Analytical Technologies in Biotechnology
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Module - 3
Chromatographic Methods
Lecture - 5
Gel Filtration Chromatography

Previous lecture, we have discussed about ion exchange chromatography. In ion exchange chromatography, the properties of charge which a particular analyte carries on itself are utilized for separation procedure in chromatography. Now, in this lecture, we are going to discuss another important chromatographic method, that is gel permeation chromatography.

Now, gel chromatography utilizes the molecular size or shape of a molecule for separation. This is also called, this has different names and this is also called gel filtration chromatography or gel exclusion or we can say size exclusion chromatography, then molecular sieve chromatography or even simply gel chromatography. So, this technique is a form of partition chromatography and it is used for separation of molecules like I said on the basis of their shape and size. Now, this technique exploits the molecular properties of a variety of porous materials.

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If we consider basic principle of these techniques, then different kinds of molecules which differ in sizes are partitioned between solvent and stationary phase of a defined velocity. Now, the separation is carried out using is a porous gel material or gel matrix in bead form and it is packed in a column surrounded by the mobile phase or the solvent. Now, if a like a sample is applied which contains mixtures of smaller, larger and intermediate size molecules compared to pores of the stationary phase matrix, then separation will occur as one where smaller molecules can enter the pores molecule and hence moves slowly through the column. This means they will spend more time on the column because they can move in and out of the beads more frequently than other molecules. So, the smaller molecule will rather move more time on the column as compared to other molecules.

Now, larger molecule which are too large to enter into beads due to do the pore size limit, they will be completely move to the stationary phase and hence elute first form the column and they will come what will call in wide volume. Then, there are molecules which are intimated to enter the stationary phase, but spend less time. It is you can say they spend relatively less time than the smallest molecules and more time in largest molecule. So, depending on their molecular size, there will be a kind relative of time spent in the charge or in the column and accordingly, they will be purified. So, all the molecules which are eluted, they are eluted in order of their decreasing size.

Now, let me explain these before we go into the details of these methods, let me explain in a brief and this method is to you on your screen. Now, first thing is that this chromatographic technique is based on size and shape. Then, another thing is since there is molecular size is the bases of separation, the column lands are usually long, so that the analyte needs to be separates can be separated, well as they will travel a longer distance that will allow them to be separated to a certain extend that peek over left. The second thing is that the column is mostly narrow board column or it has a less, it has lower what you call a diameter of column is less as compared to other normal columns, so that there is no diffusions and run gel filtration is run successfully. So, what you have is now the beads which are packed that are the most important part here.

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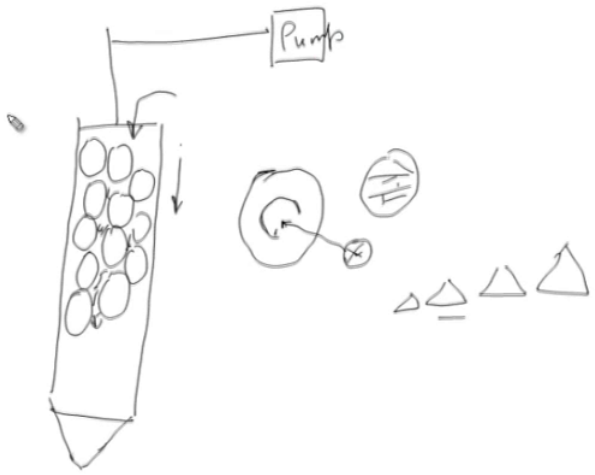

That is beads if we say if there is a column like I said and long column and with narrow board size and this column will contain beads here. Now, what is the property of bead here like what is the base the separation like we are talking about? Now, these beads, pores beads, these are hollow beads actually and these beads if I make it single bead, then these beads are totally hollow inside. And they have core actually or means this is just schematic to tell you these are cross linked polymers.

They present a gel like structure and they have particular porosity because of these cross linking, porosity just like to make you understand if this is the pores size, then what will happen? A molecule which is bigger than this pore size, if I said that this is the molecule, then this cannot enter here, but a molecule which is smaller than pore size can enter to the pores. This can go into, it can access the inner volume of this bead which is the trapped solvent or trapped mobile phase, but the larger molecule cannot enter. So, what happens then? That larger molecule will not be able to spend time. It will only spend time interstitial space or the spaces surrounding the stationary support material, in which the stationary phase contains. So, these will come very fast, but the smaller molecule will spend more time because it will go back and forth.

As it travels back in forth to the column, it will move back and forth into the stationary phase and then mobile phase. So, if you compare different sizes of molecule and if I say there is one size, there is another size, there is another size, there is larger size, then all of

these molecules according to their molecular mass or molecule weight, they will spend relatively different times; smaller the longest time, the largest the least time and in between, they will be expanding times as per their size.

So, that is how the separation occurs because they are moving down, you have a continuous flow system and they are moving down at different speeds or at different rates actually, which are eluted different rates. So, this is a very easy principle of column chromatography, of gel filtration chromatography. Now, these columns like I said are long and they are narrow board, so they could be for low performance, coarse beads or bigger beads and for high performance, liquid chromatographic, finer beads which could be packed very nicely. Now, this system mostly can only, cannot be run by gravity feed. So, it is required here if I have further things, you required a pump here which is a very important part of column so that it can constant flow rate. So, this is a pre requisite that you should have a pumping system to run column.

If you certain like you use you can put reservoir sort of over the column, but that will not give you a constant flow rate. So, this is a very in a simple method, I tried to explain the principle of this gel filtration. Now, let us get into our discussion or detailed discussion. So, what we are talking about was, what we were talking about that the smaller molecules can enter the pores, hence they spent more column and the larger molecules are excluded. So, larger molecules are excluded in wide volume and dead volume, which is the total volume of the column minus the volume occupied by the beets. So, since not getting inside the beads, it will be excluded.

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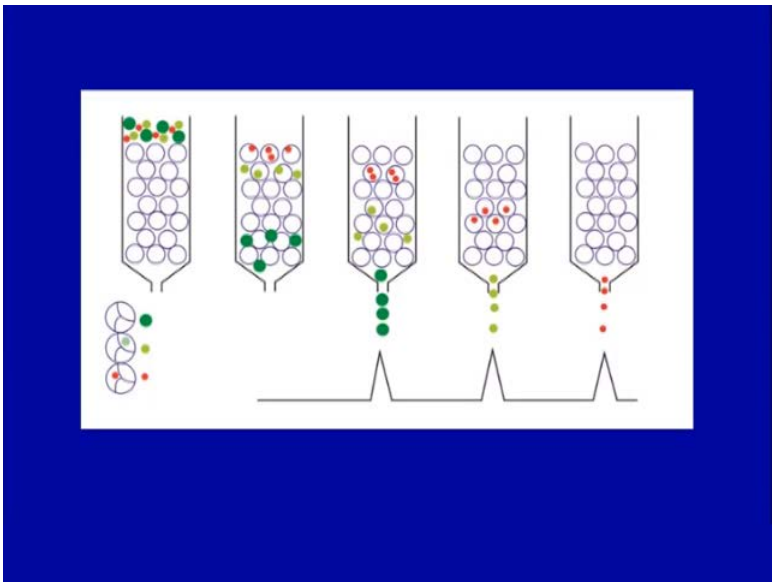

Now, here is another figure which explains it the same principle. If you can see here these are, this is a column where the beads are actually and you have different size molecules. These are larger size, intermediate size and smaller size. If you can see them, they are moving at different rates; larger moving out very fast, intermediate moving at relatively slower speed than the larger ones and the smallest ones will move at the slowest moves.

