Experimental Biochemistry
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Lecture - 27
Gel Electrophoresis of DNA and Proteins Part – I

Hello I am Professor Soumya De I have I am a assistant professor in the School of Bioscience at IIT Kharagpur. Today, I will talk to you about Gel Electrophoresis of DNA and Proteins; this is a 2 part lecture. And, in this part I will talk about the basics of gel electrophoresis. And, in the next part I will talk about some of the applications above of this method.

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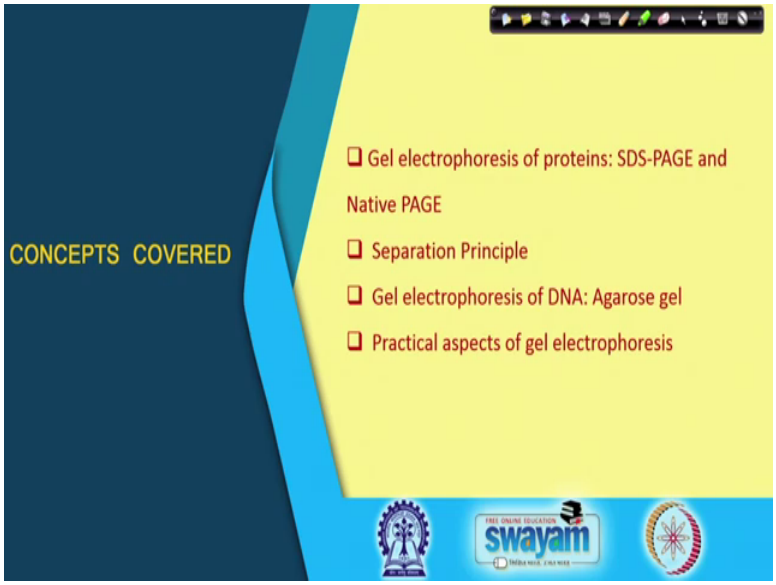

So, gel electrophoresis is a very versatile technique that is used in almost all biological labs ah. So, we will specifically focus on 2 types of gel electrophoresis; one is the SDS page or the page polyacrylamide gel electrophoresis and the other one is the agarose gel electrophoresis.

We will see how this technique works and under what conditions you will use which method. So, for polyacrylamide gel electrophoresis we will see 2 applications; one under denaturing condition SDS page and the other one native page. And, we will talk about the separation principles how this macromolecules are separated in this gel

electrophoresis. And, also I will try to talk about some of the practical aspects of this technique.

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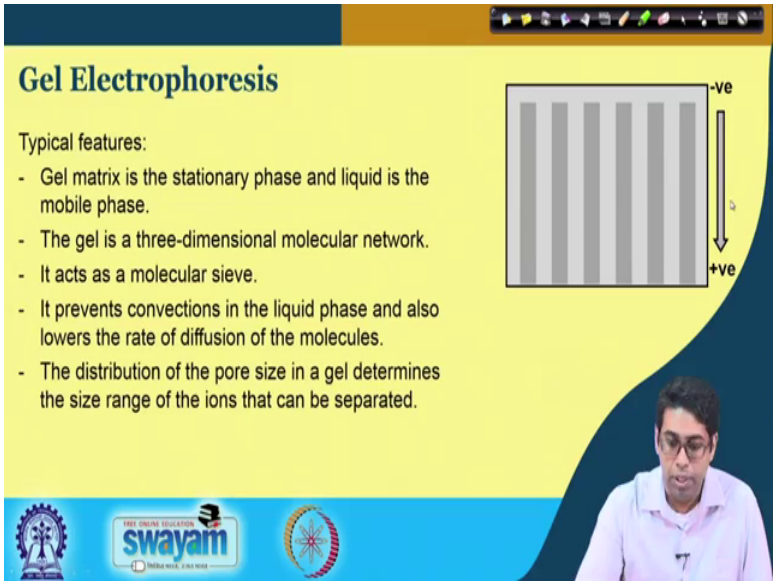

So, typically so, these are some of the typical features of gel electrophoresis that are listed here. So, there are 2 phases; one is the gel matrix which is the stationary phase and another is the liquid, which is the mobile phase.

So, liquid is basically the buffer that we use which has various ions. The gel matrix is made up of some polymer in case of polyacrylamide, it is the polyacrylamide polymer and then the other one is the agarose polymer. So, this gel acts as a 3 dimensional molecular network. And, this molecular network has various size of pores through which this molecules can pass.

So, the gel matrix acts actually as a molecular sieve it also prevents convection in the liquid phase and also lowers the rate of diffusion of the molecules. So, on the right hand side what you see is a rectangle. So, a typical gel would look something like this, the thickness of this gels are very small. If, it is a polyacrylamide gel this thickness will be something in the order of 0.5 millimeter. If, it is an agarose gel then it can be up to 5 millimeters.

