to recover the proteins that is bound to the stationary phase okay and this is based on Hofmeister series okay.

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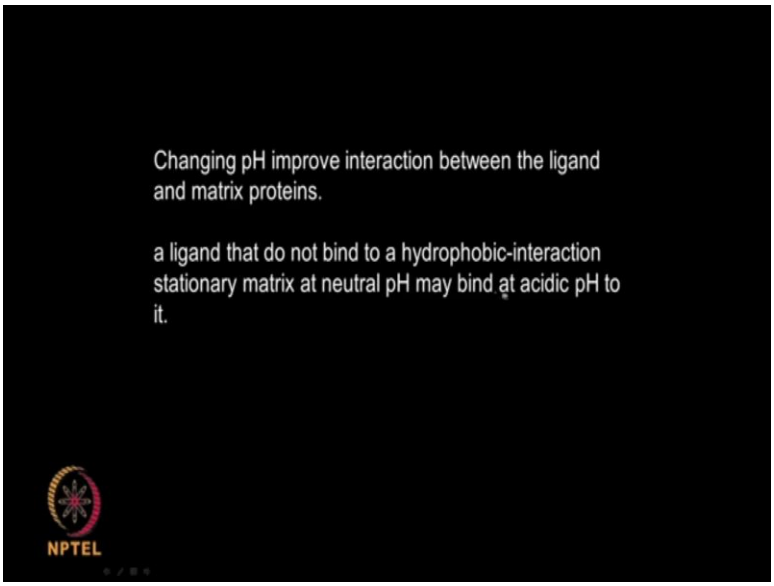

Changing pH improves the interaction between the ligand and the matrix proteins a ligand that do not bind to a hydrophobic-interaction. Stationary phase at neutral pH may bind at acidic pH to

it. So if it is not binding at neutral, then I may have to change the pH little bit, so that binds and at pH actually.

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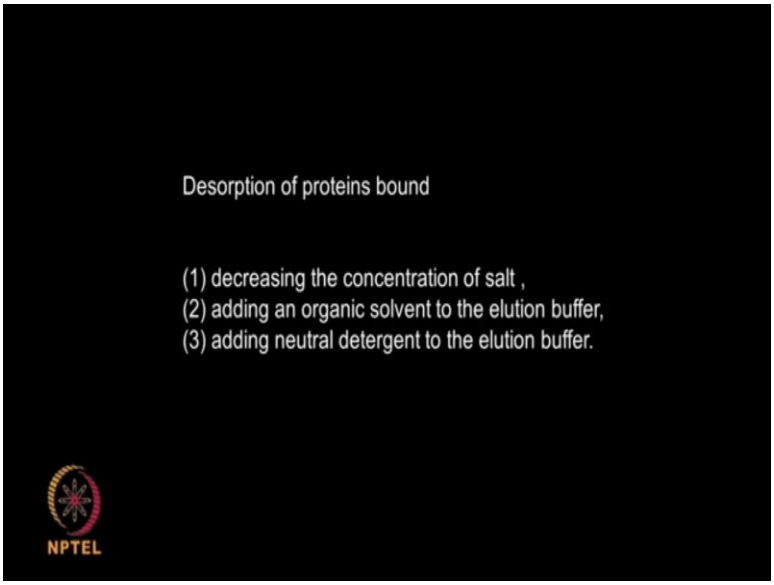

So desorption of the protein bound decreasing the concentration of the salt, like it is almost like salt again, adding an organic solvent, adding a detergent, neutral detergent or a surfactant. So all these are tricks by which you can recover whatever has been bound okay.

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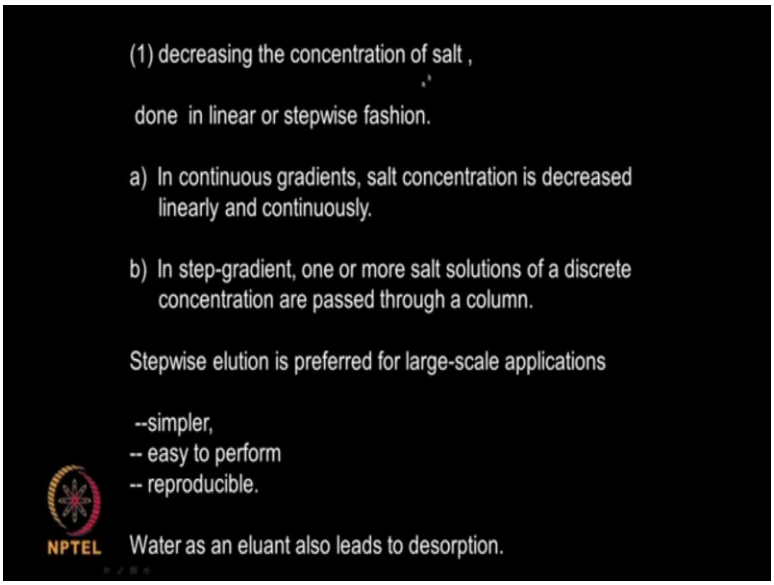

