Analytical Technologies in Biotechnology
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Module - 4
Electrophoresis
Lecture - 3
Native gel electrophoresis and SDS-PAGE

In previous lectures, we have discussed about general principle of electrophoresis. We have discussed about the factors that affect a successful electrophoretic run like electrophoretic mobility heating tool heating and the electro osmotic flow. Also, if you could recall, we have discussed about horizontal and vertical gel electrophoresis. The two most widely used matrixes are agarose gels and poly acryl amide gels. Agarose gels are mostly utilized for separating nucleic acids, where as poly acryl amide gels are mostly utilized for protein separation. Poly acryl amide gels could be utilized for DNA separation that is of very small DNA fragments.

Likewise, proteins could be utilized for the agarose gels could be used for not really protein separation, but where you can have certain analysis say immune electrophoresis or iso electric focusing where sieving effect is not required. So, there protein could be analyzed. So, we will continue our discussion on electrophoresis and extend our discussion from where we have left. So, if you could recall we left at the vertical and horizontal gel electrophoresis.

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Buffer systems in electrophoresis

Two types of buffer systems are:

1. Continuous buffer system:

2. Discontinuous system/multiphasic buffer system

Now, there are different kinds of buffer systems, which are utilized in electrophoresis buffer systems. What we are talking about is when you cast the gel or when you polymerize the gel, you polymerize them in a particular buffer. Now, this electrode electrolyte buffer plays a very important role in electrophoresis separation or in experiments. It greatly affects the separation and resolution electrolyte buffer is very important and greatly affects the separation resolution of a particular analyte.

Now, proteins differ quite a lot in their sensitivity to pH ionic strength type of ions. As we have discussed, the proteins charge on proteins will depend on what pH buffer you are taking and that will depend on what is the pH value of that protein. As proteins are amphoteric molecules, they can carry either positive or negative charge as per the pH and pi values. Now, in electrophoresis, there are two types of buffer systems. One is called continuous buffer system and another is discontinuous buffer system. It can also be called as multiphasic buffer system.

So, continuous buffer system is a single buffer system that where you are utilizing single buffer, where as in discontinuous buffer system, you will have more than one kind of buffer or may be two buffers or three buffers. Now, for nearly all electrophoresis of concerning nucleic acids, mostly continuous systems are utilized either in agarose gels or poly acryl amide gels. The electrophoresis of proteins is mostly done on a discontinuous system. So, there is a difference in here. We will discuss both types here.

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Continuous buffer system

Let us start with continuous buffer system. Now, in continuous buffer single buffer or constant ph is used in gel as well as electrode a reservoir that is a single buffer all over. Now, sample is loaded directly on to the gel where separation will occur. They are fractionated as per their mobility. So, the height or we can say amount of the sample volume you are taking will determine the band width. This will limit the resolution attained by the continuous system.

So, what will happen if you have dilute samples? Then you have to load larger amounts or larger volumes of the sample for detection of that particular molecule. What will happen is this will certainly lead to relatively, you can say broader or wide bands. Therefore, the continuous system with constant pore size gels is kind of restricted to only high concentration.

This is because if you apply sample in high concentration, then you get better results; not so broader or wide bands. In dilute solution, since you are loading too much of solutions, the bands width will be higher actually. So, this system has certain problem. Let me show you on the screen how this affects the resolution as such. If you can pay attention on your screen here, so when we are loading the samples, if you could recall I was telling about the wells or slots are created for loading the samples.

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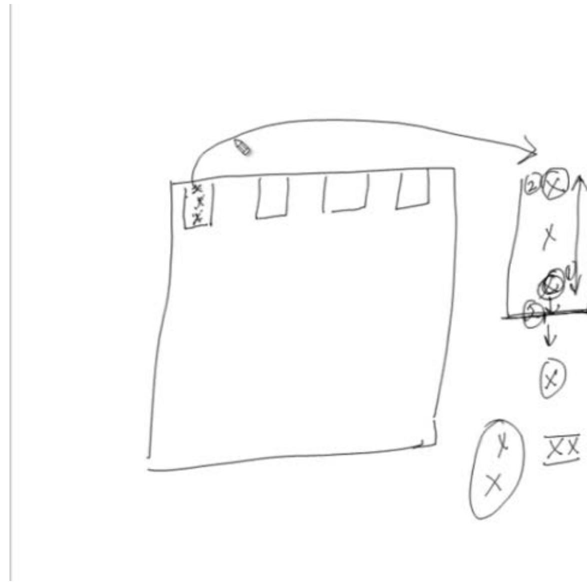

If you have samples, if you have well apparatus or slab gel electrophoresis you are doing, you have plate and you have created two plates. Between two spacers, you cast your gel. What you do is in the top of the gel, you create wells where you are going to load the sample. Now, samples could be loaded in two ways. One is like dilute solutions will be loaded here all over. Now, if I just kind of show you this here that if I have a sample here that is at this place and this place and this place, let me show it here. This is your well where we are loading this sample here. Now, you have a sample here, you have a sample here, you have a sample here. This is the boundary or this is the enter phase here where from the sample is going to enter the gel.