So, what we do is we apply an electric field across this gel. So, one side is a positive terminal and the other side is the negative terminal and this molecules are ions. So, the

negatively charged ions will start moving from the negative terminals towards the positive terminals. And, what we will also see is that inside the gel we have certain lanes. So, that the samples are at applied at the top in these lanes and when the electric field is applied, they run vertically in this direction. The presence of this gel reduces diffusion across the lanes.

So, there is no mixing between the samples across this various lanes ok. And, also the pore size of the gel determines the distribution range of the macromolecule that can be separated through that gel.

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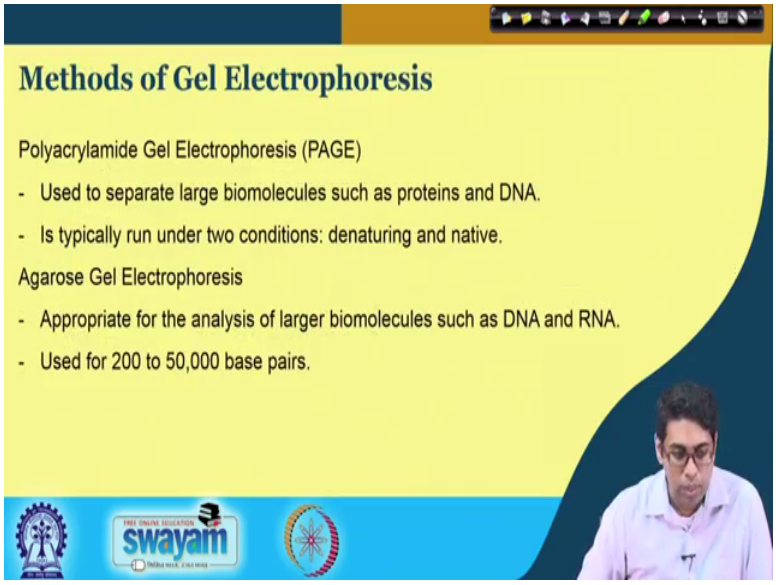

So, as I mentioned before gel electrophoresis there are 2 basic 2 types, we are going to talk there are many, but in this lecture I am going to focus mostly on 2 types; one is polyacrylamide gel electrophoresis, the other one is agarose gel electrophoresis. Polyacrylamide gel electrophoresis is used to separate large biomolecules. Such as proteins and DNA and agarose gel electrophoresis is used to separate even larger biomolecules. And, in this case it is typically DNA and RNA.

So, page is used mostly for proteins, page is used mostly for proteins and it is used for DNA if the length of the DNA is less than 200 base pair. If the DNA or the RNA is more than 200 base pair then we have to use agarose gel electrophoresis ok.

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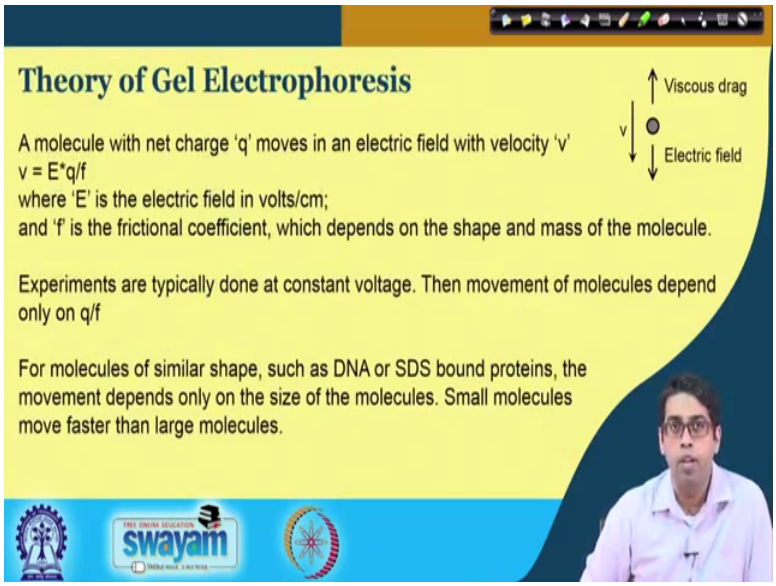

So, what is the basic principle by which these macro molecules are separated? So, typically what is done is that all this micro molecules have a are given a negative charge. So, in case of DNA and RNA they naturally have negative charge because of the phosphate backbone, but proteins depending on the P I of a particular protein can be negatively charged or positively charged based on the buffer of p H of the buffer that is used for electrophoresis.

So, in case of DNA the DNA or RNA the molecules are negatively charged, but in case of proteins the molecule is made a negatively charged by adding some chemicals such as SDS, sodium dodecyl sulfate. So, in essence what we have is a charged particle and then an electric field is applied across the gel. So, this charged particle experiences an electric field. So, this electric field we will push the molecule towards a positive or a negative terminal if it is a negative charge then it will start moving towards the positive terminal.

Now, this gels have certain pores. So, when the molecules are passing through this pores they face a friction ok. So, there is a viscous drag which acts in the opposite direction of the electric field of the movement of the proteins. And, this viscous drag is proportional to the size of the molecule.

