Analytical Technologies in Biotechnology
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Module - 3
Chromatography Methods
Lecture - 3
Low-Pressure Liquid Chromatography (LPLC) and High Performance Liquid Chromatography (HPLC)

In previous lectures, we have dealt with the basics in chromatography, that is we have discussed the basic concepts in chromatography. We have also discussed about the various performance parameters of chromatography. Now, in this lecture we are going to extend our discussion on column chromatography; that is the most widely used chromatographic method, what are the basic components of column chromatography.

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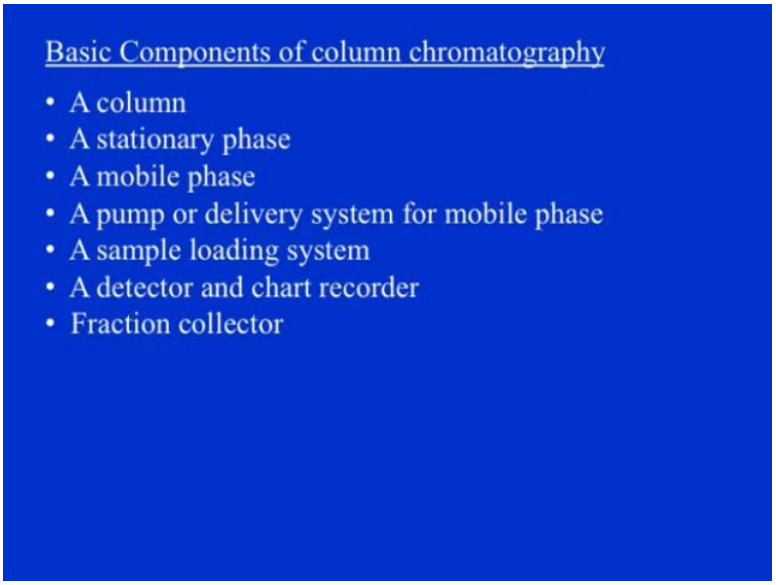

Now, column chromatography consists of a column, column could be made up of a glass, it could be a glass column or a metal column or it could be a plastic column. Then another component is stationary phase, which is packed in the column. Now, it could be simple stationary phase or stationary phase supported on a matrix or support material. Then a mobile phase, which passes through the stationary phase and due to distribution co efficient the partitioning or the separation of analytes takes place. There is a pump or we can say delivery system for mobile phase particularly in high pressure or high

performance liquid chromatography techniques, you certainly require a pump. Even in low pressure chromatography column chromatography, you can do it by gravity feed, but it is better to have a pump like peristaltic pump for constant flow rate.

Then you need a sample loading system for low pressure chromatography systems, you can load the samples manually also, but for advanced systems there has to be a injector system attach to the to the operators. Then finally, a detector and chart recorder detector different types of detectors are used as will see in chart recorder gets the redoubt samples could be collected in a fraction collector if they are required for like say you want to purify or analyse them afterwards.

So, in fraction collector different peeks can be collected and then it could be used for further analysis. Now, column chromatographic system can be classified according. To the back pressure generated within the column leading separation process. So, low pressure liquid chromatography systems generate pressure which is less than 5 bar or point 1 mega parcel medium pressure. A liquid chromatography generates pressure between 6 and 50 bar and high pressure liquid chromatography generates pressure above 50 bar.

Now, a days like both medium pressure and high pressure gives excellent resolution and the this particular demarcation has been a kind of gone. Now, both of them are called high performance liquid chromatography system. So, HPLC or high performance is preferred rather than high pressure or medium pressure.

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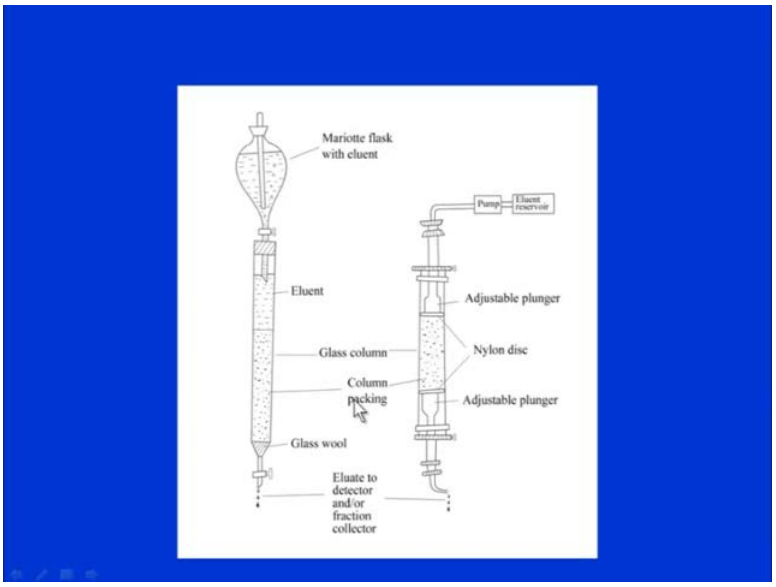

So, if you consider this is very schematic of chromatograph or equipment part, if you can see here there is a column there is a mariotte flask above this where the solvent or the mobile phase. Now, this will be run on gravity and so although this kind of maintains the pressure, but it still the fluorides are very as per the label of the mobile phase. Finally, this will go to detector and as eluent comes out it will go to fraction collector and through detector. The improve systems even for low food pressure chromatography, here you can see 1 there is a eluent reservoir and there is a pump.

It is usually peristaltic pump as we will see later on the pump kind of drives the mobile phase into the column, there are 2 plungers here and this is nylon disc or nylon filters are attached to it. So, stationary phases supported on to here. As the mobile phase passes it comes out here and goes to detector and finally, it could be collected in a fraction collector. So, that is a very simple arrangement and will discuss each of these things individually. So, let start with the columns.

Now, these columns are mostly these glass columns are used. These columns should have means of supporting the stationary phase near the base. That is important the first thing is you have to support. So, that could be staying there and another is you have to minimise the dead space below the column support. So, that post column mixing of separated analytes will not occur. Now, commercial columns passes at there a porous glass plate fused on the base of the column or a suitable devise for supporting a replaceable nylon filter net is there. So, which will support the stationary phase. So, a

column like I said even syringes could be used as columns and glass wool could be used to support the stationary phase for simple chromatography.

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Matrix materials which are used which supports the stationary phase or stationary phases attach to them. Matrix materials should have we will discuss about what kind of matrix materials are used, but there should have certain properties. It needs to have a say high mechanical stability to maintain good for its you have to have the particles or the stationary phase with good mechanical stability. So, they are not compressed of flow rates are not altered actually. It should have good chemical stability as, we are going to use different kinds of buffered solvents and other things.

So, chemical stability is also required. Then there should be functional groups on stationary on matrix to facilitate the attachment of stationary phase, because most of the time like say for attaching ironic groups or say affinity legends, you need to have certain functional groups, where these could be conveniently attached. Then it should have high capacity that is you can support large stationary phase so that amount of sample to be purified could be enhanced.

Then there should be available stationary, this matrix should be in a range of particles size and also in porous form for certain kinds of chromatographic methods. The surface of matrix needs to be inert to minimise non specific adsorption of analytes, otherwise the chromatographic separation will suffer as peek trailing rather problem might occur, due

to non selective adsorption on the matrix. Now, there are different types of matrices which are being used.