As the column runs and as the time passes, the first elute is the larger one and you get a separate fraction. That is how we can see clearly the next one. You can see how the peek resolution can take place if their molecular sizes are farther apart, far apart. They could be easily separated into different fractions and could be utilized for different applications as a purified product.

Now, what I was trying to say why you need a long column? You need a long column as they start single entity when you load these columns and as they move, if you have smaller column, then the separation column not really it is quiet separated quite a lot. Their separation will be not complete and they will have you can have a lower overlapping peeks. So, as long as the column is narrow board column for avoid diffusion, you will get better separations could be achieved in gel filtration chromatography. So, this is a very in a very simple manner, it explains plain the whole bases.

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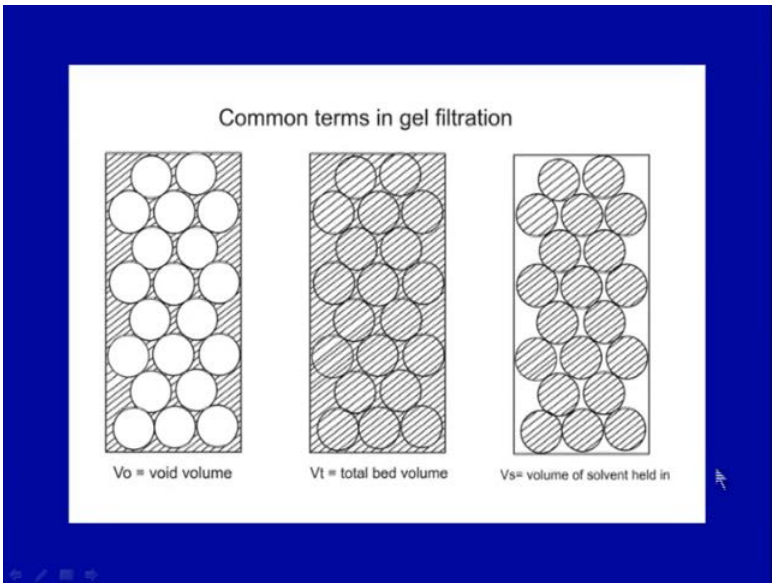

Now, if I have to say like what are different terms in terms column. If you see here the shaded part here, this is called the void volume. It is the volume outside the support matrix. This is totally volume like it is taken both the volume occupied outside the beads and the inside the beads. That is the total bed volume and then you have volume, which is inside the stationary face and that is called Vs, or volume of the solvent held in the beads. So, that is the kind of distribution or you can say that is how the void volume, the totally bed volume and the volume occupied by solvent inside the support beads is described.

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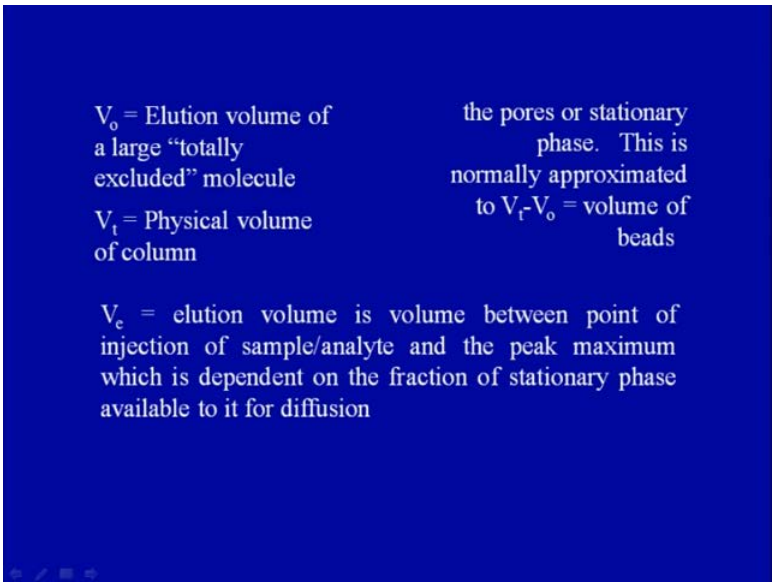

Now, here the elution volume of a large totally excluded molecule will be given by wide volume. Vt is physical volume and other like I said is the, you can divide you can sorry subtract the total bed volume by the wide volume, you can get the volume of the beads. Now, elution volume, there is another term called elution volume and that volume is the volume between the point of injection of the sample and analytes at the top of the column and the peak maximum, which is dependent on the fraction of stationary phase available to the analyte for diffusion. So, that will be like elution volume is the volume which is required for eluting the particular analyte and that will be starting from the point where you inject the sample and the point where a peek appears or that analytic is eluted.

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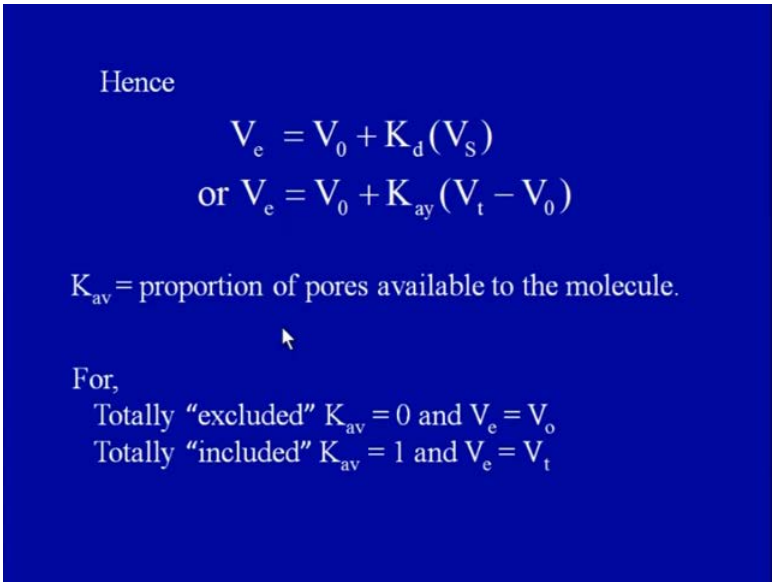

So, if you consider here for these equations here, Kd is of course it is a distribution coefficient and Kav is proportion of pores available to the molecule and both would be used. So, for a totally excluded molecule, since Kd or Kv will be 0 and so the elution volume will equal the wide volume that is for completely excluded molecule. The totally included volume will be it is like there is no fractionation happening and elution volume would be equal to the volume.

So, the intermediate would be separated, in between, they will have certain values and being 0 and 1. So, the total bed volume can be measured by determining the by elution volume of a small molecule that is which is not in the fractionation like salt or acetone,

they could be interlinked. Since, they have complete axes, they can easily move in. So, they just move in the mobile phase then the most time and that could be calculated.