Now, obviously when you have dilute solutions, this is your amount of sample you have taken and it is a large volume, then since your analyte or molecule is distributed all over, the molecule which is closest to the gel inter face will enter first here. It will also move somewhat here on application of electric field. This molecule with the same analyte will there will be a time gap when it enters as relative to this one molecule that is if I say number 1 and this number 2, then this will move somewhere. The second will enter in here.

So, what will happen? This band may be they catch up slowly later on, but still they will not be able to be like this, rather they will appear to be broader band will be seen, rather than this kind of band. So, what will happen that with dilute solutions it is not running

continuous system is not advisable. It has been seen for nucleic acids. It does not really make so much of difference. So, for nucleic acids, continuous systems are mostly run, but for proteins you get much broader band.

If it is a dilute solution, so you will run rather a discontinuous system. Then continuous system, so continuous systems are not advisable in the case of proteins. So, number 2 buffer system, which we were discussing discontinuous, we can call it multiphasic buffer system. Now, this system uses different buffer systems in the gel and electrode reservoirs. If you could recall I have shown you that on the top and bottom, there will be electrode reservoirs where electrodes are placed. You have a tin buffer or electrode buffer. These are designed to, main purpose of discontinuous multiphasic buffer system is to concentrate or sharpen the sample zones for high resolution separation.

I have shown you right a little while back that if you have dilute solutions and if they enter the gel at different times, the product bands will be obtained. So, the best thing is to make sure when they enter the separating gel or resolving gel, they enter as one entity. So, it is like the running line is same for all molecules. So, you get better resolutions during the separation.

So, to concentrate the sample in a very narrow zone, a large pore stacking gel which serves as an anti convective medium here, when you say large pores proteins will be moving unhindered. There will not be any kind of restricted migration here as large pore size will not have effect. That is only anti convective medium. Now, all ionic species are for moving fronts here where buffer ion front leads.

So, what are ionic species? Here, ionic species will be certainly when we have multiphasic buffer system, one ionic species is your protein and the other ionic species is like buffer, which is gel buffer and then the electrode buffer. So, these are ionic species. So, if you say all ionic species when electric current is applied, they start moving front actually. So, where what happens? A buffer ion front leads as it is ionized completely and as it moves ahead of the sample molecule.

So, buffer ions we will see what are the buffer ions and details here. We will see electrode reservoir ion fronts trails behind sample. We will see why it trail trails behind and the sample ions are between the two fronts that is buffer ion front and the trailing front reservoir ion front. So, what happens is these sample molecules or sample ions are

sandwiched or they are compressed between into two fronts into a very narrow sharp zone in order of their mobility. If there is a mixture of the sample, then in order of their mobility, they will settle down. So, sample molecules step in a very narrow region.

So, what you can say that it can be a few micro meters thick or may contain a very high concentration of proteins say 100 mille gram per ml. So, stack samples are moves through the stacking gel in very narrow zones regardless of their initial sample volume and when they enter resolving gel. Now, resolving gel contains small pore size. So, what happens? These stacked samples will be un stacked. They will move according to the size and charge in the resolving gel. So, what discontinuous system is essentially doing it is putting two gel systems, where stacking gel is to concentrate the sample, so that you can get good separation and good resolution here.

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So, discontinuous gel electrophoresis was like it was first developed by Ornstein and Davis in 64. They were first to develop a high resolution poly acryl amide gel electrophoresis for the native proteins. Now, it was discontinuous and non denaturing. So, there was no denaturing agent in the gel electrophoresis system. This is the extensively used technique in gel electrophoresis with certain modifications. We will see the modifications here.

Now, the system is made up of four interrelated components here, which includes a stacking gel. Again, stacking gel will contain a buffer resolving or separating gel with a

buffer concentrations and an electrode buffer that is twist like seen buffer and the sample buffer. The sample will also be put in a like it could be simple like stacking gel buffer or particular kind of concentrations.

Now, stacking gel has a lower acryl amide concentration. It has a large pore size around 4 percent. It is cast on the top of the resolving gel. The resolving gel can range from say 5to 20 percent or even up to 30 percent, but up to 20 percent is mostly utilized. Now, stacking has a lower ph and ionic strength. So, stacking gel has trigs C l whose ph is 6.8. It has a lower ionic strength. Also, there is separating gel, which has a ph of 8.8 and it has a higher ionic strength around 0.35 molar trigs C l as compared to 1 to 5 molar in stacking buffer. Now, the ph difference in two gels regulates the mobility of glycinate ions from cathode reservoir chamber. The concentration of buffer is also derived from electro chemical consideration.

So, the main aim is to concentrate the sample actually and accordingly the ph and concentrations have been derived or designed. You can say the stacking gel with large pore size mainly functions as anti convective support as we were saying and helps in formation of sharp zones without impeding the migration of proteins. The separation of proteins will take place in the separating buffer. So, remember stacking gel is only for concentration and not really for separation here.

Now, the sample buffer has less ionic strength. Again, it is 0.065 molar trigs C l. In Ornstein and Davis design, ph is same 6.8 that of stacking gel or stacking buffer. Now, this is loaded on wells as I have shown you present in the stacking gel. So, sample is loaded or located between the stacking gel and the electrode buffer. Now, electrode buffer is 0.25 molar trigs and 0.192 molar glycine and ph automatically comes to 8.3. So, the ionic strength of sample solution is lower than the buffers above and below. Let me show you like on the screen what is the situation here.