So, the velocity of the molecule is given by this equation, where E is the electric field q is the charge divided by f which is the frictional coefficient and this fictional coefficient depends on the shape and mass of the molecule. So, typically these experiments are done

at a constant voltage, which means that the velocity of the molecule depends on the ratio of the charge and the frictional coefficient ok. Now, if you think of DNA it is easier to think DNA molecules in terms of this gel electrophoresis. These are typically long molecules all DNA molecules have very similar shape and the charge distribution is also exactly the same for all DNA molecules.

So, it turns out that they have similar shape and they have similar charge to mass ratio. So, under these conditions these molecules move depending on their size. So, only the size is the one that determines whether a DNA molecule we will move faster or slower. Smaller molecules we will more faster because they can pass through smaller pores as well as the larger pores and the larger molecules we will move slower, because they can pass only through the large pores and not the small pores. So, similarly we will see that we actually use a trick to make uniform charge to mass ratio for proteins by using a molecule like SDS.

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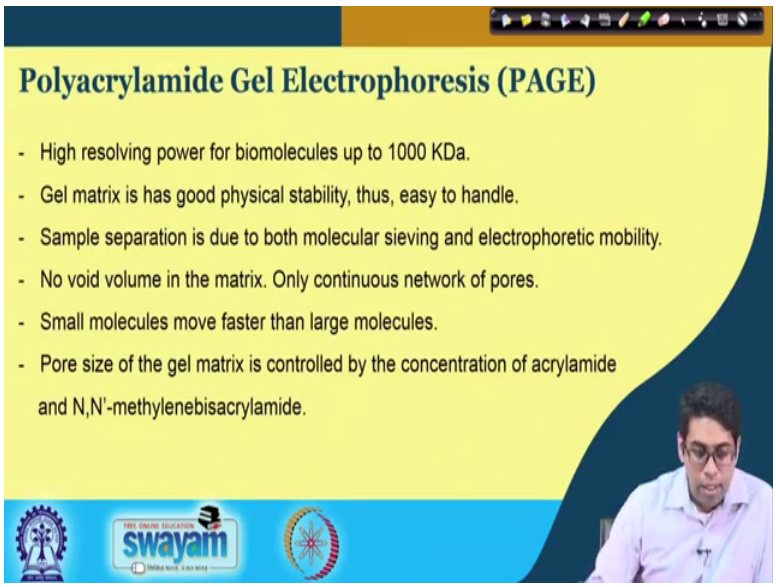

So, the first gel electrophoresis that I am going to talk about is Polyacrylamide Gel Electrophoresis or in short it is called PAGE ok. So, this gel has smaller pore size compared to the other one the agarose gel. And, hence it has a very high resolving power for biomolecules up to 1000 kilodaltons. If the biomolecule is bigger than this then we will have to switch over to agarose gel.

So, some of the physical qualities of a PAGE gel are listed here, one is that it has very good physical stability and thus is easy to handle. It means that after running a gel you will have to take the gel out from the glass plates, you will have to stay in it is. So, a lot of handling is required. If, the gel matrix breaks easily then it becomes very inconvenient.

PAGE even though it is very thin a very thin gel is used it has a very good physical stability, which means that it can be easily handled without damaging the gel. The sample separation is due to both molecular sieving and electrophoretic mobility. So, molecular sieving is larger molecules we will move slower, slower smaller molecules we will move faster. And, electrophoretic mobility means that molecules with higher charge we will move faster and lower charge we will move slower.

So, the mobility is because of the charge on the molecule. At this point it is important to point out an important difference between this gel electrophoresis, and the size exclusion chromatography that you have learnt about in previous lecture is that, there are no void volume in the matrix of a gel.

So, in this SDS page gel and also in agarose gel they have only continuous network of pores, which means that in case of size exclusion chromatography larger molecules can enter the void volume. And so, they can quickly reach the end of a a c c column, but in this case all the molecules will have to in pass only through pores since there are no void volumes.

So, small molecules can move through both small and large pores. So, they move faster and larger molecules move slower. So, this is opposite of what we have seen in case of a size exclusion chromatography. And, the pore size of the gel matrix can be controlled by the concentration of acrylamide ok. So, we will see that since this is a polymer we use 2 types of monomer; one is acrylamide, the other one is N, N-methylenebisacrylamide. So, by varying the concentration of these 2 monomers we can change the size of the pores.