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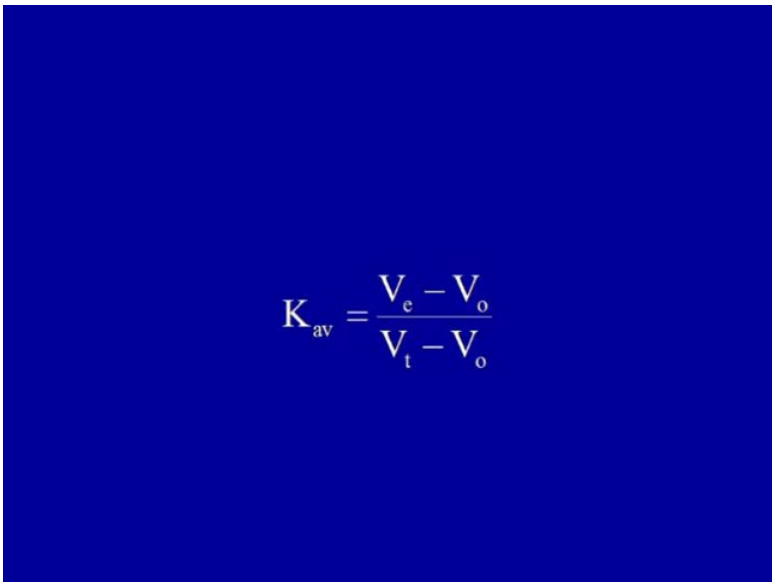

Elution volume could be calculated the utilizing a very small molecule.

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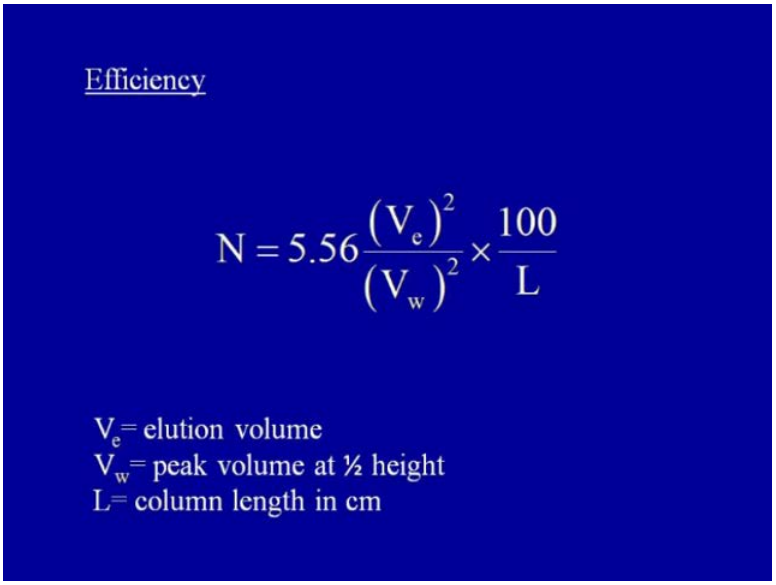

If we can consider the efficiency of a column or a gel filtration column, if you could recall we were talking about the theoretical plates actually. Theoretical plates, more is the theatrical plates, better is the resolution and better is the separation of the wall of column. So, here efficiency is determined by calculating number of theoretical plates per

meter. You can, this equation can be utilized for calculating the theoretical plates where Ve is elution volume, Vw is the peak volume at half the time peak and L is the column length.

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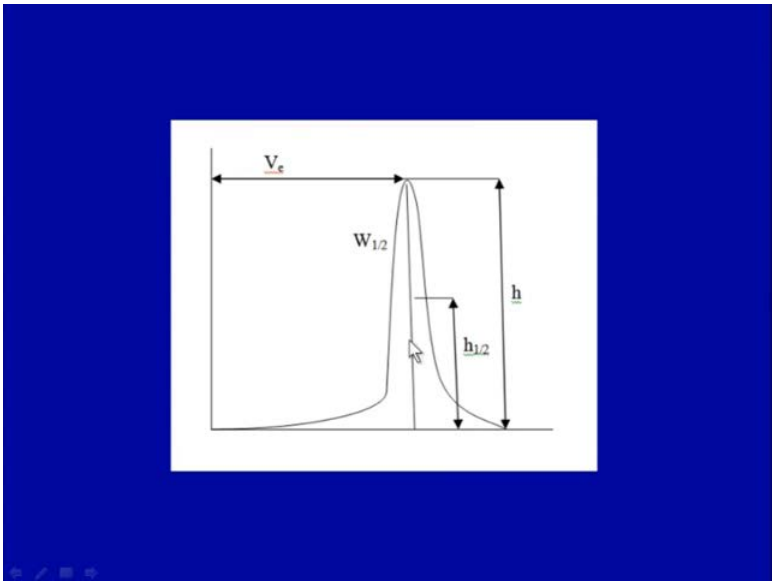

So, it is like here, half the height of the peak and this is the elution volume. So, you can calculate the theoretical plates. Remember if you compare low pressure and high performance liquid chromatography, though the length of the high performance liquid chromatography columns are not that long. But they still contain more of theoretical plates because the material, which is packed to the stationary phase or support matrix is much finer material.

So, they can pack in many or more material in a small area. They were providing more number of theoretical plates and enhancing the resolution at the separation power of the column. Let us discuss all different aspects of the gel filtration chromatography. First is gel permeation media. Now, when you take the media, there should be certain properties of this media that is column material. One is that this should be completely inert with respect to the molecules being separated. If it is not inert, that non specific binding or absorption might be a big problem.

The media or the column material should be completely inert for better separation. There are a lot of different kinds of media or the gel material, which are utilized. If you could recall, we discussed about this.

As we go along in terms of gel filtration, there could be lot of different support media gels available like dextrin. Dextrin is a homo polysaccharide of the glucose residues and it is prepared with various degree of cross linking to control pore size. If you remember here, the sieving effect or filtration effect, it is a kind of filtration which is taking place. So, pore size is very important as on particular pore size of the gel material, the different kinds of molecules will be separated.

So, it is like cross linking is controlled for pore size. It could be obtained as dry beads or beads were when water and it could be the beads, beads can be spelled and when water is added we will see how that is the name, this trade name is sephadex. It is mainly used for separation of small peptide and develops proteins which are small to average molecular mass. Now, when we say we have swell it and the swelling is done like you take the dry beads which are supplied by commercial, commercially.

Now, those could be put in the water and buffer initially in the water and left for certain period of time and say you can leave them over night or so and let them swell. The swelling could be observed by you can look into the under the microscope and you will observe the un swelled and swelled beads could be clearly observed. It could be enhanced by you can put these beads with the water in an oven at higher temperature. They will swell faster. So, that could also be done.

Then, there is poly acryl amide. These gels are prepared by again cross linking with the acryl amide with a clean the acryl amide. The pores size is determined by the degree of cross linking. The separation properties of this material are mainly same as the others like dextrins and they are supplied. For example, bio gel p, they are available in wide range of pore sizes. So, you can isolate or purified raise of material or analytes.