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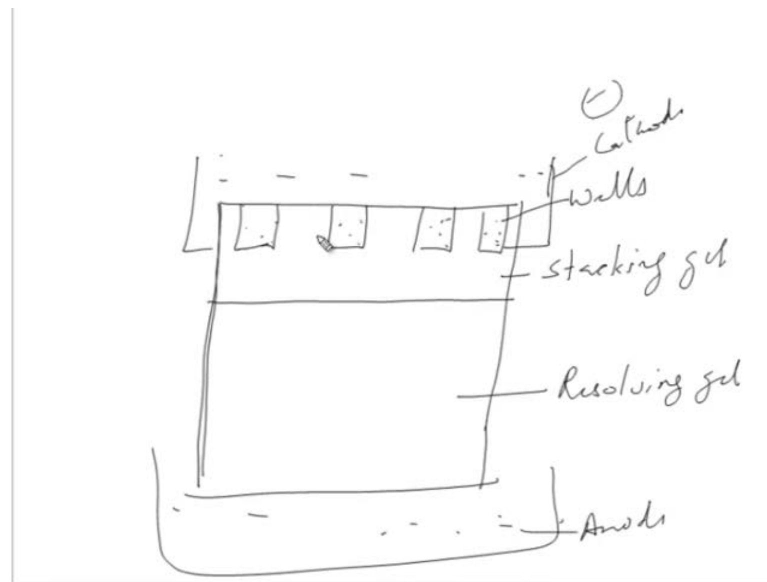

So, what you have is on this gel, you have two gels actually. So, what is this? This is stacking gel. This is resolving gel. So, automatically when you are casting the gel, first you must put pour resolving gel. Then after resolving gel is polymerized, then you put stacking gel. Actually, these are wells where sample is loaded. So, your sample will be loaded in these wells as per like how many samples you have. On top of this, there will be electrode buffer. On the top and bottom, there will be electrode buffer. This is cathode chamber and this is anode chamber.

So, this is how the whole thing is in here. So, you can see the sample is between the stacking gel and the electrode buffer here. So, let us move on. So, power is applied. What will happen is a voltage drop develops across the sample. It helps in driving the sample into the gel that is stacking gel. Now, as the current begins to flow like I said buffer ions leads actually, so here chloride ions will lead and the gel. As the current begins to flow, these chloride ions will be first to move. Then proteins in the sample with the negative charge will move. Then the glycinate ions in the electro buffers will start moving towards the anode.

So, three things, which are moving in here is one is chloride ions. Second are the chloride ions in the gel. Then it is protein in the sample with negative charge. The positive charge protein will not move towards the anode and glycinate ions in the electrode buffer moves towards the anode. So, what is the pattern here? So, all cations,

which I am saying trigs or proteins with positive charge will move towards cathode. So, it is a hard thing to separate. So, it is like you have to change buffer conditions or certain things. We are talking about this continuous system.

So, here you will not be able to see cationic proteins as such. Now, chloride ion with higher mobility move out of the sample and a localized low conductivity and high field region is created behind. So, it will accelerate the proteins in the sample and the glycinate ions from the electro buffer to save velocity as the chloride ion. Now, the effective mobility of the glycinate ions will be less than those of chloride ions proteins as the glycinate ions are not completely ionized at lower ph. Remember ph of stacking gel is 6.8 and even in this electro reservoir, it is 8.3 around.

So, glycinate ions are not fully ionized. So, they move like little slower. So, they trail behind actually. So, what moving boundary is formed here with chloride ions in the front and glycinate ions in the rear with the proteins sandwiched between them? So, it is compressed between the two fronts, one is leading front and other is trailing front. How the protein molecules concentrate here as individual thin zones? It is in order of their decreasing mobility.

If you have more than one protein, one kind of protein or one type of protein molecules, then different protein molecule as per their mobility, they will concentrate as thin zones. They will concentrate here between two fronts, leading and trailing glycinate ions. Now, when the moving boundary region will reach the inter face of stacking and resolving gel, so in stacking gel, what is the situation? It is that due to the ph differences, ionic differences, one front that is chloride front leads and the glycinate ion front trails. In between, proteins are sandwiched and they are compressed.

Finally, sharp zones are formed. This particular process is known as iso techophorersis. So, when these are concentrated, they reach the inter face of stacking and resolving gel. So, they will concentrate at that inter face because they are going as thin zones. Here, they will enter into the resolving gel as one entity. So, as they reach there, the chloride ions and the glycinate ions leave behind the protein. Now, here, if you remember the ph of resolving gel is 8.8 and as the glycinate ions move, the ph is much higher of the resolving gel. So, the glycinate ions are not fully ionized. They move as per their velocities. Proteins now enter low pore size gel.

So, there will be a restrictive migration as per their size and charge. So, protein will enter the resolving gel. They will experience a sharp retardation due to the resolving gel pore, smaller pore size of the resolving gel. These protein zones, which we were talking about, which were stacked actually that is why it is called stacking gel. Now, they un stack since there is no hindrance or movement in stacking gel. Small, large or other proteins will have to move according to the pore size.