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Some commonly used matrices are example Agarose. Now, this Agarose is a polyacrylamide made up of dig electros and 3 6 an hydro 1 electro units, it is the unbranded polyacryed jeans are cross link with agent such as 2 3 die bromoproponol and to give gels that are stable in peach range from 3 to 40 and examples are like sapharose and bio gel 8 which are frequently used. Second material could be polyacrylamide it is a polymer of acrylamide cross link with be acrylamide. We will see this is a quite used both Agarose and poly acryl amide are used in electrophoresis in a next section. Polyacrylamide is also predictable in p h range of 2 to 11, an example is bio jell p, that is a commercially available.

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Then cellulose could be used as matrix material it is a polyacrylamide of beta 1 for linked cellulose units and crossed link with apicloro hydrant. It has good p h stability and very good flow properties and is hydrophilic in nature. Dextran, that could be another polymer, it is a polyacrylamide containing alpha 1 6 linked glucose units. This will also be cross linked with chlorohydrant, it is less stable to acid hydrolysis and it is stable up to ph say 12 it is hydrophilic.

One example is sulphide which is commercially available in different forms then polystyrene the disantardic sought of material could be utilised it is a polymer of steering crossed link with di vinyl jean. It has a good stability over all p h and mostly used for exclusive and un exchange chromatography. Then there is another one called silica, it is a polymeric material produced from arthoslices acid and it is stable in p h range of 3 to 8. So, these are some of the examples of the matrices which have been used.

Now, on these matrices attached is stationary phase, which is involved in the purification process. So, the chemical nature of the stationary phase depends upon the particular form of chromatography to be carried out. Say if it is iron exchange you will be in mobilising ironic groups, if it is affinity and so on. Now, most stationary phases are available attached to the matrices in a range of size and shape commercially. So, a both properties of size in shape are important because they influence the flow rate and the resolution characteristics.

Now, column packing once you have selected the matrix with stationary phase, the next step is to packed the column with the stationary phase. That is very important part of chromatographic separation. Now, packing a column is normally carried out by gently pouring a slurry of the stationary phase in the mobile phase into the column. Column is gently debt to ensure that no ear bubbles are trapped and the packing settles evenly. Now, poor column packing will give rise to un even flow and reduced resolution.

Once a column has been prepared it is very important that it does not to make sure that it does not run theirs. So, it should be all the time kept vat actually or mobile phase should be there, if it gets dries dried then it will break or crack that again you have to pack it again. So, let me show you how column is packed and how sample is applied on to the column on your screen. So, please see on a screen.

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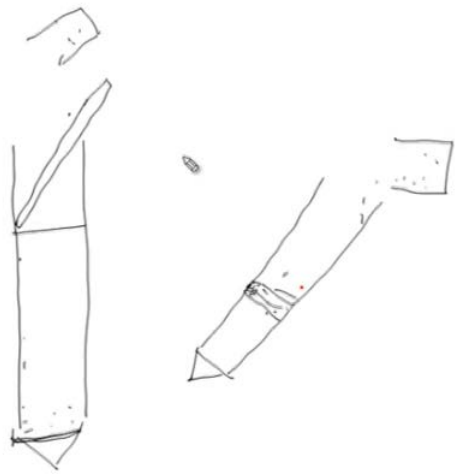

So, what is done if you have a glass column or any other column and you can see there is a support at the base, which will stabilise the stationary space. Now, what happen is, your stationary phase is filled in through a glass rod or either you have to have a glass rod here and you will pour your stationary phase onto the a through this glass rod. So, that it will pass and it will settle down. It will go to the base through the walls of the column. Now, advantage is that you will and it will filled from the base that if you suddenly at this to the column the air bubbles will be trapped, but with this method air bubbles will not be trapped at the base of the column.

Other way could be that you can tilt the column rather than, if you do not want to use the glass rod then column can be tilted and material could be could be poured in through the sides of the wall. So, this way what you can do is you can pour and see that you when you are pouring this martial you do it evenly, like continuously without creating gaps or demarcations like for examples, there could be a column which could be packed in this way that there is some unfilled areas could be there. This will again give a uneven flows and will have hampered the chromatographic separation. So, these things has to be looked into. Now, how sample is applied on to the column let us see that. So, when you have to apply the sample onto the column, what is done is again you have a packed column.

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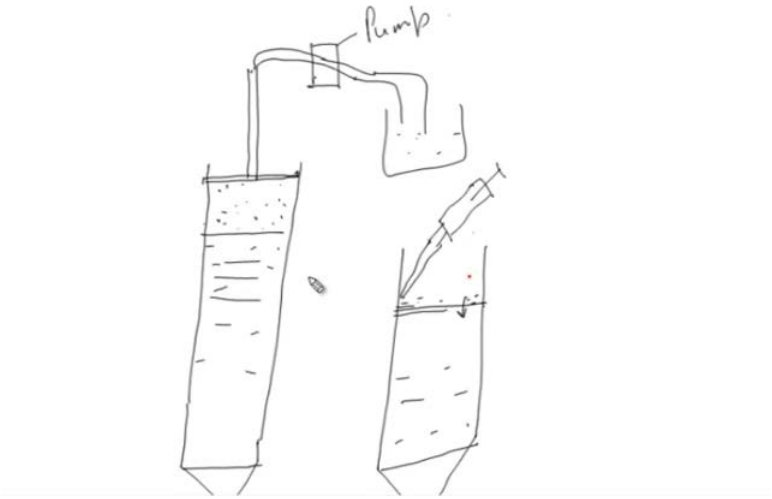

Once you have packed, and you have make sure, here we acrylrated the column with the particular buffer in which chromatographic separation needs to be done. Then you have acrylrated the column or stationary phase with that. Now, what you will have is, you have a stationary phase filled in here and you have your mobile phase which is here. You will either have closed it with plunder or with a certain crock with tubing in that. So, if we tubing which is attached to your mobile phase, it may be through pump or it may be without pump.

So, you can do it either by gravity or may be without gravity. Now, how do you load your sample one is if you have lot of sample and it is like iron exchange

chromatographic things it could be directly loaded through peristaltic pump, but other methods could be that you have to remove this top linger or scar, which have whichever is there and then what you have to do is then once you have you have this material. So, you have to remove this mobile phase from here a very slowly without disturbing the stationary phase. Once you have removed then very nicely with a micro syringe or a pipe it, you can inject the sample or you can load the sample in here in a smaller volume.

Then very slowly let this sample get into the column. Once it gets into the stationary phase, then you can put the mobile phase again and then put the plunderer on and start the chromatographic procedure. Another method could be, you could have high density solution like you use sucrose density sucrose or greased all in your sample which gives them high density. Then with you do not have to really remove this mobile phase because you can put in when your mobile phase is there you can put in the material and since it has high density it will settle in here without any problem.

So, you would be able to do it without removing the mobile phase. So, these are few ways you can do it, like say for high concentration of protein solutions are quite dense and they could be done like that. Let us come back to a word discussion. So, this was about the column packing and remember poor column packing will certainly rise to uneven flow and will certainly reduce the resolution.