Then, there is agarose. It is a linear polymer of dialectics see three and hydro one electrodes. It forms a gel that is held together by mostly hydrogen bonds. The concentration of the material in the gel determines the pore size. So, the pores of the agarose gel are much larger than those of bio gel p and it could be utilized for separation of large globular proteins or non liner molecules such as DNA. Dextrin and agarose beads are biodegradable. So, many times, you have to use microbes like sodium acetates for storage.

Another important part as we were discussing was large globular proteins and long and linear molecules like I said this will also affect like shape of the molecule will also affect separation. Remember when we consider these things will ideally takes spherical shape, but may be a two molecule of the same molecular mass, but different in shape like see like a molecule which is spherical, which is elongated.

Elongated molecule will certainly will not be able to enter the beads as compared to the same molecular mass is farcical material. So, those things have to be, has to be taken into account while purification. So, all when you are externalizing things most than the spherical molecule. So, when you are considering gel matrix like different materials we have discussed, there are certain factors.

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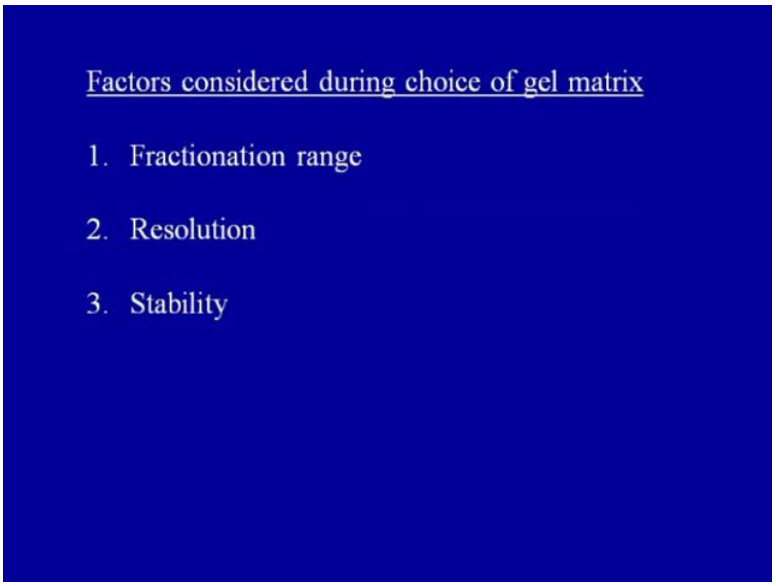

They need to be considered while you are choosing a gel matrix. One is the fractionation range that is if all proteins in a mixture are to be separated from relatively low molecular weight solutes like say you have molecules are large molecules. And you separate a smaller molecule from the mixture, you have to separate your smaller molecules.

Then, you can have small packing with the small pores to gel material and this kind of a process is called desalting. The proteins are total excluded because you are using gel material which is of very small pores size. So, your material or protein does not enter and smaller molecules will enter and you will be able to easily purify. For example, Sephadex g25 or bio gel p 6 or p 10 could be utilized for these purposes. For resolution

of different proteins which are more closely related in size, there is a range of size actually.

Then, you have to take a matrix, where it is like molecules do not get excluded in wide volume or they are not eluting in wide volume. So, there is fractionation range of the gel that is of a particular pores size and it allows all the analytes to enter; unless you want specifically one of them, it is excluded, from the excluded and does not fractionated actually. Then, you have considered resolution. For higher resolution, smaller beads should be utilized. They will give better resolution because molecular diffusion is slow, there is lower is less zone broadening.

However, smaller particles give higher resistance. So, you have to use a high performance chromatography or very good pumps for that purpose. Rigid and larger beads allow faster performance and times they are used for large scale application. So, this is another factor that what kind resolution your seeking for, and you can use low pressure chromatography or high performance chromatography.

Now, another factor, very important factor is stability of the matrix. The matrix needs or should be stable at different ph values or in different buffer and organic solvent conditions because you are going to use buffers of solvents during the run and the matrix needs to be physically fit for these solvent. The matrix needs to be perfect; necessarily proteins or any analysts which needs to be purified should not be affected by this particular problem. So, this is another important part that is stability. Now, if you consider this instrumentation of gel filtration, it is a very simple, a very basic sort of thing as we have already discussed earlier in for low pressure chromatography and again for high performance liquid chromatography.

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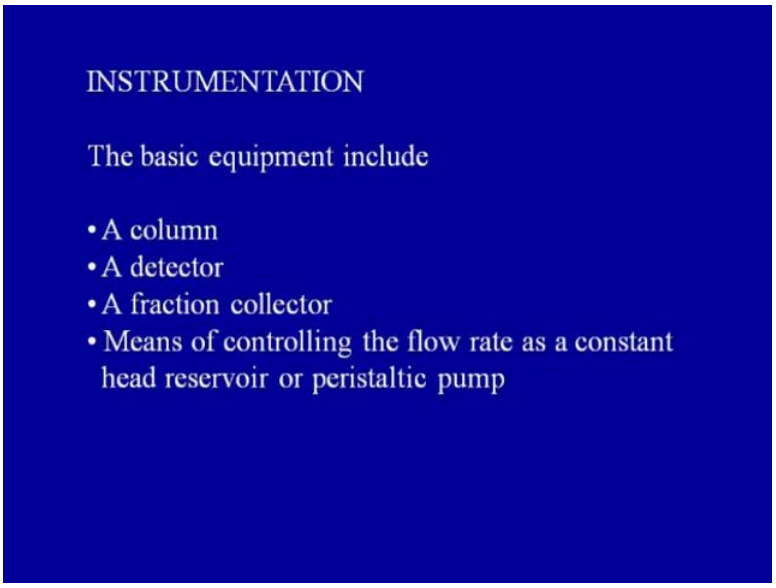

In instrumentation, the basic part is you require a column which is like I said it is a long column and narrow board column. You require a detector so that you can go through the peeks which are coming and you can see the purification visually. Then, you require a fraction collector to collect those fractions or different peeks separately. Then, this like flow rate is to be controlled and it is mostly like you can constant head reserve, but that is not very suitable. What you require a pumping system. For low pressure, peristaltic pump, for high pressure, it could be like constant displacement or other pumps could be utilized.

Now, the system should be arranged in the monitor connected as close as possible as to the column outlet. This is to prevent mixing the longitude mixing in the tubing. Similarly, connecting tubing and flow cells should have narrow dimensions to prevent mixing. So, that is a very important part the column needs to be like when you are purifying these. The aim of the gel filtration is to obtain the greatest possible resolution of the molecules of interest.