So, they will unstuck and separate according to their size and charge. So, what you see is that all these factors will result in the proteins becoming compressed inter face of the gels. Therefore, they give you very high resolutions in these experiments. So, this is this continuous system is quite useful system for higher resolution for protein separation.

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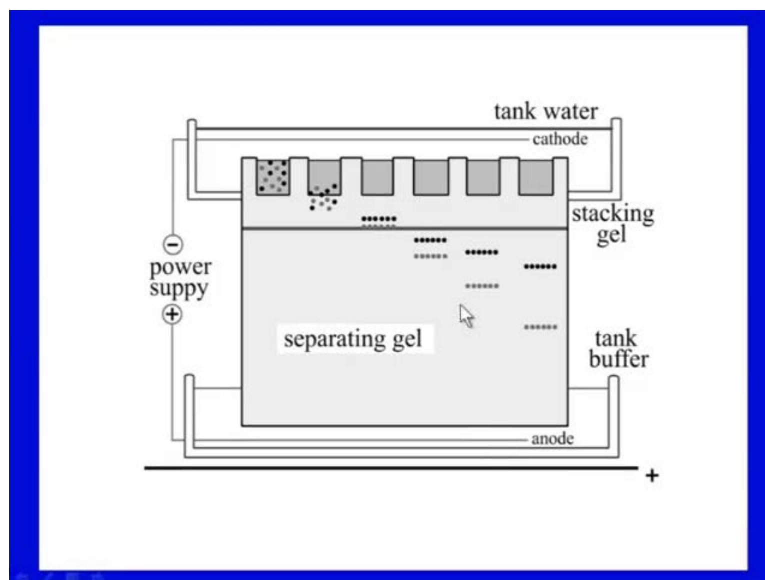

Now, this figure shows the things very clearly. Here, what you see here? There is a tank buffer, which is cathode electrode is negative electrode. There is tank buffer tank below the reservoir below, which is also filled with reservoir buffer. So, there is reservoir buffer here. Here, there is a separating gel, which is larger, which you can see most of the plate is covered with separating gel.

There is a very small part is stacking gel. Here, you load your samples in these wells. If you see these samples are distributed here big small. They form boundaries here thin zones. When these thin zones enter like one here, one here, when they enter the gel at the inter face to the resolving gel, they will separate according to their size and charge. So,

this is how discontinuous system works actually and it is will very well depicted in here. So, this was about two buffer systems, which were widely used, one continuous system and other discontinuous or multiphasic systems.

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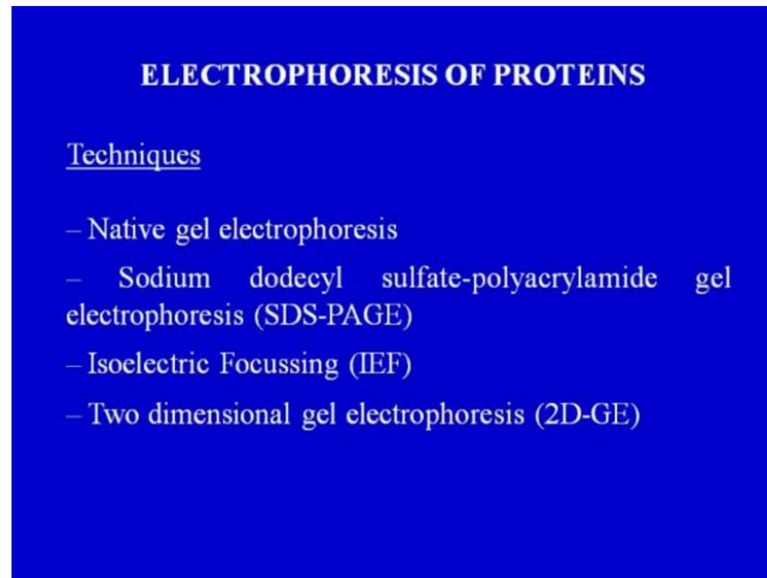

We will start with the electrophoresis of proteins. Now, there are many variations of electrophoresis, which are commonly used. There are lots of techniques, which are used for different applications for analysis of proteins. We will discuss some of them. Native gel electrophoresis is one where analyte separate is according to the difference in apparent mobility and protein is not denatured here. Then there is SDS page or sodium dodecyl sulfate poly acryl amide gel electrophoresis, where what you see is that the analytes are denatured because of STS and they are separated according to size.

Then, there is iso electric focusing. In iso electric focusing, the separation is on the basis of the pi values of proteins. As proteins are amphoteric molecules, they carry a net charge. The charge is 0. The net charge is 0 at their iso electric points. That becomes the basis for separation. Then there is 2dimensional gel electrophoresis. It is used in mass spectra techniques. You can separate large number of proteins sample in particular portions. You are isolating it from certain cells or tissues. Then they could be analyzed by mass spectrometry.

So, we are going to discuss all these techniques. Today, we will be discussing about native gels and SDS page. So, as native gels as we were discussing in the continuous and

discontinuous system like Ornstein and Davis system, it was for native proteins actually where you have not put any denaturing agent. So, in native gels, non denaturing conditions are used to detect the proteins in its active form, particularly in case of enzymes where you would like to see the activity of the enzymes.