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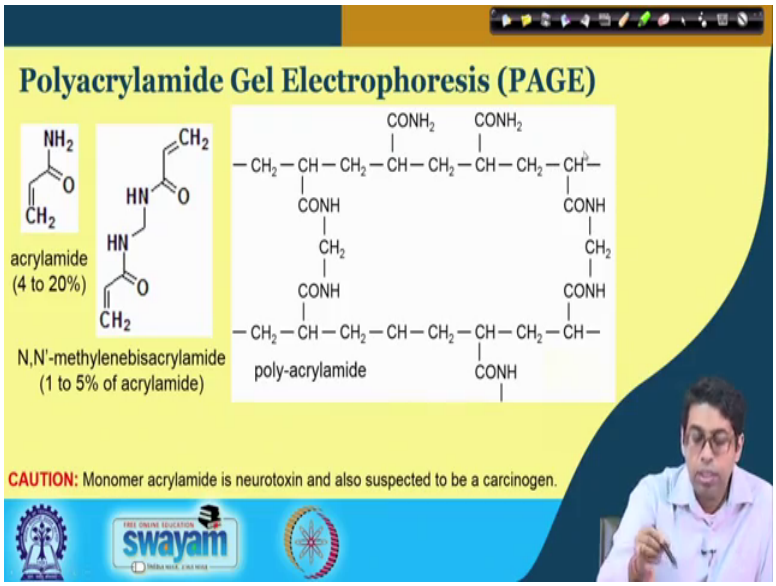

So, the chemical structure of these 2 monomers are listed here acrylamide and bisacrylamide. So, what acrylamide does is if you polymerize this then polymerization happens across this double bond. And, what we will get is a linear chain of a polymer? Ok. But, this linear chain of a polymer will not give us a 3 dimensional network to get a 3 dimensional network what we need is this bisacrylamide. So, bisacrylamide can connect 2 such linear chains and provide a 3 dimensional network of polymer. So, a a student forgets to add bisacrylamide and polymerizes only acrylamide then what you will get is a highly viscous liquid, but not a solid gel.

If, you see carefully bisacrylamide is nothing, but this acrylamide moiety. So, there are 2 acrylamide moieties this and these 2 moieties are connected by a methylene group. So, that is why it is n prime methylenebisacrylamide. And, as I mentioned before the pore size of a molecule of a gel can be controlled by varying the concentration of these 2 components.

So, a typically acrylamide is used somewhere in between 4 to 20 percent weight by volume, and bisacrylamide is used somewhere in between 1 to 5 percent of the concentration of acrylamide ok. So, if we use higher concentration we will get smaller pore size, if we get lower concentration of these 2, we will get larger pore size. And, also one question these monomers the monomer acrylamide is neurotoxin and it is also suspected to be a carcinogen.

So, one has to be very careful while handling the monomers, one see polymerize into a gel like this then it is not harmful, but the monomers are harmful. So, we have to be careful when we are handling the monomeric acrylamide solution ok.

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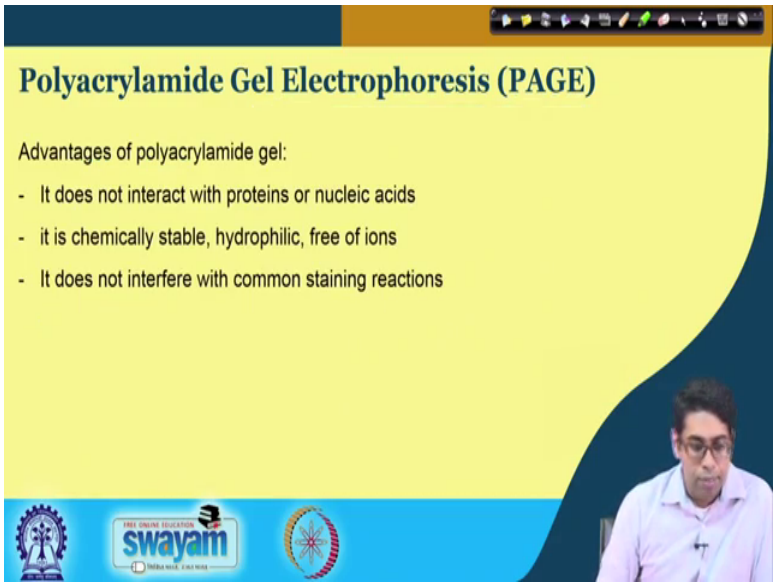

So, the advantages of polyacrylamide so, there are certain advantages; one is that it does not interact with proteins or nucleic acid. So, there is no reaction it is a very inert medium. It is chemically stable hydrophilic and also free of ions, which means that all the electrophoretic mobility that we get is because of the ions that are either supplied in the biological sample or in the buffer. This gel itself will not contribute any ion in that electric current that passes through the gel.

Also it does not interfere with the common staining reactions. All these solutions are colourless. So, at the end of a gel electrophoresis, we have to stay in the gel in order to see the protein or the DNA. Now, these polyacrylamide gel it does not interfere with the common staining methods that are used, which means that only the protein or the DNA bands are stained and the rest of the gel remains clean, which makes it easy for us to detect the protein or DNA or RNA bands.