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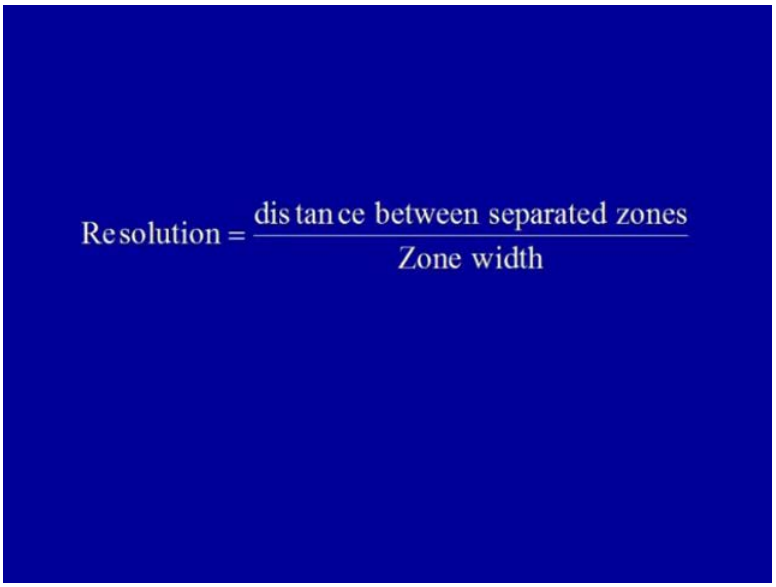

The resolution is improved by the distance between the separated zones and the zone divided by zone width. So, what you have is like I said earlier longer the column, better is the resolution because you give more time for the analytes to separate. So, when they travel through the mobile and stationary phase and the smaller columns, the peaks will overlap. But, as the columns length goes up, then they will have more time to separate from each other and separate peeks could be obtained.

Then, columns if you have longer columns up to 100 centimeter columns could utilized, the internal diameter like I said, it is very smaller like 1 or 1.25 centimeter or so. Mostly, what is done is it is taken if you take, then the length should be twenty to forty times longer than the wider. So, that has to be, you have to have longer columns, smaller diameter, internal diameter of the column.

When you are preparing the gels for column the gels for chromatography, what is commercially provided is and then either you will get dry powders like sephadex, or others and or you can get the swelled or in certain medium liquid medium that particular matrix. If you are provided with dry powders, then dry powders have to be kept in a beaker or container. It has to be swelled in the water like I said you swell it overnight, or you swell in oven which could be faster. And you can check for whether beads are swelled uniformly by observing them in microscope.

Gels which are supplied will be pre swollen like sephacryl and others. What you have to do is you require is that you have to exchange them with the buffer which is the proper eluents buffer for use. Then, when you are again restoring them again, you have to store them in particular solvent like methanol or others for long term storage.

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So, this is very important. Now, packing is another important part of running a column and here packing of a gel filtration al column is not that easy if you compare to the ion exchange columns. Ion exchange columns are smaller columns and you do not have to worry about length and width, the diameter so much. But, here these are long columns and it is a narrow or a smaller internal diameter. You have to very carefully pack the column so that column is packed uniformly without here bubbles without any gaps or like say, they have certain cracks so that run could be proper.

So, the gel should consist of slurry when you are packing these approximately 75 sector gel and around 25 superannuated liquid. Now, suspension will be done at constant operating temperature like I said that if you have different buffers can change the ph and even there could be certain other properties changed due to the temperature. So, that has to be taken care. Like for example, like if you take it suddenly take to a cold room conditions certain things or could shrink. Those could be other problems. So, suspension should be constant before and during packing. That is very important.

So, if you want to do the experiment in cold room, then you can swell the gel in cold room, swell the gel first and then pack it. Now, air bubble formation and other things should be prevented by column packing and which can be monitored by using color test samples such like for example, blue dextrin. Blue dextrin could also utilized for determining the void volumes as it will not enter the beads and it is completely excluded from most of the gel matrix.

Now, the packing is mostly done with pump like so you have all the material is in particular container or what the column it is packed marry flask. It could be connected to a pump and it could be packed properly. Manual packing might not be proper good or idea so it has to be packed with peristaltic pump or other pump at constant flow rate. It is packed properly. Remember low pressure beads, if you put too much of high pressure, they will flatten and then no separation will take place. So, that has to take care that they are packed properly. They are not subjected to very high pressure which could be like totally damaging them and no proper purification will take place.

Then, the choice of eluent for running the column is important and when you are choosing an eluent, it should be the one which can make your analyte stable. You can add various co factors or metals or other things without any problem, without any interference. So, that is very important.

If the sample is to be freeze dry, then you can use volatile buffers like if you say liaflize the sample after the purification, then if you use directly use velocity buffer, you really do not have exchange the buffer later on. Like ammonium bicarbonate could be utilized or water is volatile or other buffer could be utilized. That is a very important part. It is used to utilize. Now, one of the most important parts of gel chromatography is sample size.

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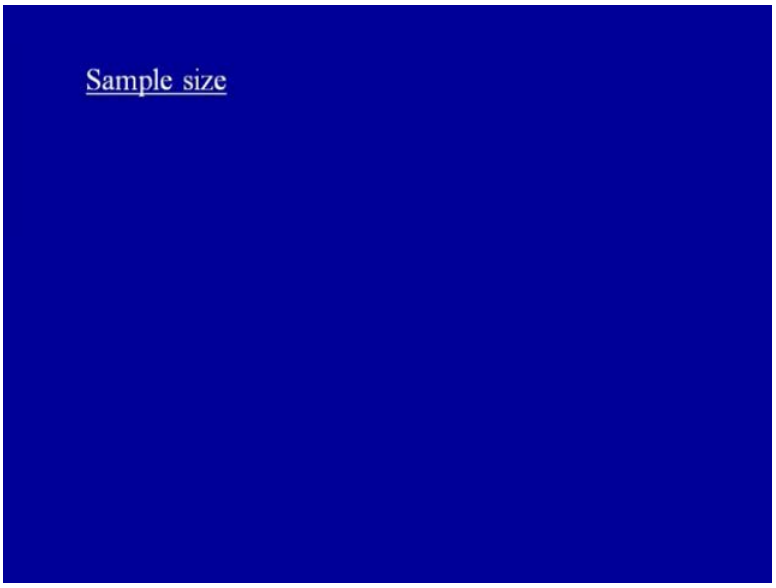

Now, one of the most important parts of gel chromatography is sample size or how much amount needs to be loaded on to the column. Now, sample volume should be as small as possible and must be lower than the separation volume between compounds in an order to get higher resolution. Typically, it should be 1 to 5 percent of the total void volume.

So, larger volume will not give you separation. I will tell you why. So, you have to load the sample into in higher concentration, but maybe what you call smaller volume actually. For group separation where you are not so much worried about separating analytes, you can maybe use little larger volume. So, when you sample when you have is when have a gel filtration is dependent on sample mass. So, high concentration could be utilized here without any problem and you can get high resolution. However, the concentration can be generally limited by the viscosity.

If it is a too viscous sample, there could be a problem relative to the eluent. So, high viscosity will result in its zone stability and in irregular flow pattern. So, the protein concentration of the sample applied to the column could be around 10 to 20 milligram per ml. Now, let me first tell you why the sample volume is very important in here as compared to other chromatography techniques where larger volumes could be neutralized in the column. Now, let us understand this.