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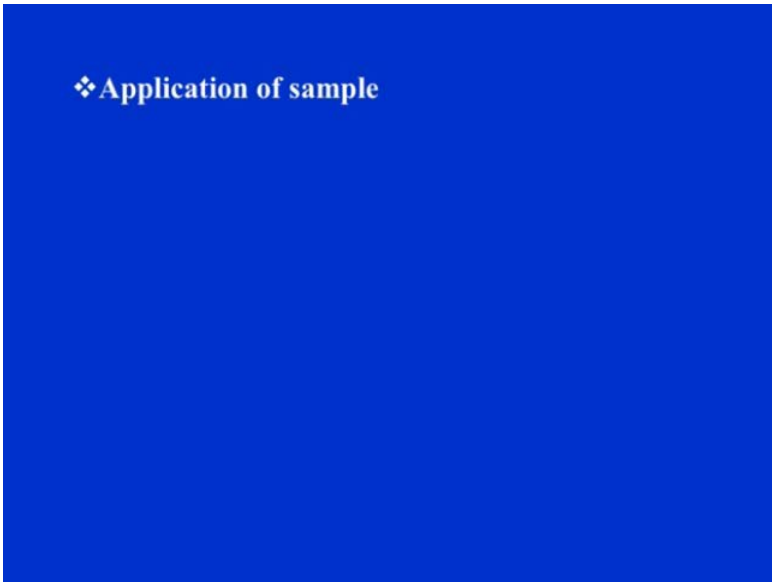

So, it is very important that no air bubbles no gaps, no uneven settling of the stationary phase and all these things has to be taken care of. Then application of sample, as we have discussed is very important part that we can do manually or through an injector system. Mostly, low pressure chromatography you can do it manually like I have shown you like you can remove the mobile phase or you can use high density like sucrose or gloeustrol or you can use peristaltic pumps. So, once the sample is loaded then you have to develop the column and elute analyte.

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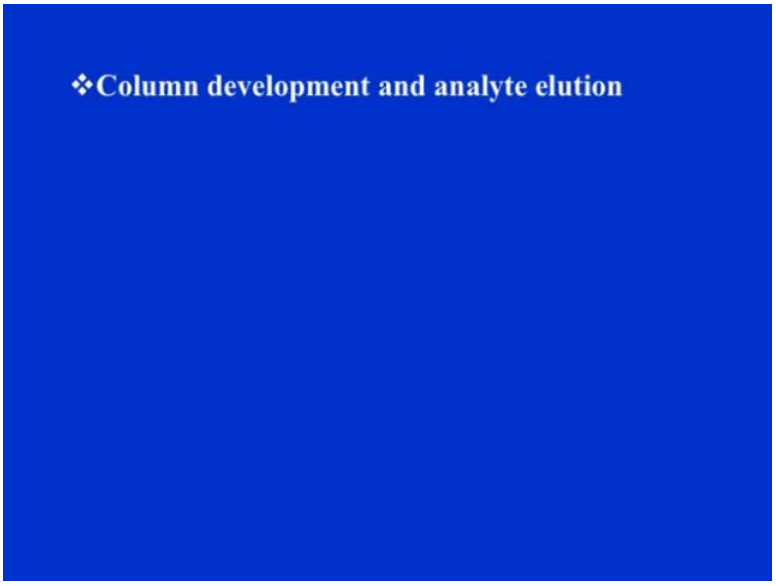

So, the components of the applied samples are separated by continuous passage of the mobile phase through the column. Now, separated analytes will be removed from the column by illusion. This illusion process is essentially that the flow of the mobile phase maintained at the constant rate and in sudden systems mobile at the column sudden systems the analyte as per retention time will stay in the column. Then slowly will be moved and since they have different co efficient did be separated, they will retained separately or differ a very differently they will be removed.

So, it is a isocratic illusion, they could be also gradient illusion where you are utilising an alleviant a containing other substances. Like see for example, salt illusion you might use sodium chloride and that replaces the analyte and then analyte is eluted a. So, there you can have a gradient illusion or that could be step gradient or that could be continuous gradient elution could be done. There are gradient makers which are available to make

the elution and to make the gradient. To produce the suitable gradient 2 or more elements has to be mixed like for example, like you can have 0 molar initial in a buffer and you can have say 7 molar initial in that buffer and though two can be combined in correct proportion prior to entering the column. This could be achieved by using two different peristaltic pumps, to delivered thus a separate ill vents and they will be mixed in a chamber before entering the column. Gradient could be a leaner gradient or it could be a convex or concave gradient. Convex gradients give better resolution initially, where as concave give better resolution at the end.

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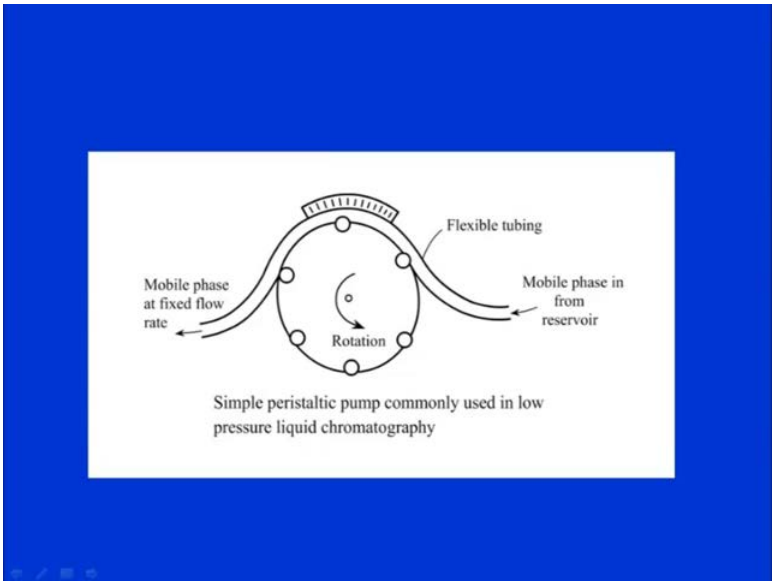

This is peristaltic pump, very simple schematic of a peristaltic pump. It is a roller type peristaltic pump and you can see this rollers here, what they do is, they compress the tubing and as they revolve, it is this revolution occurs these rollers move compressing the tubing and due to the compression the mobile phase is sucked in from the reservoir. Then will be at fixed flow rate it will be supplied to the column. Now, this has to be standardised in a sense that in a different tubing's will give you different flow rates as per the revolution of this disc actually. So, that has to be standardised, but that is a very simple peristaltic pumps there pal entry type also are available and both of them can be used for providing even flow or a constant flow this figure shows.

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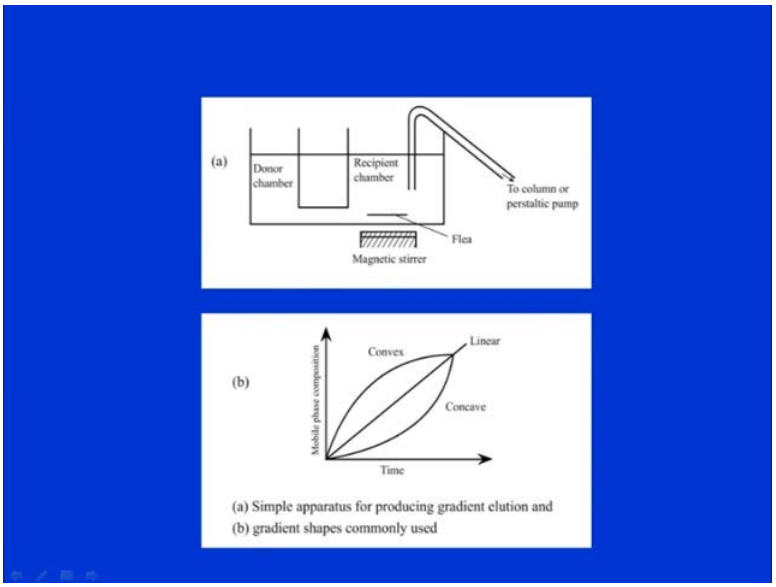

This is a gradient maker you have one solution here, that is donor chamber one is recipient chamber. Then as it could be mixed with a the magnetic stirrer and a gradient could be marked in a different proportion. This is like a linear gradient or you can have convex or concave gradient as per your requirement. So, this a very simple method of elution, where you get your analyte out and you can collect it in a fraction collector. You can detect it through a different kinds of detectors.