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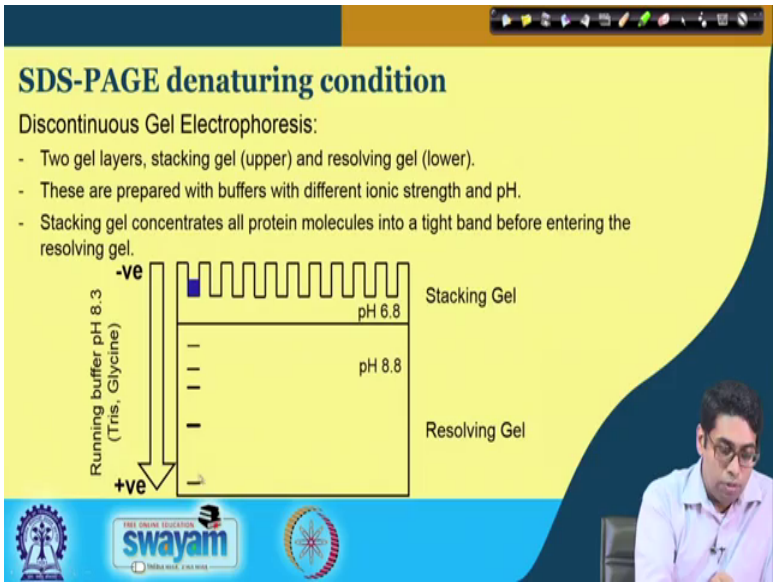

So, now we are getting into the details of this gel electrophoresis, what I am going to talk about now is the is something called SDS PAGE denaturing gel.

So, in this case as it says denaturing it means that. So, this is typically used for protein samples and denaturing means that we are going to denatured the proteins and run it on to a gel. This method is very useful if you want to check the purity of your protein sample. And, also if you want to estimate the molecular weight of your protein sample, if the molecular weight is not known you can actually determine it from this method.

So, in this method there are certain features that I would like to point out, a typical gel would look something like this. You can see there are 2 gels the upper one is called a stacking gel and the lower one is called a resolving gel and these are called the lanes. So, this is where the protein samples is applied.

So, in this setup we can actually apply 10 different samples and run it simultaneously on the gel. A, typical experiment will be will have 9 samples in this 9 lanes and the first lane will have a molecular weight marker. So, it is a mixture of standard proteins with non-molecular weights and, when they run here they will show up in different places and from that we can actually estimate the molecular weight of other unknown proteins in this different lanes. So, we can make a direct comparison between samples in each lane.

So, as I mentioned before there are 2 gels which are stacked on top of each other, the top one is the stacking gel, the bottom one is the resolving gel. And, we use 3 different buffer systems in this discontinuous gel electrophoresis. The stacking gel is prepared using a buffer of pH 6.8, the resolving gel is prepared using a buffer of pH 8.8 and the running buffer. So, this is the buffet which actually passes through the gel and is responsible for the electric current that goes through the gel, this buffer is at pH 8.3. So, we have 3 different buffers with 3 different pH values.

The stacking gel has a very important function. So, the resolving gel as the name suggest is the one which resolves all the protein molecules that are as a mixture in a particular sample. So, suppose in this particular lane, we have 5 different proteins and this resolving gel will resolve them into 5 different bands according to their molecular weight. The stacking gel performs a completely different function. So, if you imagine a race where 10 different athletes are running. So, to start that race what you have to do is all the athletes should come to the same starting line and when the gun is fired all of them should start running simultaneously, then only we can say whose running the fastest.

Similarly, in gel electrophoresis what we need to do is we should have all the protein molecules at the same starting line. So, if this is the resolving gel, then all the protein molecule should be at the same point, but when a sample is applied for example, this one these samples occupy a certain volume. So, we typically at something in the range of 10 to 15 may be up to 20 microliters of sample in each of these lanes. So, if this sample volume is 15 microliters, it means that all the proteins are distributed in this volume. So, the protein that is down here the molecular is down here, we will enter the resolving gel first and the molecule that is up here we will enter the resolving gel later.

So, there will be a diffused band and it will be very difficult to tell which protein is running faster and which protein is running slower. So, it is important to get all this molecules in a very small tight region. Before they enter the resolving gel and that is the function of a stacking gel that is why it is called a stacking gel. So, once we run the gel this is exactly what we would see if this particular sample has a mixture of this 5 proteins. Then, these 5 proteins we will separate out in that lane according to their molecular weight. The smaller one will be at the bottom and the largest one will be at the top.

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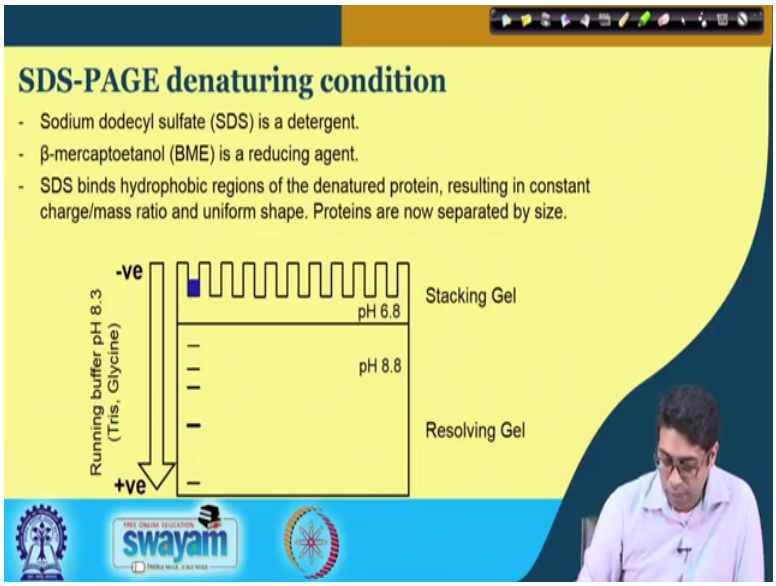