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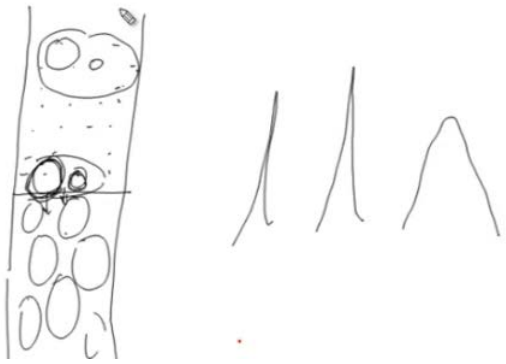

Supposing you have a column here, this is your beads here in the column. I am just trying, this is a schematic, this needs to be longer column. Now, this one you have your sample say if I have a larger volume of sample why I am saying you require a small volume and you have a different size things here. Now, what happens is that when you have a larger volume of column, this bead here which is entering in the column or this large size analyte, which is entering the column and this analyte is entering at the same time.

So, they will be separated accordingly. But, what happens is this, when they have entered here in this beads, this will pass through interstitial space. This will go through this into the bead and expand in certain time, but there will be time gap between these two entering and these two entering actually.

So, what will happen? As this one has traveled, but it will travel further, but this one since this is like this addition to the void volume, if suppose this is excluded in addition to the void volume. So, there could be a problem when you many analyte of peek overlaps or peek broadening because peek broadening will not get a sharp peek or this sort of peek rather you will get a spread peek. What is the problem? It is because this will be entering in different times. So, the purification will not be proper.

So, it is very important that sample is applied in a very small volume so that all of them, all analysts almost enter the gel at the same time and then they separate according to

their retention capacity or retention time volumes as per their molecular size. So, that is a very important factor actually which needs to be looked into. So, this is another part which is important.

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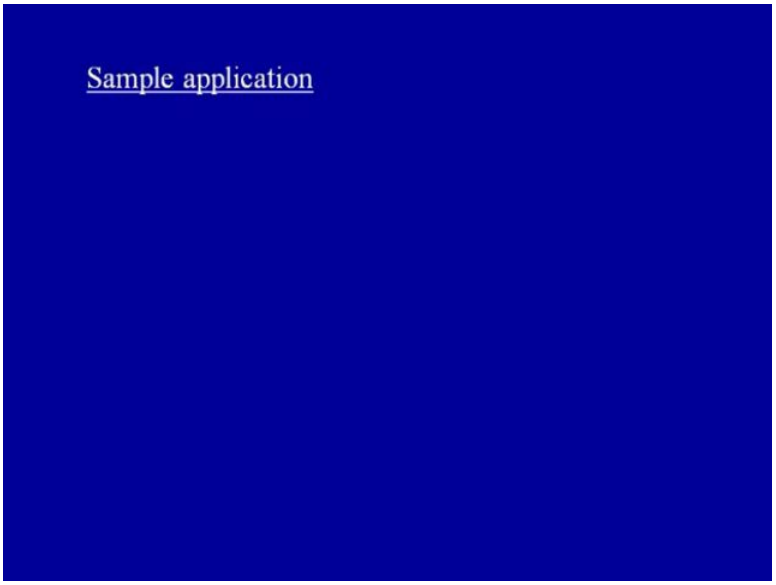

Now, when you are applying sample application here, sample application as we have discussed in ion exchange chromatography, here also you have to take a lot of when you are applying the sample. So, care should be taken when applying the sample to the surface of the bed and one is that the bed should not be disturbed. If you are disturbing the bed, then again you have to wait so that it settles down and it might settle down not so uniformly.

Now, when samples needs to be applied, first is you can remove the or drain the bed surface on the top without disturbing the bed surface and then slowly, very carefully load put your sample on the top of the bed. Then, you can layer the sample under the eluent. If it is viscous like see high concentration proteins will not unless you mix very harshly, they will not mix and evenly it could be layered in the eluent like I have told you in ion exchange chromatography technique also. You can use a loop injector or three ways wall could utilized for this purpose as we have discussed in high performance liquid chromatography. So, they could be different ways.

So, if you are applying it like mainly, then the above plunger needs to again mobile phase needs to be put in after the sample has entered the bed or the stationary support

material. Then, you can layer the mobile phase and close the column and connect it to the pump. So, the maximum resolution is obtained like I said using long columns, using slow flow rates and using concentrated, but less volume of the samples.

Optimum flow rate could be like 2 ml per centimeter square per hour, but it could go very high for spectral and like 30 ml per centimeter square per hour. But, remember for low pressure chromatography, if you use higher flow rate, then beads could be flattened because of much higher pressure. So, those things have to be taken care of. Once you have utilized column here, analyte are done, you re equilibrate the column for next run.

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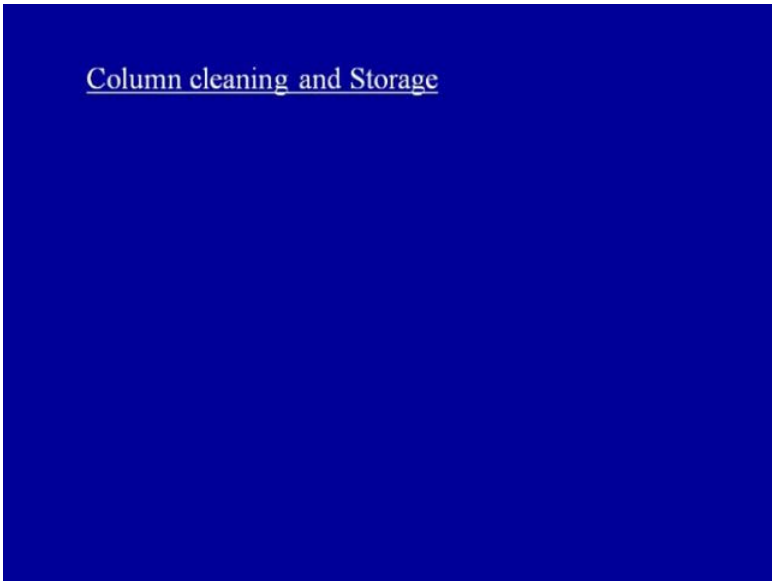

Otherwise, gels maybe cleaned up. You can treat with 0.2 molar sodium hydroxide or certain non ironic detergents, clean it up and then you can store it in the presence of anti micro will agents like sodium aside which could be put in very small amount, say 20 percent ethanol also be utilized. So, this way you can easily store your material. So, we will see the bases and mechanism and working of the gel filtration chromatography.

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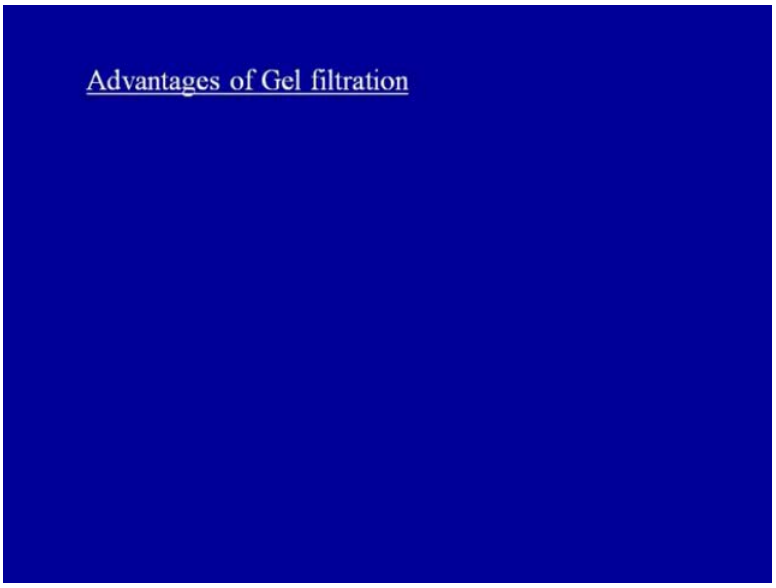

Gel filtration chromatography has lot of advantages, a lot of applications. Now, before like when you are utilizing this method, it is the best method for separating molecules, which are differing in molecular weight or molecular size. So, here like temperature, ph ironic strength, buffer composition etcetera will not be making a difference. Like in ion exchange, you have to have a particular buffer condition of ph condition because they will be charged accordingly. Here, only you have to worry about whether your analyte or protein is stable.