So, method of detection and collection of will be discussing later on with HPLC section. As many things are very common, only difference is a detectors are for HPLC are more sensitive. They are more quick to a detect certain things, rather than a low pressure chromatography system, where a simple you we monitor could be utilised. Alright this was about, the low pressure chromatography system. Now, let us shift to high performance liquid chromatography and how you will see, how it is different from low pressure chromatography.

Now, the resolving power of chromatographic column increases with column length, as you were discussing earlier and it depends on number of theoretical plates per unit length, but there is a limit to or there is a practical limit to length of the column because of peek boarding under the problems. So, what you have is that you cannot really increase the length after certain particular length of the column. So, you cannot increase it at to, like there is a limit to increase of the column length. So, what has to be done to

increase the theoretical plates, what could be done is, that in a smaller or in a particular length, you can have a stationary phase with support particles of course, with a smaller particles size.

So, if you have a smaller particles size of a stationary phase, the better resolution could be achieved because you will have higher number of theoretical plates. As you can compare of, there is a course material or higher size particles which will pack in the column and little certainly have loan number of theoretical plates, but if you have very small particles size they will pack much better. They will have higher number of theoretical plates, but problem is that as they pack very nicely there will be lot resistance due to smaller particles size, which will create back pressure in the column. Simple pumps are not really sufficient to drive the flow and many times this matrix collapse actually because of high pressure.

Now, this problem has been solved lot of advancement where development of small particles size is stationary phase which can withstand these pressures very high pressures could be with this stand these particles. Now, this development has resulted in faster and much better resolution. The HPLC or high performance liquid chromatography has emerge, the most popular very powerful and versatile chromatography. It also very quick, does not take much time as the low pressure chromatography because it is done at very high flow rates and very high pressure limits.

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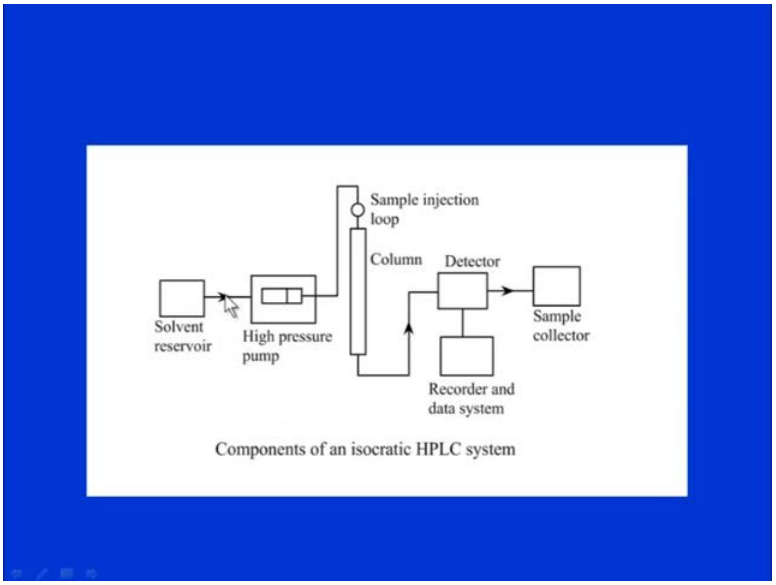

A very simple schematic of different components of isocratic HPLC system if you will see what you have is reservoir or eluent reservoir. Now, solvent reservoir then it there is a high pressure pumps. So, we will discuss these are different from simple peristaltic pump, then a you have from pump it is the solvent directed to the column. Many times there is a guard column here which kind of cleans up the system and remember all mobile phase has to be degust and filtered. So, that it does not like precipitate things in there and leads to generation of high pressure because of the chocking of the column. A column which is small column, will see how big these column are, but length is certainly less than normal columns, because you can pack more number of theoretical plates in here.

It goes to the detector and then to fraction collector, say you can collect it you can detect and read out could be seen. So, this is very simple schematic of isocratic HPLC system, if it is a gradient, you have to have two pressure pumps and gradients mixture, which will allow you to supply gradient and elute the sample. So, let us different things, different parts of this HPLC. First thing is and most important is column.

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Now, conventional column used to HPLC are generally made up of stainless steel. They with resistance pressure very high pressure up to say 5.5 into 10 is to per 7 Pascals. Columns are generally 3 to 50 centimetre long and 4 millimetre in internal diameter with flow rates which could reach up to 1 to 3 ml per minute. There are also micro bore open

tumbler columns and they have internal diameters of say 1 to 2 millimetre. They are generally up to 50 centimetre long, can sustain flow rates of 5 to 20 micro litre per minute. So, there are advantages also of micro bore columns, like they elute consumption is reduced as due to surf flow rates ideal for inter phasing with mass excerptor meter due to reduced flow rate, increase sensitivity and higher concentration of analyte could be used here.

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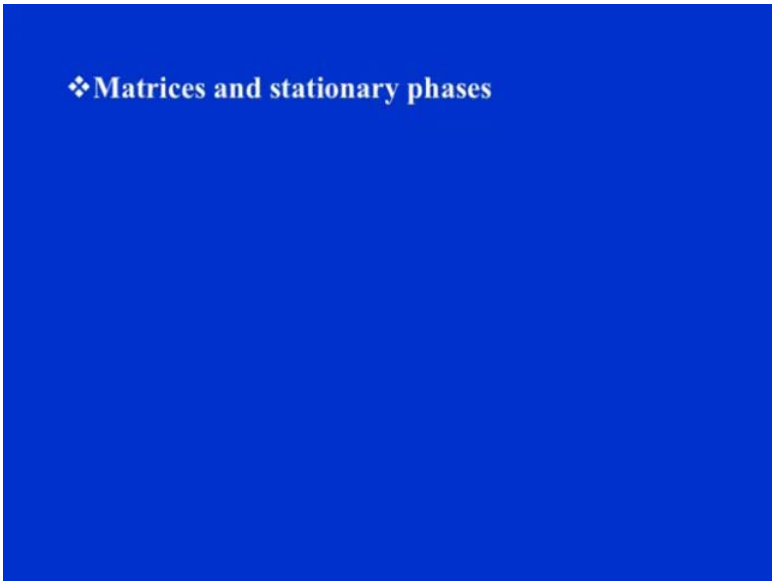

Now, matrices and stationary phase if you see, the three forms of stationary phase material are available. These are micro porous support, where micro pores ramify through the particles which had generally 5 to 10 millimetre in diameter. Then there is a pelicular supports which is pores particles coated onto the inert solid coat such as glass bead of about 40 millimetre in diameter. Then there is a bonded phase, where the stationary phases chemically bonded onto the inert support says silica.