So, in case of SDS page denaturing gel there are 2 more chemicals that are added; one is sodium dodecyl sulfate or SDS which is a detergent and the other one is beta mercaptoetanol or BME, which is a reducing agent. So, BME breaks all the disulfide bonds that are there in a protein. Once a protein is dissolved in the loading solution which has SDS and BME it is actually boiled at 100 degree centigrade. This boiling unfolds the protein. And, once the protein is unfolded SDS will bind the hydrophobic regions in this unfolded or denatured protein.

Once it binds this denatured protein what happens is that the complete protein is now coated with a negatively charged molecule, because SDS is a detergent which has a long hydrophobic chain and a negative ion.

So, it does not matter whether a protein has a low P I or a high P I at these conditions all these proteins are denatured and their uniformly coated with a negatively charged SDS, which means that all proteins have now been converted to negative charge. And, it also ensures that all proteins have a constant charge to mass ratio, which means that these proteins will now be separated depending only on their size, because they have a uniform shape, they have a uniform charge to mass ratio.

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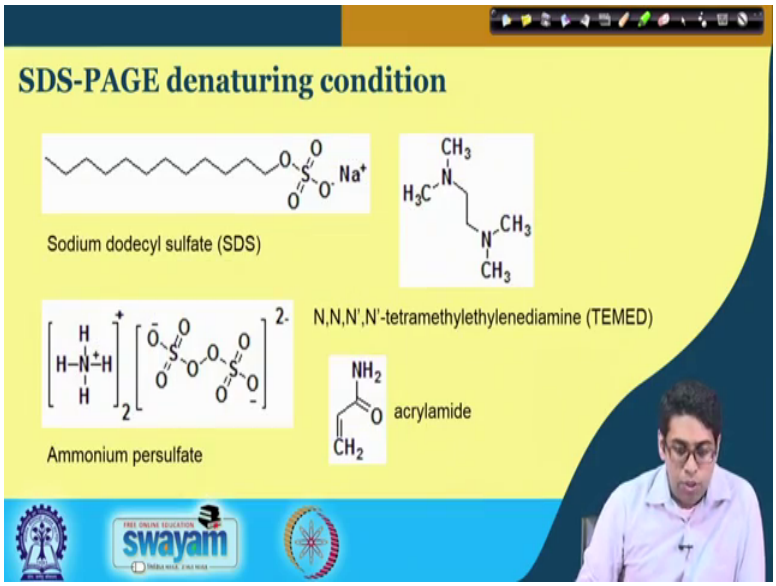

So, some of the chemicals that are used in SDS page gel electrophoresis are listed here. So, this is sodium dodecyl sulfate you can see that it has this long hydrocarbon chain and this negative charge. So, this hydrocarbon chain binds to the hydrophobic regions of the protein and this negative charge provides the negative charge overall negative charge of the molecule. 2 more chemicals are used one is ammonium persulfate and the other one is TEMED. If, you remember the structure of acrylamide it has this double bond and the polymer is formed across this double bond.

So, this reaction is mediated by the formation of free radical. And this free radical is supplied by ammonium persulfate which acts as a catalyst. So, when ammonium persulfate is dissolved in water this peroxide bond is broken spontaneously and it forms free radical, but it turns out that the free radical formed by ammonium persulfate is not very efficient, in producing free radicals on the acrylamide double bond.

So, that another compound is added which is N, N, N prime, N prime tetramethylethylenediamine or in short TEMED T E M E D. Ammonium persulfate free radicals can easily generate free radicals on the TEMED and these are very efficient in starting the polymerization of the acrylamide. So, this is the catalyst this TEMED is the initiator and together they start polymerization of the acrylamide bisacrylamide mixture. One very common mistake that students make is the forget to add one of these two components they will either forget ammonium persulfate or they will forget TEMED. In

either case what you will get is that, even after waiting for hours there will be no polymerization of your gel.

So, in that case the only option will be to pore out the solution mixture from between the glass plates, but remember that unpolymerized acrylamide is harmful. So, you should not dump that into the lab saying what you should do is take it out in a beaker, add some ammonium persulfate and TEMED, let it polymerize and then you can scrape it out and dispose it.