Decreasing the concentration of that salt can be done in linear or step wise fashion continuous gradient salt concentrations decreased linearly and continuously. Step-gradient one or more salt solutions of a discrete concentration are passed through a column okay. Step wise is simpler easy to perform reproducible. So water as an eluant also leads to desorption that means water without any salt right.

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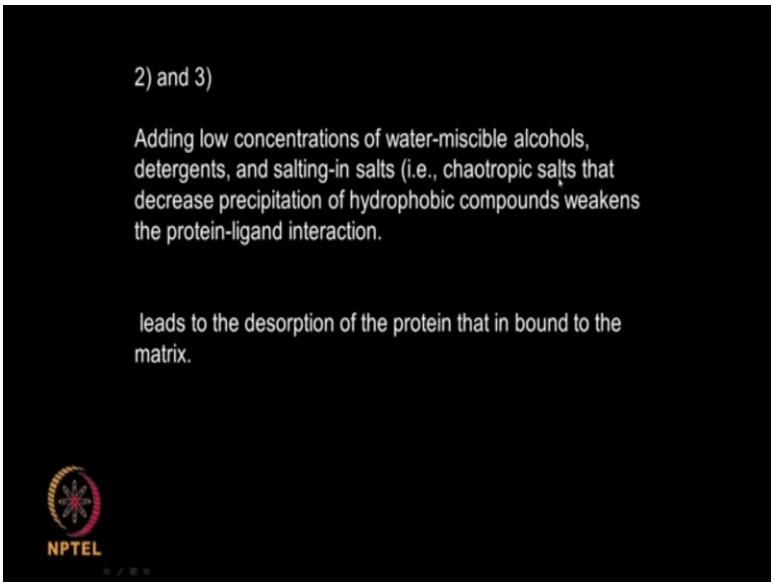

Adding low concentration of water-miscible alcohols, detergents help in salting-in like chaotropic salts and so on… These all leads to desorption of protein that is bound to the matrices.

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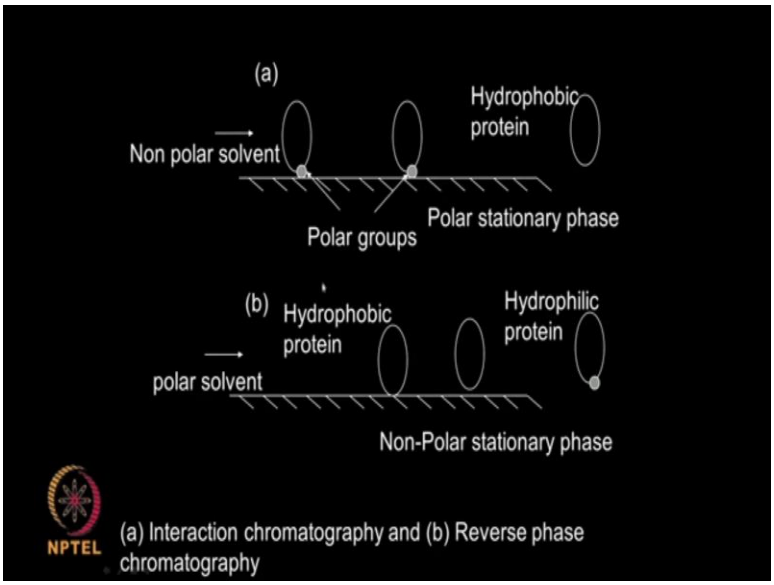

There is interaction chromatography. So this is you are looking at polar stationary phase. Okay. Then you are using a non polar solvent, okay non polar solvent. What is a non polar solvent? It could be a hexane could be toluene and so on…So polar stationary phase this is a normal chromatography. Whereas if you look at the reverse phase chromatography, you have a non polar stationary phase. That is the hydrophobic interaction chromatography and you are having a polar solvent.