Then, you might want to use the native gels. Now, in native gels, poly acryl amide gels are used. The SDS or any other denaturing age agent is absent. So, you do not denature the protein in any way here. Since, all the proteins in the samples being analyzed carry their native charge at the ph of the gel proteins will separate it according to their electrophori mobility and the sieving effect of the gel and various types of mechanisms in native gels. There is no restriction on the ph of the buffer.

You can very well design or you can as per your requirement, you can use certain buffers for native gels. Then you can detect it while various methods, which we are going to discuss in later lectures. So, this was about SDS page. Actually, this was about native gels. Now, this is one of the main techniques, which is widely used in lot of research labs and in teaching labs. It is the SDS page or sodium dodecyl poly acryl amide gel electrophoresis.

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So, we were discussing about Ornstein Davis discontinuous and non denaturing system if you could recall. Now, this particular system has been modified by lammily to a discontinuous, but a denaturing system. Now, lammily system made it possible to

determine the molecular weight or the molecular mass of the proteins. How it is done is what we will be discussing. So, what happens here is rather than having like in Ornstein Davis where no denaturing agent was used in this system of lammily are denaturing agent, which is 0.1 percent SDS that is in anionic detergent will be utilized in buffers and also it will be introduced.

One more step will be introduced that is denaturing step sample preparation or sample treatment. So, what happens if you treat the samples, which contain around 2 percent SDS? Also, heating step is introduced here, which will completely denature the sample proteins to their constituent polypeptides before the run. So, here sample treatment involves heating in particular buffer. This buffer is like mostly stacking gel buffer like I told you earlier, but this buffer, the sample buffer, is which we were discussing.

Ornstein and Davis system will also be having 2 percent SDS and also reducing agent like 5 percent beta mercaptoethanol. So, the proteins are completely denatured and polypeptides will take a uniform charge to mass ratio, which is imparted by SDS. Also, reducing agent will break any disulphide bonds between polypeptides or within polypeptides gels. So, SDS can be interacting with all parts of the polypeptide gel many times.

What will happen when disulphide pressures are not broken? There are regions where SDS is not accessible and may be erratic patterns could be seen in here. So, poly acryl amide gel electrophoresis in the presence of SDS is the most common form of protein gel electrophoresis. Now, here lithium dodecyl sulphate that is an alternate, which could be utilized. SDS is the most widely used detergent. Now, SDS disrupts some of the non covalent interactions that is stabilizes the protein quaternary and treasury structures and which facilitates denaturization.

What happens? SDS has negative charge. What it will bind to the protein in a constant mass ratio that is mass ratio of 1.4 gram of SDS binding to 1 gram of polypeptide. So, that total amount of detergent bound is directly proportional to the molecular weight of the protein. It is 1.4 SDS per gram of poly peptide protein. So, what it does is SDS coats the poly peptide completely and coating of negatively charged SDS overwhelms the inherent charge of protein molecule and gives them a uniform charge to mass ratio.

So, this is like now protein will carry a net negative charge as per its molecular mass because of the interaction of SDS. Now, remember many times though it happens, but certain proteins with say lot of basic residues say lichens or others may there might be certain discrepancies to charge to mass ratio. There might be certain problems in running.

In general, this is the rule where you have a particular mass to charge ratio actually. So, this allows protein to be separated on the basis of their relative size because of the charge that is negative charge according to their molecular mass. So, you are essentially separating it on the basis of size actually.

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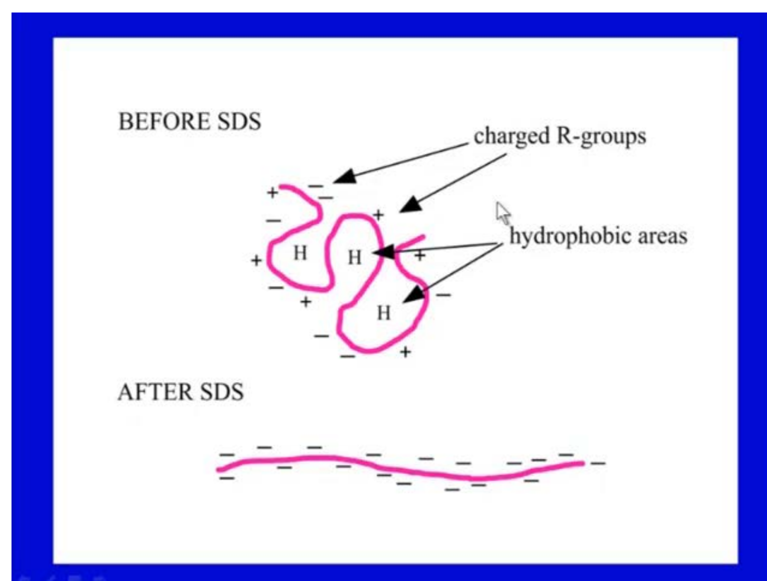

So, what happens is as we were discussing about what happens to proteins, so a protein is folded here. It has interactions. So, there are hydrophobic areas. There are other charged groups, hydrogen bonding, all those things. Now, when you treat with SDS protein is extended and it will over, all covered by the negative charge. So, that is what many times, this proteins will have different shapes.