So, these are some of the stationary phases available. For adsorption chromatography, adsorbents such as silica or alumina are available as micro pores are pelicular forms with a range of partials size. For partition chromatographic system the stationary phase may be coated on to the inert micro pores or particular support.

For iron exchange chromatographic system crossed linked micro porous polisteryne resins are widely used. Pelicular resins forms are also available and bonded phase exchanger covalently bonded to crossed linked, a silicon network are also used. Now,

application of sample is very important in this HPLC. If you could recall and low pressure you can do it manually, but in HPLC which is advanced an enclose system there should be very like very accurate or automated system of application of sample.

So, application of sample on to HPLC columns is done through, like for successful separation it is done through loop injectors actually and not manually. Now, this consists of a metal loop of fixed small volume and they could have be of wearing volume from very small to very large and that can be filled with the sample. Then eluent from the pump is then generate through those loop, by means of walls searching system and then the sample is fleshed on to the column while the loop out let, without interrupting the flow of the ill event to the column. So, that is the very important part. Now, let us see let me show you on your screen this loop injector. So, you can appreciate how does it look like and how this whole thing is happening alright. So, loop injectors like I said there are very important part. Now, in loop injector is simply they are different ports.

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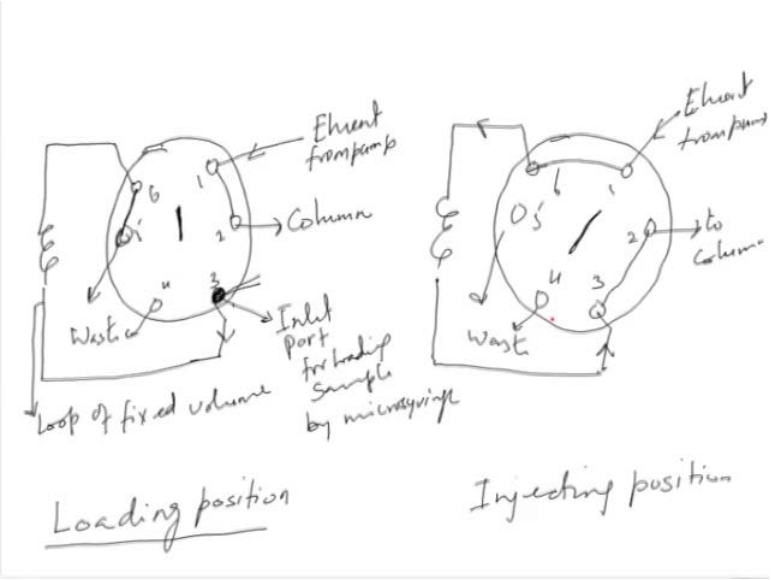

Let me make this for you here, I said this sort of and you have different ports here. We can numbered them like say 1 2 3 4 5 6, then there will be a loop which is connected, if you see there is a loop here which is off. So, this is a loop here which is being formed. Now, there is a two position, one is loading position, if I could say this is loading position. Then what will happen, loading position means you are loading your sample onto the loop.

So, what is the configuration here, configuration is that you have a particular eluent or the mobile phase from the pump. This is going to the a port number 1 and from the port number 2 it is going to the column. So, still you have not loaded. Now, you this is the loading position where you can load your sample. If you I say this is now which would has to be shifted then this is the loading position. This is your loop, is fixed volume now this could be like I said it could be like 200 micro litre 2 say a few m l. Now, what will happen?

So, you have to load this is the port where you will be loading the sample actually. So, this is inlet port, for loading sample by micro syringe alright. Now, once you have loaded. So, once you have put in your micro syringe and you are loading you sample through a micro syringe in this, you have to fix a micro syringe. Then what will happen, this will go here your sample will move from into this loop and it will fill this loop here alright. So, what will happen, say if this is 200 micro litre loop. So, if you load 250 micro litre then 200 micro litre will be contained in this loop and rest of it will go to through the port number 5. Like it is connected 6 loop is connected to 6 and 6 is connected to 5 here. So, this will go to waste, these are both things will go to waste.

So, what will happen you have loaded the loop extra sample will go to the waste alright. Now, this is loading position Now, let us see that you have to inject it onto the column. So, what will happen what is the difference here. Now, what is these are all things are same that you have. these ports here which is 1 2. Now, this is your loop here and this is connected here. So, what was the situation here then here, it is from eluent from pump is coming here and this is 2 column. So, what will happen, now here if you see this knob will be shift to injecting position.

It will be you can just rotate it to this position. Now, the configuration will change. The configuration will change in the sense that, the eluent which was here going to port number 2 directly rather as you change this knob, this will go to port number 6. Now, port number 6 and 5 are not connected. So, what is happening the eluent is going to port number 6 then it goes to the loop and from the loop finally, it goes through port number 3 to port number 2 and then to the column.

So, you can see the change here, as you port is from loading to the injecting position the eluent is directed through the loop and. So, the eluent pushes the sample onto the column

and then this run could go on. When we have to inject again what you will do, again it will be brought to the loading position and then again you have to do the same thing. These will go to the waste actually both of them. So, this is the very good system injector loop which could be used for loading the application of the sample alright. So, let us return to our discussion alright. So, we were discussing about the application. I have shown you this is how the application will be performed application of sample will be performed.

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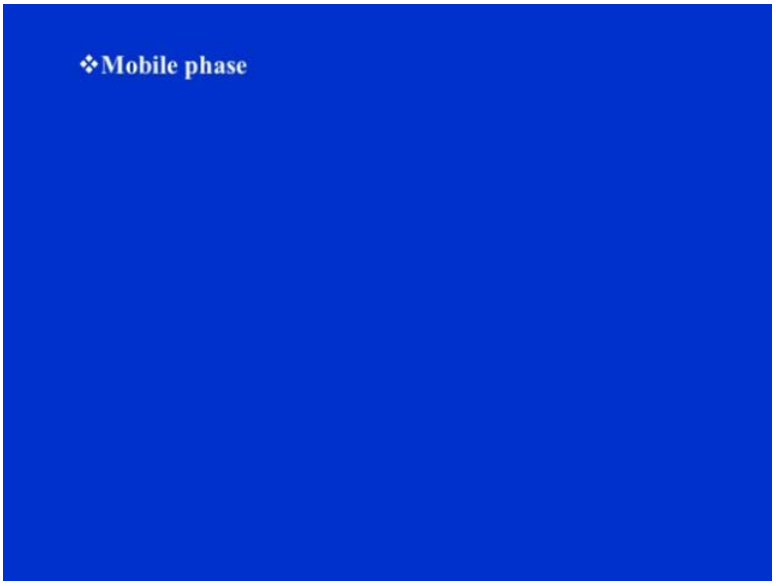

Now, mobile phase depends upon what separation needs to be achieved. If it is a isocratic elution it could be made with the single pump using the single eluent or 2 or more eluents, premixed in a mixed proportion, but if would say gradient illusion then you have one needs to use 2 pumps and they will be gradient mixture of programmer has to be used. All eluents must be specially purified because traces of impurities can affect the column and inter phase with the detection system.