Okay like water, it is a polar solvent. Okay. So here in a polar stationary phase, hydrophobic proteins will come out first whereas in non polar phase, hydrophilic proteins will come out first.

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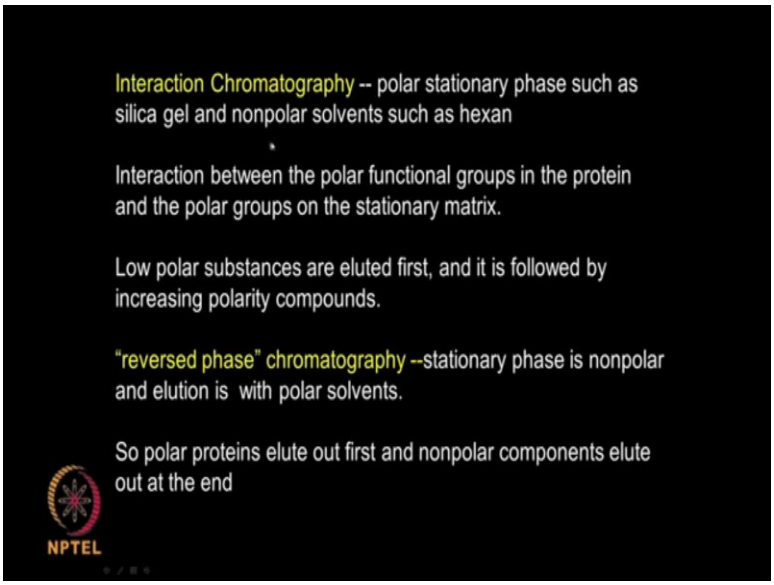

So interaction chromatography polar stationary phase like silica gel and non polar solvents like hexane whereas reversed phase chromatography stationary phase is non polar and elution phase is polar solvents like water.

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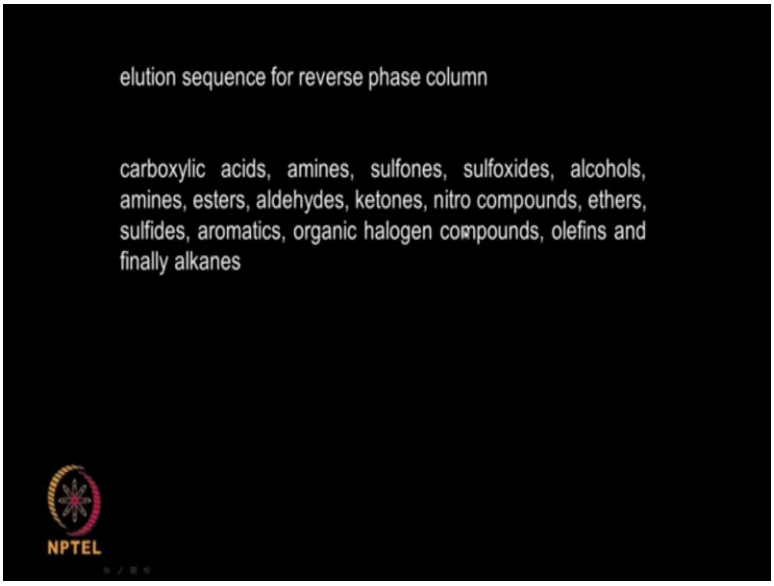

So elution sequence for reverse phase carboxylic acid, amines, sulfones, sulfoxides, alcohols, amines, esters and so on actually and finally the alkanes are there actually.

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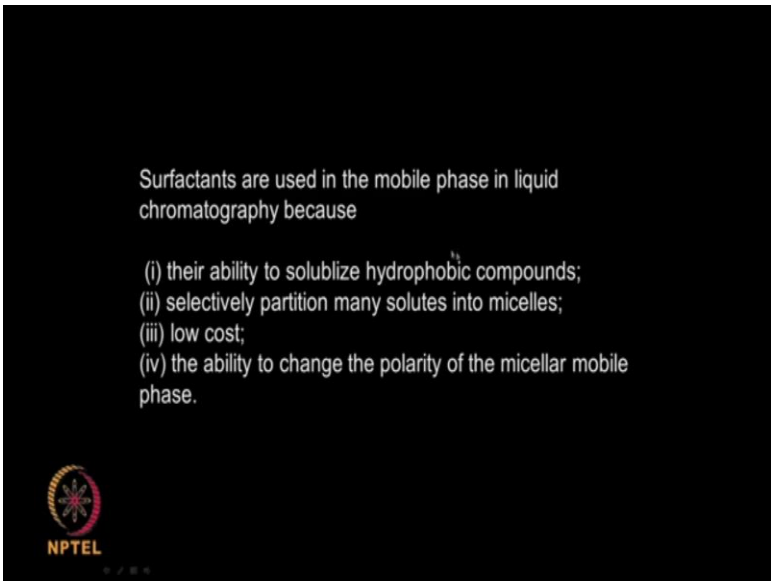

Surfactants are used because in the mobile phase, if you want to recover whatever has been because surfactants have the ability to solublize hydrophobic compounds. They selectively partition many solution to micelles cheap and it can also change the polarity of the mobile phase by adding that.