So, that is the important part and separation can be carried out under any conditions mostly iso critic flow like could be in the presence of ions or co factor detergents and other particular conditions could be utilized. There is very little adsorption because this are very inert columns materials, it is less zonal spreading. Then, other techniques, elution volume is to the volume weights like I said, so this could be utilized for determining molecular mass.

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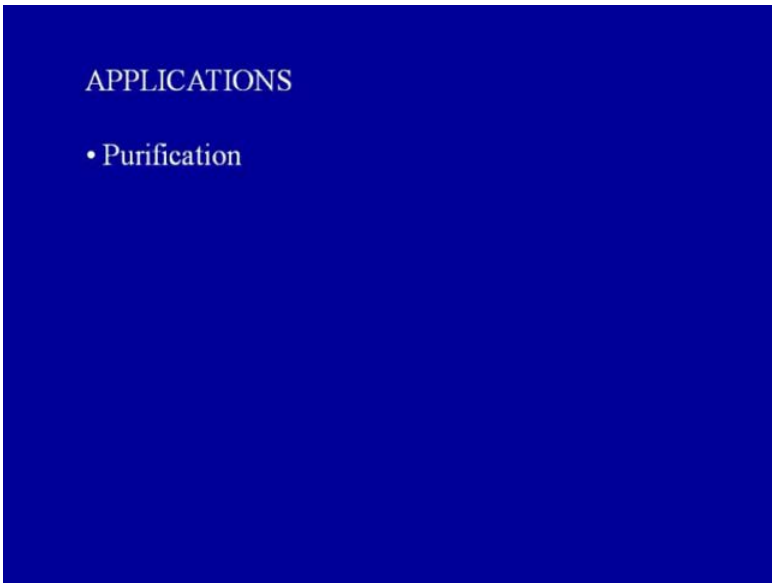

If you consider the applications of gel filtration chromatography, there are many applications of gel filtration chromatography. Let me list you some of them. One is that like we are discussing purification. Purification is the most important application of gel filtration chromatography. Now, it is like main application and you can separate them on the bases of their shape and size proteins, enzymes, hormones, antibodies, nucleic acids, polysaccharides, many more could be separated or purified on this chromatographic column. In general, gel filtration is considered a low resolution chromatography, and it is not the first step in chromatographic procedure when you purifying an analyte rather it is the final step in certain cases. It depends from case to case you can use it before; also mostly it is the final policy step.

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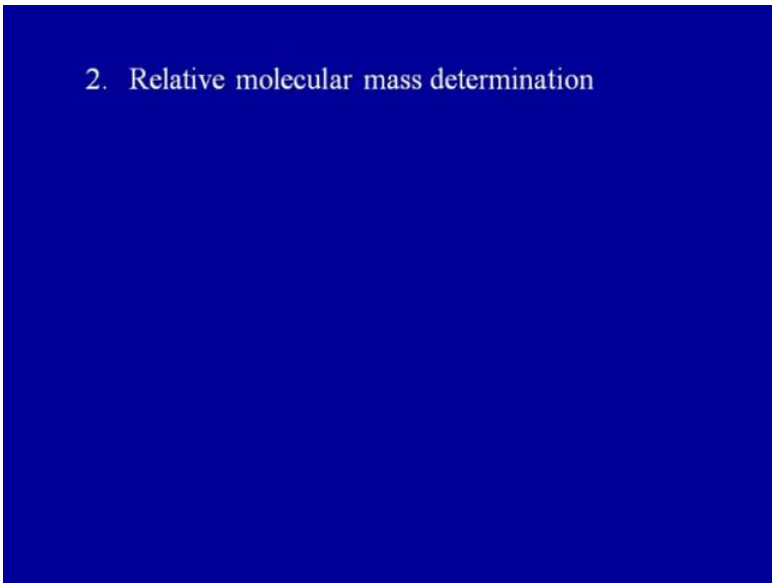

Relative molecular mass determination could be performed on gel filtration column. So, what happens is like I said elution volumes of globular proteins are a characteristic value and they could be determined by their relative molecular mass. So, if you run standards, certain standard proteins, which you will know the molecular mass and you will know the elution volumes, and then you have a standard graph plotted.

Then, an unknown sample could be run and through the calibration curve, the proteins of similar shape or like spherical proteins ideally, their molecular weights could be determined. So, that is a very good technique determines the molecular mass. Only thing is that all the standard conditions, which have been maintained for running the standards, need to be maintained for running the unknown sample.

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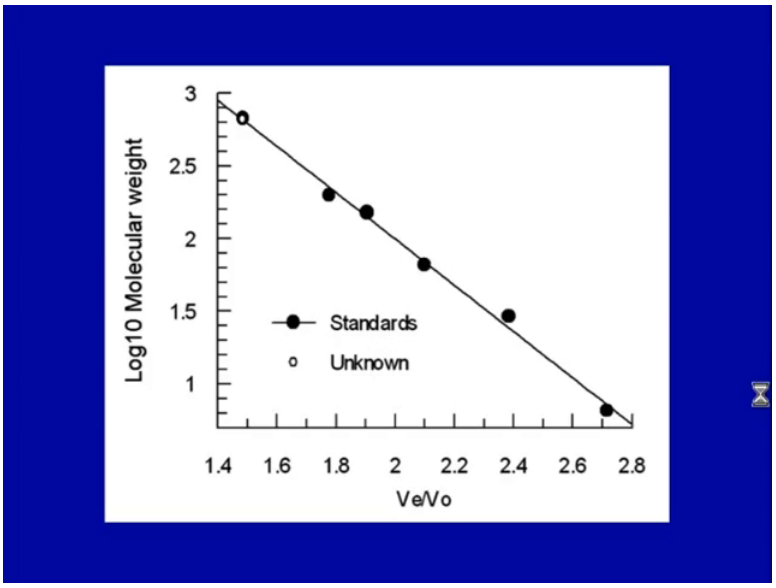

So, this is how you can plot log of molecular weight and the elution volume. There will be a plot. From this plot, you can determine the unknown sample. You can concentrate certain samples here; say you have purified sample or other. So, what is the done is sample of high molecular can be concentrated by simply adding sephadex g 25.

Now, earlier as I told you, this is not purification, it is a kind of salting. So, what will happen when put the dry water? It will swell and absorb a lot of water and so low molecular substance will be in the gel, but high molecular subs will excluded and they remain in solution. So, after a certain period of time, you can add very low speed and you can centrifuge them and you get the solution or the solvent, which contains high molecular weight analyte. It is concentrated because a lot of water is absorbed by the material.