Now, they are now extended molecules and heating also helps in that. Now, many times also, you can use other denaturing agents. For example, urea is quite commonly used denaturing agent, which disrupts the hydrogen bonds. It is widely used for nucleic acids as we will discuss later on. Pro proteins also it can be used, but SDS is the most widely and heating. SDS is the most widely used method.

Now, advantages here of this particular method are that all poly peptide gels are now forced into extended confirmations because of SDS. SDS treatment eliminates the fact of differences in shape. Shapes are now all extended.

Then, individual poly peptide gels migrate like negatively charged SDS protein complex through the porous poly acryl amide gels. The speed of migration is proportional to the size of the proteins. Smaller poly peptides will run faster because they will have less restricted references. Larger poly peptide will run slower because the pore size will be smaller. There will be kind of resistance, which will be higher for larger poly peptides. So, these are you can say the advantages, which SDS provides. SDS treatment provides for running the electrophoresis of proteins in denaturing conditions. Now, here you can see in here in figure.

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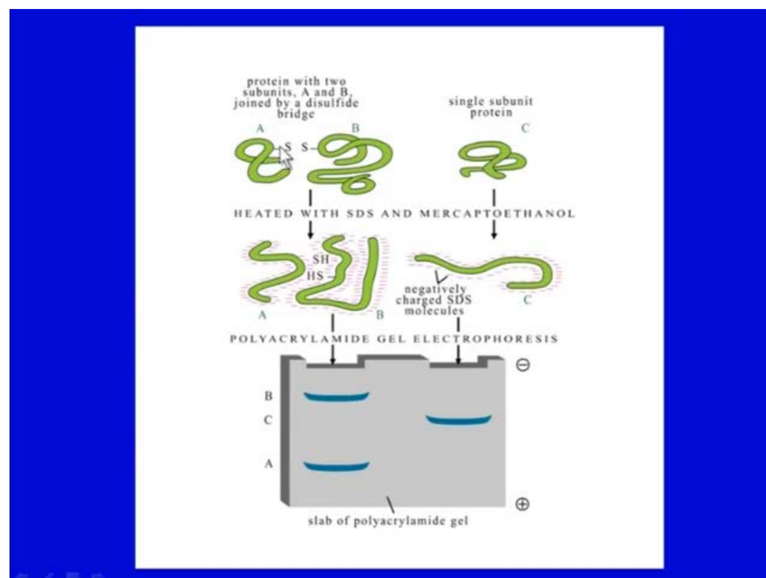

Here, you can have 2 samples here like 1 protein, if you can see here is having disulphide linkages. One is without disulphide. When they are heated in the presence of mercaptoethanol, these disulphide links are broken. It is a reducing agent. So, it reduces that. So, when single protein though it is made of it is made of 2 poly peptide chains, but it will be seen as 2 separate bands here in reducing gel.

Now, to know whether it is a single protein, always you can run non reducing gels. Also, in non reducing gels, these disulphide bridges will not be broken as mercaptoethanol will not be included in the sample buffer. Single band could be obtained in here.

So, both ways, you can say if you have proteins say certain disulphide bridges between the poly peptide chains, then if you are utilizing reducing gel certainly, you will get 2 bands. You will not be able to know whether it is pure or not. If you are testing the purity, then you must run a non reducing gel that is sample buffer not having mercaptoethanol. If single band comes, then you will be sure that it is same protein. It is a single protein made of 2 poly peptide or more poly peptide chains. So, how this SDS page is per formed the practical aspects of this?

Now, the first step in discontinuous electrophoresis is to pour the separating gel like I told you in earlier lecture. I have shown you how to cast the gel. So, there what you are going to do first thing is resolving or separating a gel will be casted first. Then separating gels will typically contain 6 to 20 percent of acryl amide. The size range of proteins being separated has to be taken into account desired. Resolution amount of sample is being applied. All these factors need to considered while choosing the acryl amide concentration once you have casted the resolving gel.

Then, once it is polymerized, you will put the stacking gel and with comb in it like I have shown you earlier. So, that wells could be created. Now, there could be resolving gel where you could use gradient gels also and these gradient gels. It is a pore size gradient gel. We can say because they will be either decreased in the pore size. As you move down the glass plate, so it will give better separation. It could be step gradient or continuous gradient gel. In many situations, where you have to examine both high and low molecular weight proteins on the same gel, gradient gels are more advantageous.

The stacking gel is poured after the separating gel polymerizes and just before electrophoresis to is done. Once these are polymerized, you have to take care that both resolving gel, when it is polymerized, you can overlay it with say organic solvent, so that surface labels properly. Then you can remove that. Then pour that stacking gel and put the comb. Now, it is done. You will load your samples into the wells of the gel and the current will be applied. Now, samples for SDS page are prepared in sample buffer as we have discussed earlier that is 0.625 molar trigs, pH 6.8. It contains 2 percent SDS and 5 percent mercaptoethanol. If it a reducing gel, 10 percent glycerol is present.

Now, mercaptoethanol is added just before the experiment. Glycerol will provide density for under laying the sample on the stacking gel below the electrode buffer. So, since the

buffer is already put in, if you have a glycerol, it will not diffuse and properly settle down in the well. A tracking dye that is bromophenol blue around 0.0 to 5 percent is also included in the sample. This dye reaches the bottom of the gel. So, what you will know is when to turn off the power that is fastest.