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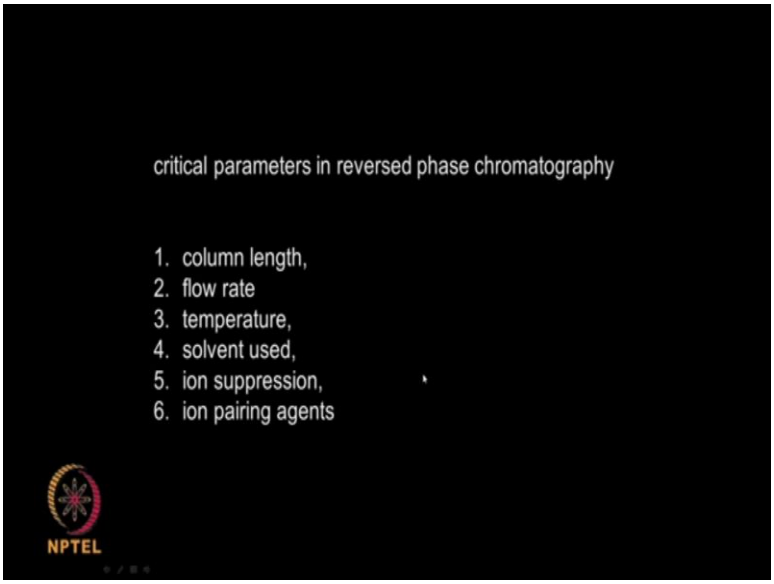

So what are the parameters column length flow rate temperature solvent used ion suppression sometimes, if the proteins has ionic groups then, it will appear like a polar. So we need to suppress that, so the hydrophobic part gets interacted with the stationary phase. So we need to

have some ion suppressing agents, sometimes you need to add ions varying agents also basically. These are done to suppress the ionization of the protein.

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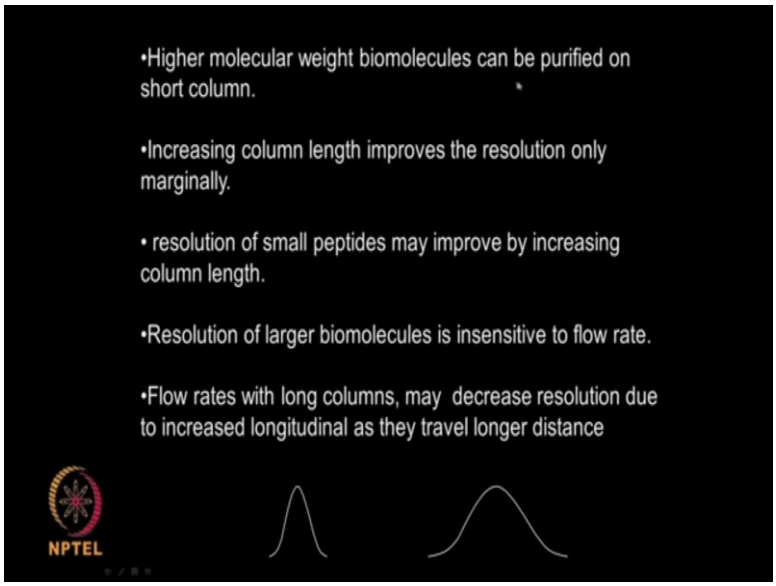

Higher molecular weight biomolecules can be purified on short column, increasing column length improves the resolution only marginally, resolution of small peptides may improve by increasing column length. Resolution of large biomolecules is insensitive to flow rate. So if I am doing flow rate with long, it may decrease resolution due to increased longitudinal diffusion as it travel longer. So if I have a small column, you may get like this.

If I have a large column separation may be good but it can go the peak may go very broad actually because of the longitudinal diffusion that is taking place. So we will continue in the next two classes more on the chromatography. Thank you very much..