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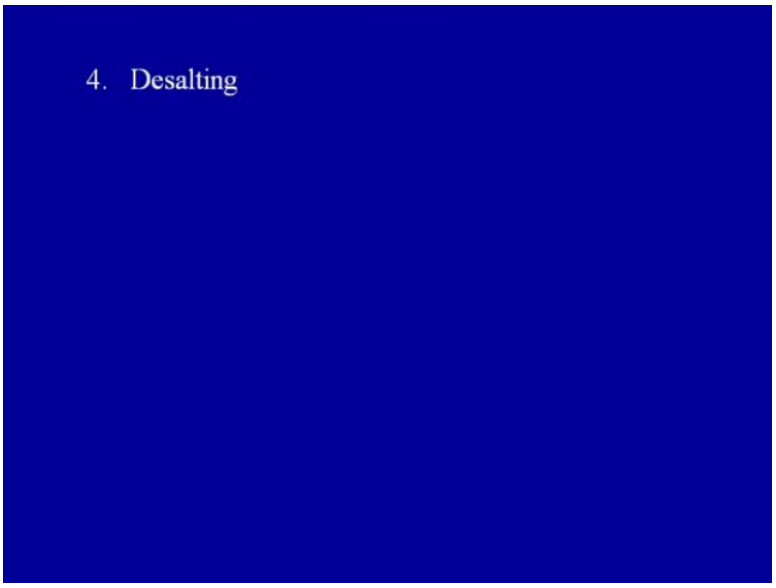

This could be utilized. It is frequently utilized for desalting. So, by the use of sephadex g25 solutions, high molecular mass compounds may be desalted where you can pre swell the gel in this case. So, the high molecular mass substance will just move in the wide volume whereas the salts will be distributed and will be in the stationary phase. So, this could be another important part. It is very fast and more efficient effect than dialyses which is a little cumbersome.

There are a lot of applications of this particular method. You can remove like for example, removal of phenol of nucleic acid preparation, it could be utilized, it cud be utilized for removing of ammonium sulphate form, protein preparation, it could be utilized for removal of salts forms samples eluted from ion exchange. Like for example, ion exchange will contain a lot of salt and to take it to the next level, you need to remove the salt in certain cases.

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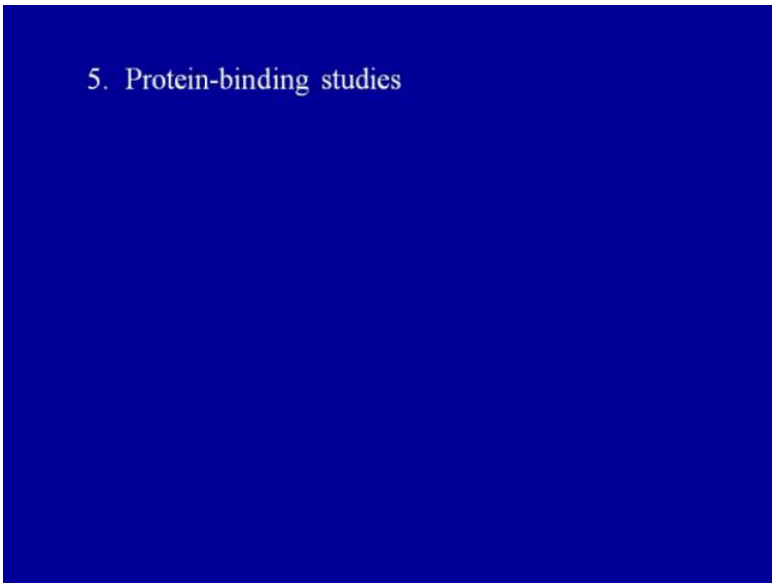

You can do protein binding studies in here. Protein binding studies like you can use gel filtration to study reversible binding of a ligand to a micro molecule. There are a lot of combination like receptor hormone or proteins and anisettes. Likewise, it could be utilized, body antigen and this could be like when will a separate ligand and protein, they will a different elution volume.

But, when they combine and if they are interacting with each other, then elution volume will be different because they will eluting earlier as per increase in the molecular mass. So, there is a procedure and you like go through that and even experiment could be repeated as the series of ligand concentrations and you can appropriately, the binding constants can also be calculated. So, it is a very useful technique.

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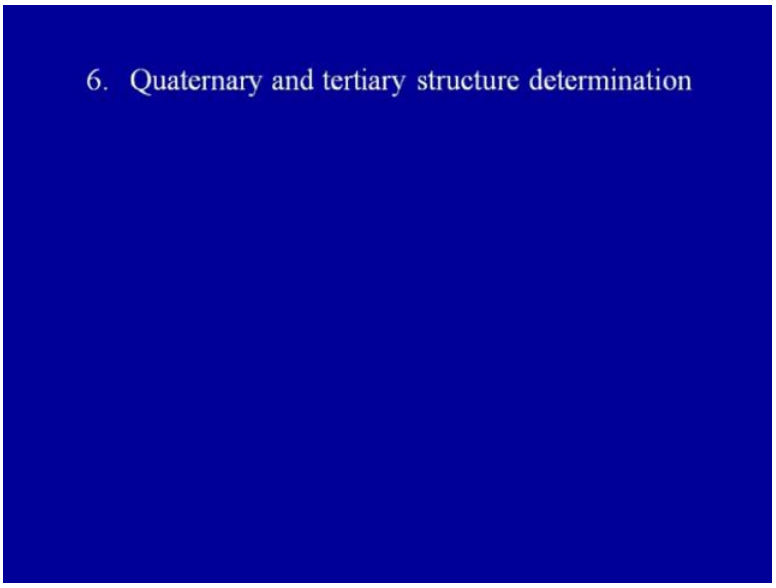

You can also determine quaternary and tertiary structure here. So, what is done? when you have purified proteins and if you run through the gel filtration to column through elution volume or through the retention time, their hydro dynamic radius of typical protein will play a role and there will so change in elution volume. So, two forms could be separated as well as you will have an indication certain change in the quaternary and tertiary structure of structure a particular protein.

So, that is there another way. Many times in polymer synthesis, it could be utilized are measure size and the poly dispersity of a synthesized polymer. So, that is ability to find the distribution of the size of polymer molecule and if standards of its own size are run preciously, then the calibration can be created to determine the size of polymer molecules of interest in the solvent. So, these are different applications of gel filtration chromatography technique.

So, as you see here in this, this is a very useful technique and it has many applications. It is on bases of the size and shape like I said ideally we talk about spherical size, but if it is elongated shape, then certainly same molecular mass protein will be excluded faster than spherical protein.

So, this is very nice technique can be done in low pressure chromatography or high performance liquid chromatography with different types of columns with more number of theoretical plates as finer particles are packed. It is like one of the techniques where

you do not have to worry about certain factors, physical properties except for size here like ph or other thing. The only thing to worry about is stability of the protein in a particular buffer condition. So, this completes gel filtration chromatography. In the next lecture, we are going to discuss about another different technique, which is affinity chromatography.

Thank you.