So, you do not want to go after that, otherwise your samples might over run a common way to detect proteins. After electro electrophoresis is performed, stain the gel. That we will be discussing later on before staining gels are usually fixed with acetic acid methanol solutions like 10 percent acetic acid, 40 percent methanol or so. What happens is it fixes the proteins or precipitates the protein into the acryl amide matrix. So, they do not move or diffuse. You can easily do the staining.

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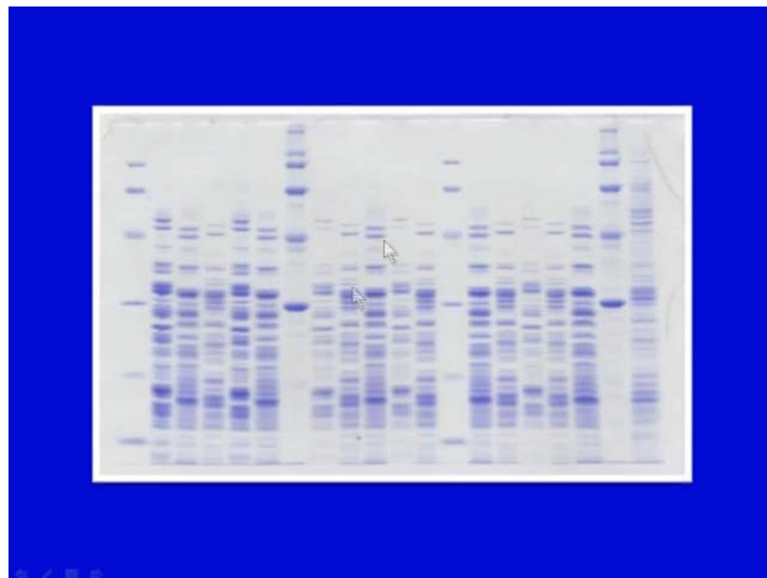

After staining, the gel will look something like this. You have like here. These are molecular weight markers. These are different samples, which have been done. You can see these blue bands here, which is stained with camasses brilliant blue. This is you can get very good. This is how you can see your gel and different band patterns. Now, I was saying this gel here, which I have shown you.

Mostly, it is for the high molecular mass proteins. When you have to run a very small molecular mass proteins, then it is better to have rather than glycine buffer, you can have tricine buffer. Tricine buffer is better for smaller poly peptide chains. They will stack.

They will separate better in tricine buffer. SDS page is widely used for like calculation of molecular mass.

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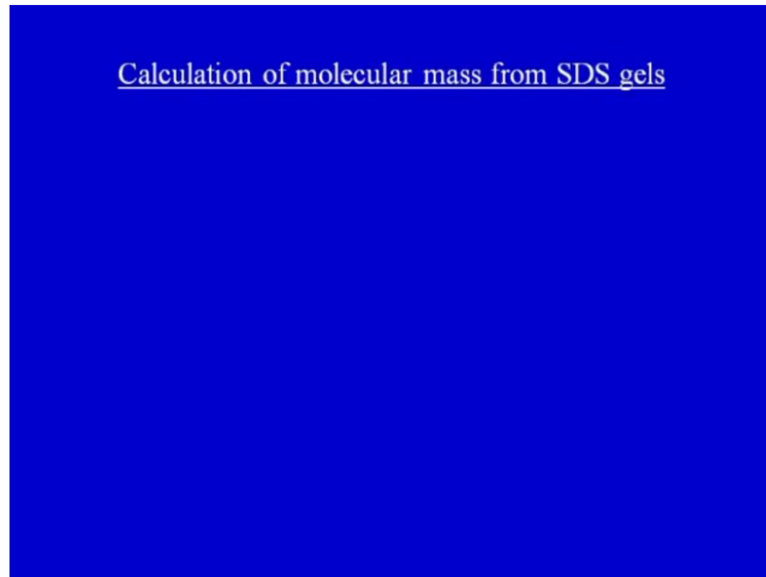

We say calculation of molecular mass. So, you can identify the protein and get some information about it. Now, molecular masses of proteins can be estimated by comparing the migration of proteins of interest to standards of known size. If you could recall like I told you earlier that gel is very random mesh work. It precipitates and fibers are formed. Then they form a random structure. Many times, gels, these things cannot be repeated actually, as every time you cast a gel, it might be different.

So, you have to really compare it with standards actually every time you run your sample. So, the relative mobilities of the standards are plotted against the log of their molecular masses. Then you can compare the size of the unknown protein and then extrapolate from the standard curve. So, here what you do is relative mobility or r f value is calculated. if you could recall when we were talking about the chromatographic techniques, particularly thin layer and paper chromatography, the r f value is very important factor. So, here also, it could be like calculated by dividing the distance the protein migrates to the distance total length of the gel or the di front actually. So, what is done?