It is also essential that all elevates be degust and there should be filtered for high resolution. The next component important component of HPLC is the pumps actually. Now, main feature of good pumping systems in HPLC is that, it is capable of output of at least say 5 into 10 per 7 Pascals. Ideally there must be low pulses as this may affect the detector response. So, there should be like your flow capabilities of at least like say 10 centimetre cube per minute or up to 100 centimetre for preparative systems.

Now, the pumps which are used in here are mostly constant displacement pumps and reciprocating pumps like. Now, all pumps which are used in here the incorporate pulse deepeners to minimize the pulsing effect. They have in built safety cut out mechanism to inactivate the pump automatically. A pressure within a chromatographic system changes from p set limits.

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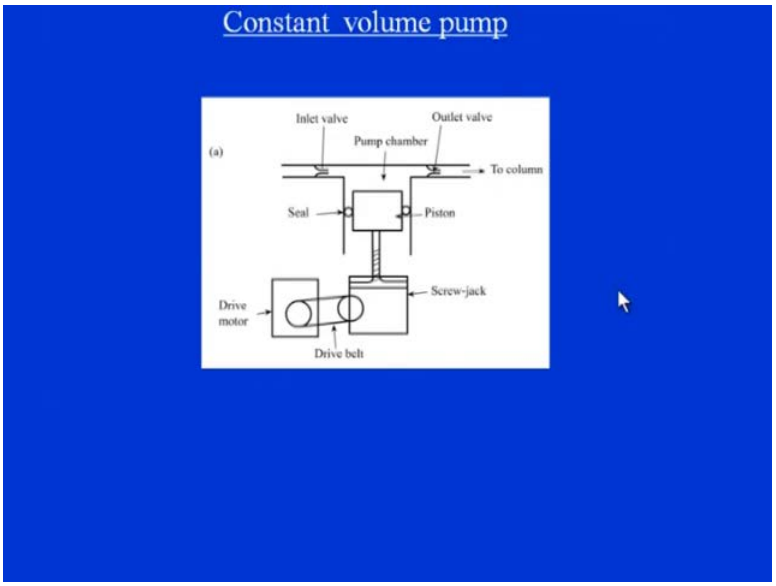

Now, here is one example is that is constant volume pump, if you can see here this constant volume pump, displacement pump it maintains a constant flow rate through the column irrespective of the changing conditions within the column. This is which is like constant volume pump, it is the motor driven pump. We can say it is a syringe type pump that delivers a fixed amount of volume to the column by piston driven by a motor.

So, what happens is this pump contains a screw jack driven by a stepper motor. So, on the delivery stroke what is done the eluent from here goes and it is delivery to the column at a constant rate. So, once it is done then these wall should be then accordingly they will be closed are open. Like say for when it has to be the eluent has to come from the reservoir this will be opened then in an next, when it the syringe pump strokes and like when it has to deliver this displace this elements onto the column this valve will be open and this will be closed. So, this is like constant volume pump and it is quite used in the HPLC systems.

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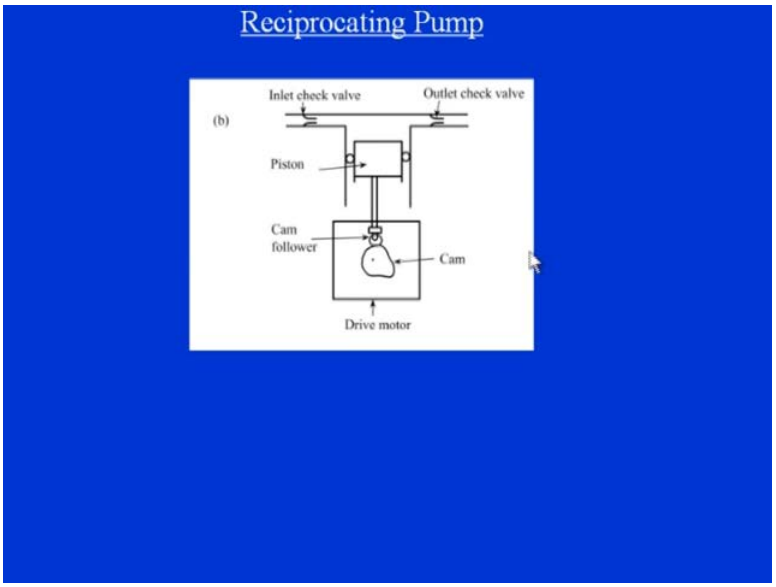

There is another one reciprocating pump. Now, in reciprocating constant displacement pump. Motorised crack drives the piston and check walls, regulate entry of eluent to the column. On the compression is stroke like here this, one driven drive motor and there is a cam crank driver, motorised cam which drives the piston. So, what happens is on compression stroke, they eluent is forced from the pump chamber.

So, eluent will be forced from the pump chamber to the outlet wall and to the column. During the return is stroke the exit check wall closes. The eluent is drawn in other entry wall that is a inlet wall. So, these 2 strokes that is compression and return stroke, one will let the eluent coming to the system, other will force the eluent into the column. So, these pumps produce a small pulses of flow and pulse stumpers as I said are employed to minimise the effects.

So, these are the two kinds of pumps which are utilised constant displacement pumps, which is constant volume pump and reciprocating pump. So, the pump here are important in the sense, they will maintained the constant flow rate irrespective of the conditions in the column. There is a cut of limit that if the pressure goes higher after certain set limit then pumps will automatically will switch off.

Another important component is detectors, there are lot of different kinds of detectors which are used. Now, the quantity of material applied to this columns is normally small and it is a very important that sensitivity of the detector is sufficiently high and stable to

respond to the low concentrations of each analyte in the eluent. Now, somehow we are taking some examples of detectors, most commonly used detector is the variable view length detector. It is a u v visible detector based on a u v visible spectrometry. It is capable of measuring absorbance down to 1 19 meter and can give full skilled deflection for, as little as 0.001 absorbance unit. Sensitivity is almost like 5 into 10 is to power 10 gram per centimetre cube and linear range is 10 is to 5.

So, they allow continuous monitoring of the column eluent. There could be others detectors like scanning wave length detector and it has facility to record the complete absorption spectrum of each analyte and display a plot on computer screen in real time. Then there are fluoresces detectors which are extremely valuable for HPLC because of their greater light sensitivity. Like in order of 10 is to minus 12 gram per centimetre per cube. Then you u v detectors, but they have reduced linear range. They could be used, but limitation is that not many analytes floras, so that the limitation so there used for specific analytes.

Then they could be electro chemical detectors which have selective for electro active analysts and the potentially highly sensitive. There are two types which is empromatic and coli metric. They have a empromatic detector have higher sensitivity and then the coli metric and these are also have a greater linear range. Then also you can attached to HPLC mass spectrometer and HPLC like here you will enable the analyte to be detected. It is structure determined by mass spectrometer, it is like mechanism like identification of different kinds of analytes and also you can see over leaping peeks here.

Then they could be like sudden times you can use n m r spectrometer directly attached to HPLC. It can again give structural information about the analyte, that is complimentary to the obtained by HPLC MS that is master spectrometry. Then they could be refractive index detectors and they depend on change in the refractive index of the eluent emerges on the column. It will respond to any analyte in any eluent and the change in refractive index being either positive or negative. Commonly used in for say analysis of carbohydrates and other eluent and other analytes.