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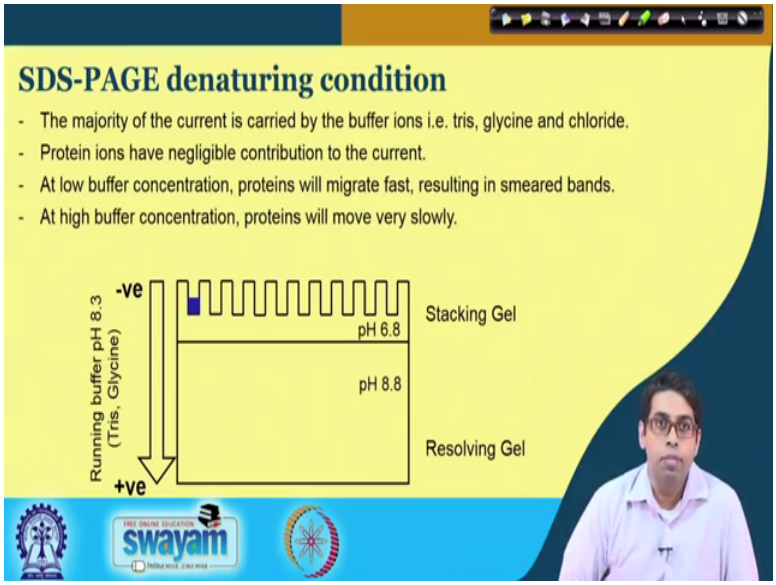

So, coming back to the SDS page denaturing gel again this is our setup. So, what we have at this point is a stacking gel, a resolving gel, we have added our sample to this lanes and we have applied and electric field across it. Now, all our protein molecules are negatively charged, which means that they will all migrate towards the positive terminal and the running buffer is also there which has a pH of 8.3.

So, the majority of the current that passes through a gel is carried by the buffer ions for example, tris glycine and chloride. These are the 2 negative ions which will migrate with the proteins towards the positive terminals.

Protein ions even though they are negatively charged they have negligible contribution to the current. So, it turns out that the concentration of the buffer is very important. Again a typical practice in any lab is that we make 10 x buffer solutions. And before using or

before running a gel that 10 x solution is diluted to 1 x and the gel is run. If someone forgets to dilute it to by 10 x and makes it let us say 20 x or even more, then the buffer concentration will be too less.

Now, if the buffer concentration is too less then, the current that passes through the gel also results in faster migration of the protein. Because if the there are not enough ions then the protein ions also start contributing to the total current of the current passing through the gel which means, that these proteins will migrate fast and it will result in smeared bands.

So, we will not get nice crisp bands and it will be difficult to determine where our protein is if the buffer concentration is high. So, if I just use 10 x buffer and forget to dilute it, then the proteins will move very slowly then the proteins will move very slowly. So, it is important to have the right buffer concentration.

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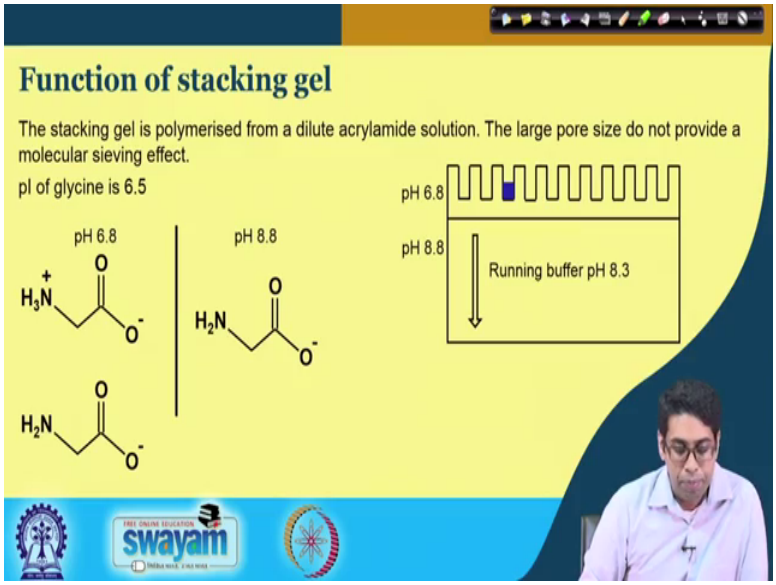

So, that brings us to the function of the stacking gel how does the stacking gel stacks all the protein and brings them at the all the protein molecules and brings them to the same point that is the most important thing.

So, this part is the stacking gel p H 6.8 and this part is the resolving gel p H 8.8. The trick is that what we use is glycine, this is the structure of this amino acid glycine it has a P I of 6.5. So, at p H 6.8, which is very close to it is P I glycine stage in these 2 different

forms. This one is the zwitterionic form where the carboxylic group is negatively charged and the amino group is positively charged. So, it is neutral and a small portion of the glycine will be negatively charged, but in the resolving gel where the p H is high 8.8 almost all glycine will be negatively charged.