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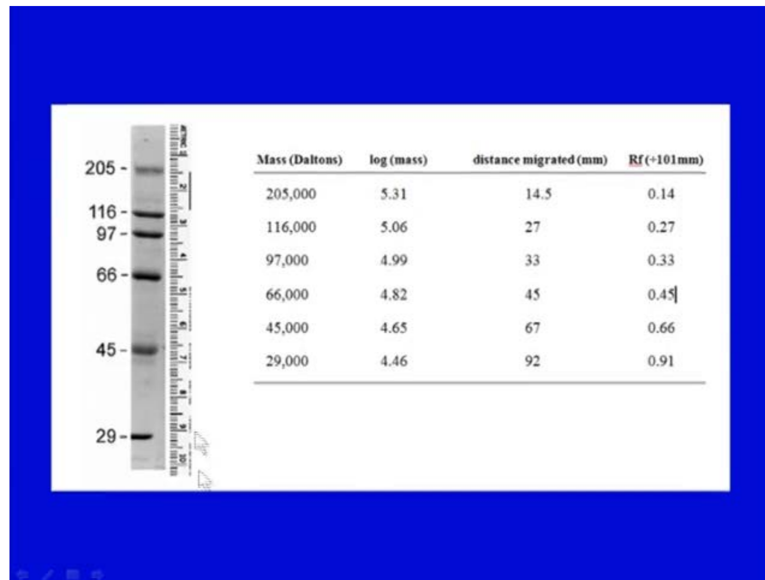

For example, this is your standard here. So, the migration of the standards could be taken. So, you have taken these are the molecular mass here. You can calculate the log of molecular mass. Then here distance migrated. So, you have distance like 14.5 mille meter for this band and likewise, 92 mille meters for this band last band. Total gel length will be 100 mille meter. So, r f value can be calculated by dividing by total gel length.

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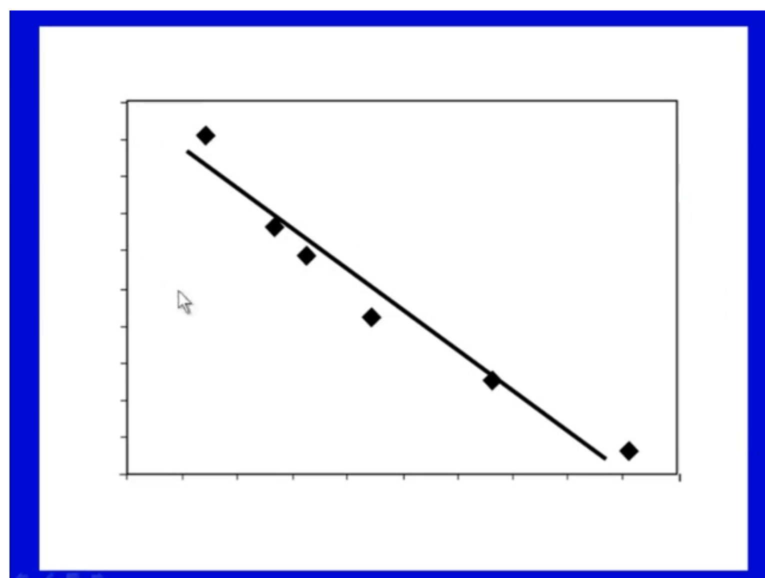

Then, you can plot the r f value along the molecular mass against the r f value. This standard curve can provide you the size of the unknown sample. So, this is how very

easily molecular mass it is approximate. I would say approximate molecular mass could be calculated here. So, SDS page is a very useful technique to be used. So, in this lecture to summarize, we have discussed about the continuous system, the buffer system and the discontinuous buffer system.

The continuous buffer system utilizes a single constant pH buffer all over like in electrode buffer gel buffer, but where as in discontinuous system, there is multi phase buffer system like stacking gel buffer. You have resolving gel buffer, electrode buffer, sample buffer. So, Ornstein and Davis made this for poly acryl amide gels first time. It gives high resolution because you can concentrate the protein stacking gel. When they enter as 1 entity into the resolving gel, family improved it further for molecular mass determination by making it denaturing gels. He introduced and he put 2 percent SDS. It is most widely used electrophoresis technique in or routinely used technique in lot of labs here or bio chemistry labs.

So, 2 percent SDS page in sample and 0.1 percent SDS in buffers denatures through heating and mercaptoethanol reduces the disulphide bridges. So, poly peptide is completely extended and all the different proteins are completely extended and overlaid with negative charge. So, they move according to their size actually because charge is the ratio is same mass to charge ratio is same. So, that way you can compare with standards proteins. You can calculate the molecular mass of an unknown sample or unknown protein. Here also, SDS page gives you a clue about the purity of the sample whether say we have gone through a purification procedure.

Then, first thing to do is whether your purification is done or not, you have accomplished the purification. The SDS page is run to see that you get a single band. Now, remember even if you get a single band, it is not 100 percent purification. There might be impurities, which are not seen because you are utilizing a particular kind of detection system like say camasses brilliant blue can give you to a certain extent as we discuss later on. Silver staining then further gives you that is much more sensitive and can tell you about impurities if you want to know.

So, in the next lecture, we are going to discuss about 2 more techniques that is iso electro focusing, where pi is taken for separation and 2 d gel electrophoresis to separate many samples in 2 dimensions. We are going to discuss that in next lecture. Also, we will be

discussing about detection methods of proteins and preparative gel electrophoresis in the next lecture.

Thank you.