Then there could be evaporative light scattering detectors, which realised on the vaporisation of the eluent operation of eluent and quantification analyte by light scattering could be done. It can quantify analytes in flow rates up to 5 centimetre cube

per minute, very useful for the detection of say factory acid lipids and carbohydrates. So, these were some of the detectors which could be used, but mainly it is a u v based detector, which are the most common. For say carbohydrates refractive detectors could be based on refractive index change could be utilised. Then fraction collectors is utilised to collect the fractions of the analytic purified fractions. Now, first studying in wrist analyse in the eluent is to be collected and studied further.

That could be divided into different fractions. Automatic fractions collectors which have programmed along with the whole system and they could be designed, either to collect a selected volume of eluent or to collect the eluent for a pre determined period of time. So, both way it could be done. So, this was above the high performance liquid chromatography. You could see that there are lot of changes in low pressure and high performance liquid chromatography in terms of practical size, in terms of flow rates, in terms of types of pumps utilised, in terms of a high sensitivity detectors utilised and in terms of purity and the high resolution. So, there are whole lot of differences and HPLC are widely used for high resolution work.

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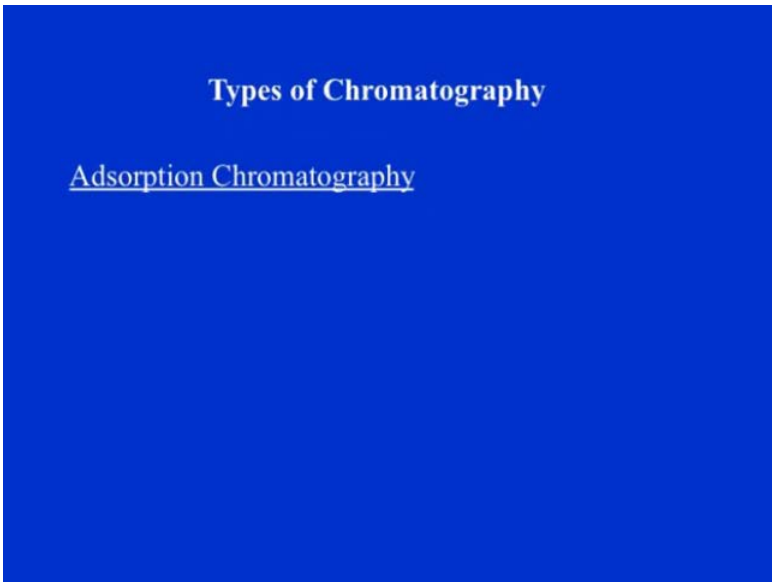

So, both low pressure chromatography or high performance chromatography, there are whole lot of different chromatographic methods have been developed for both of these techniques. That is low pressure and high performance, I will just give certain examples

here a of certain chromatographic methods which have widely used and will be also discussing some of them in the subsequent lectures.

One is adsorption chromatography. Now, adsorption chromatography is probably one of the oldest types of methods. It would utilises a mobile liquid or gaseous phase that is odd adsorb on to the surface of a stationary solid phase. The equilibration between the mobile and the stationary phase accounts for the separation of different studies. Like you can see here there is a adsorption there surface on which a material a solute will adsorb to. This is the basis of separation.

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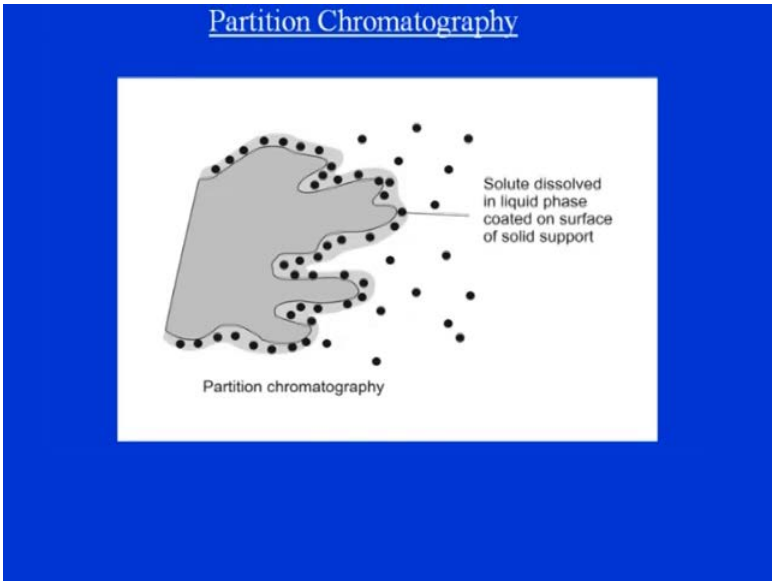

Next is partition chromatography. Now, this form of chromatography is based on a thin film, you can see here thin film formed on the surface of the solid support by a liquid stationary phase and the solute equip rates when the between the mobile phase and the stationary phase. You can see here solute is dissolved and in a liquid phase coated on surface and mobile phase passes on and this becomes basis like for example, reversed phase chromatography and other a systems.

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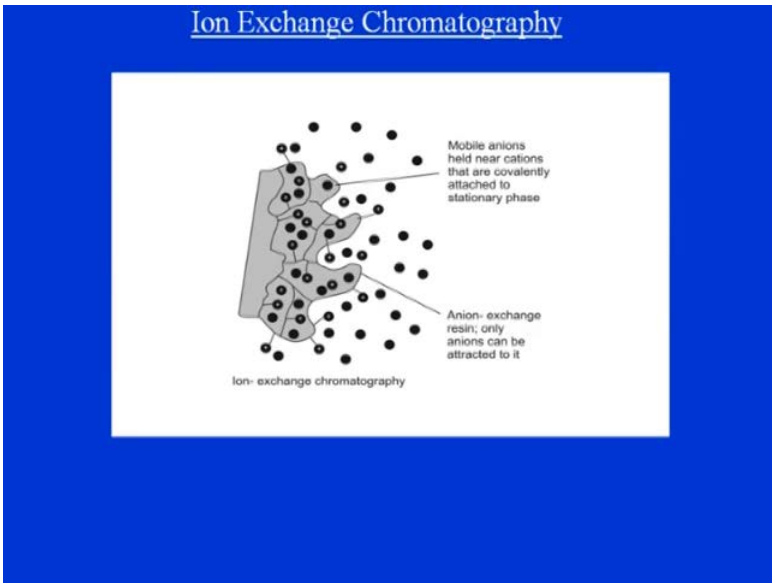

Then there is ion exchange chromatography, here what is happening you have a mobilised ion actually. Like the use of this it is a covalently attached an ions or cat ions on a resin. The solute ions of the opposite charge will we attracted will be binding to stationary phase. Then you will have to have a they will be binding through electro study force and then you have to have say salt some other eluent elevate containing those material to eluent the analyte. So, this is another important chromatography which we are going to discuss.

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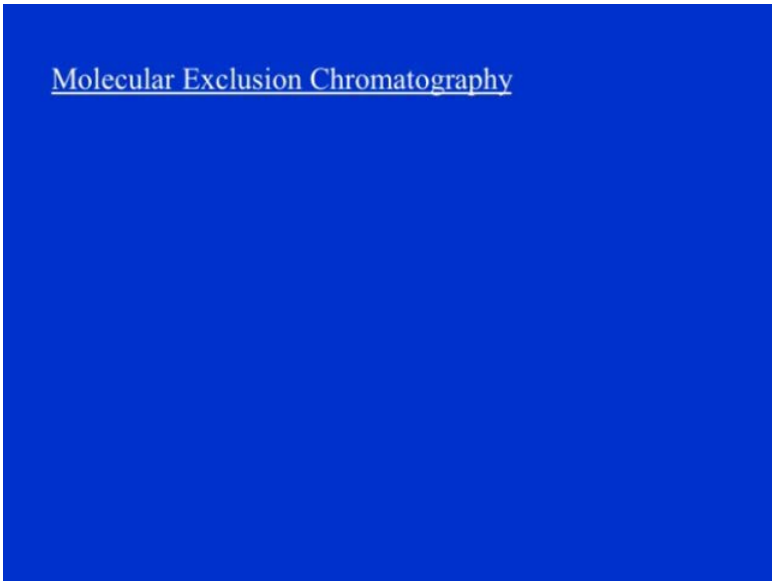

Then molecular exclusion chromatography, it is also known as jell permission or jell filtration chromatography. It relax an interaction solve of thing between the stationary phase and the solute, but what happens is here like the separations occurs through a porous jell. The stationary phase is the solvent or the mobile phase trapped in the pores.

So, pores are normally small and exclude the large solute molecules, but allows water molecules a smaller molecules to enter the jell causing them to flow through a larger volume. This causes the larger molecules to pass through the column at a faster rate than the smaller ones, because larger molecules will not be able to enter the pores and will not be retained for a longer period to prime as compare to smaller molecules.

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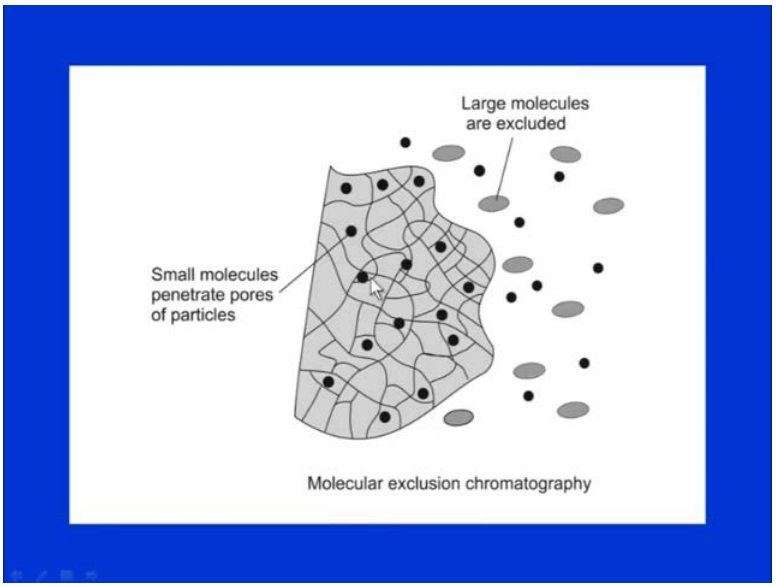

You can see the pores and these molecules are kind of entering and going out. So, they spend more time in the column as compare with the bigger partials which will not be entering these pores.

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Then there is affinity chromatography, it utilises the specific interaction between one kind of solute molecule. Second molecule that is mobilised on a stationary phase for example, they mobilised molecule may be an antibody to a specific protein that is antigen. When solute contain a mixture of proteins are passed by this through this chromatographic stationary phase, only the specific proteins will bind and others will not bind to it. So, you can see like this legend which is here and this specific protein will only bind to that legend a mobilise legend.

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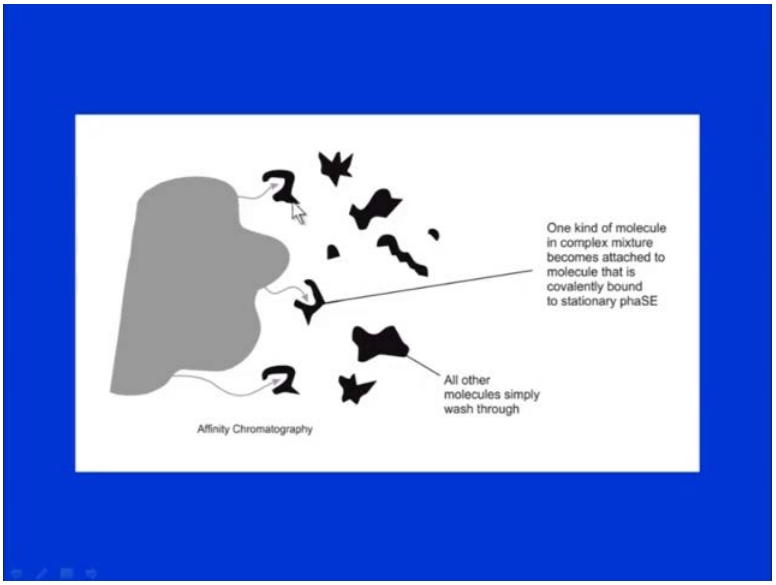

So, that is another form of chromatography which we are going to discuss.

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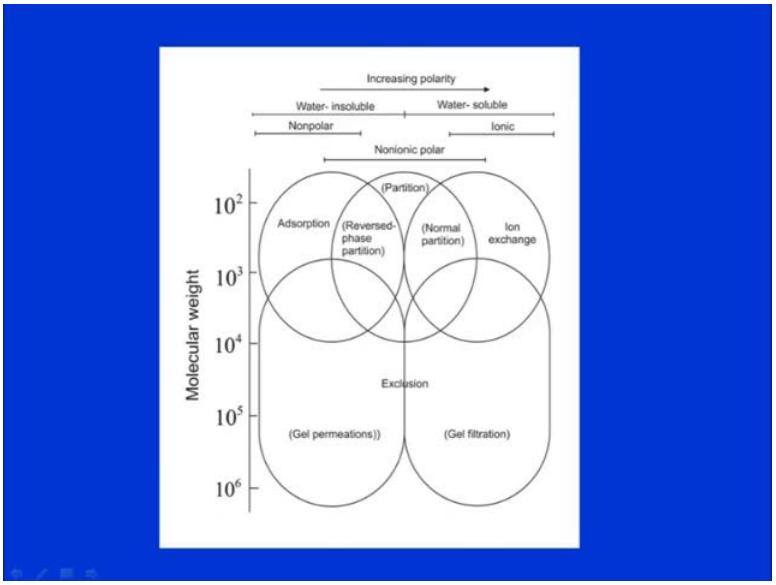

Now, if you see in this figure, it is summarises sought of things. Where you have certain chromatographic like gel permission, adsorption, reversed phase partition, ion exchange. Here you could see increasing polarity like more polar ion exchange chromatography could be utilised, for say non polar adsorption and other chromatographic system could be utilised. Jell filtration of permission can be utilised for any kind of molecule because it is legs the particular interaction of that sought.

So, this completes our section on introduction to column chromatography, where we have discuss the column chromatography or liquid chromatography and high performance liquid chromatography. We have gone through different components of both the systems, where we have discussed about columns pumps stationary phase mobile phase detectors and so on. In next section we are going to discuss, will start with is specific chromatographic method, which we are going to discuss about ion exchange chromatography, jell filtration or exclusion chromatography, affinity chromatography and also will be discussing about gas liquid chromatography systems.

Thank you.