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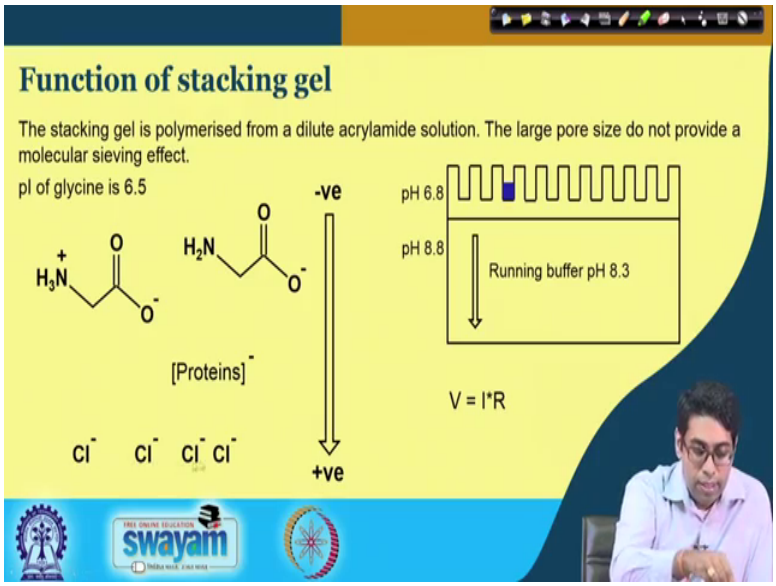

Now, this is our stacking gel, the chloride ions have very high electrophoretic mobility. So, in this stacking gel when the voltage is applied the chloride ions move very fast and what they do is they actually from the front. So, there is a very tight chloride ion front that is formed in the front of the gel ok, because they are moving the fastest. Glycine at this pH 6.8 most of it is neutral and very small amount of glycine is negatively charged. So, it is electrophoretic mobility is actually quite less. And, also the dearth of ions in this region results in an effective increase in the resistance, because we have less ions which can carry the current.

Now, remember that the current that flows through the gel is constant, because there is no accumulation of charge all right. So, if the current remains constant, if the resistance increases then from ohms law we can tell that the voltage will also increase locally in this region. So, when the voltage increases in this region, the protein ions start moving faster. And, one trick that we use for the stacking gel is that a very dilute acrylamide solution is used, which means that the pore sizes are very large. So, there is no sieving effect, small molecules, small proteins, large proteins all can easily pass through this gel.

So, all of them pass through the gel through the pores quickly and since the local voltage is high in this region they will quickly reach the chloride ions. The moment all these proteins reach the chloride ion the concentration of chloride ion is very high here, which means that the current is high right. So, the effective resistance goes down. So, the voltage also goes down. So, this protein ions will stay right behind the chloride front. So, the effect is that all the protein ions are stacked into a very narrow band right behind the chloride ions. And, together they reach this intersection and the enter the resolving gel as a night nice tight narrowband.

The moment this glycine chloride ions and proteins they enter the resolving gel the pH of the resolving gel is 8.8. So, now, all of the glycine molecules are quickly converted to this negatively charged ion and they start moving fast. So, this concentration effect that we saw in the stacking gel is now lost in the resolving gel. Also in the resolving gel a higher concentration of acrylamide is used which means that, now the sieving effect takes comes into place and it will start resolving the protein according to their size.

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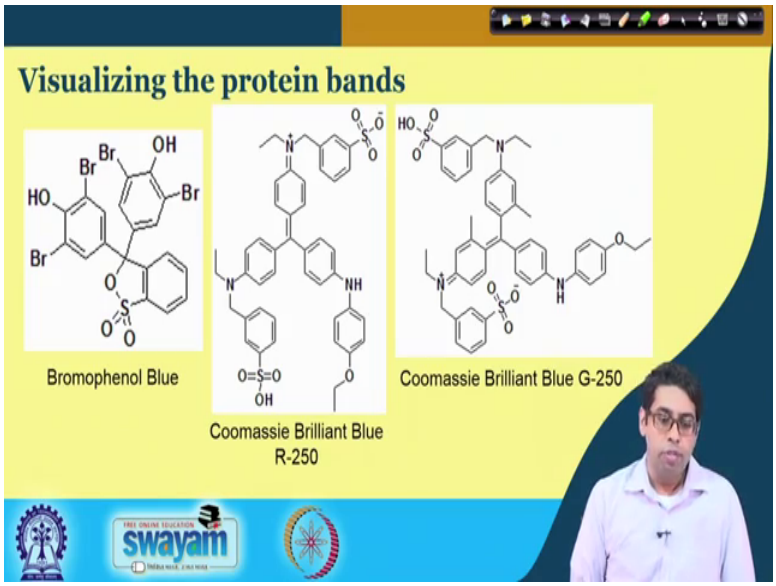

So, once the gel is run we need to visualize them. So, while the gel is running all of these are colourless so, we do not see anything. So, in order to ensure that we know when running the gel is complete a tracking dye is added, typically bromophenol blue. So, it has negative charge and it has very high electrophoretic mobility. So, it runs the fastest. And, once this tracking dye reaches the bottom of the gel we know our gel

electrophoresis is done. And later on to stain the proteins typically one of these 2 or a mixture of these 2 molecules coomassie brilliant blue is used.

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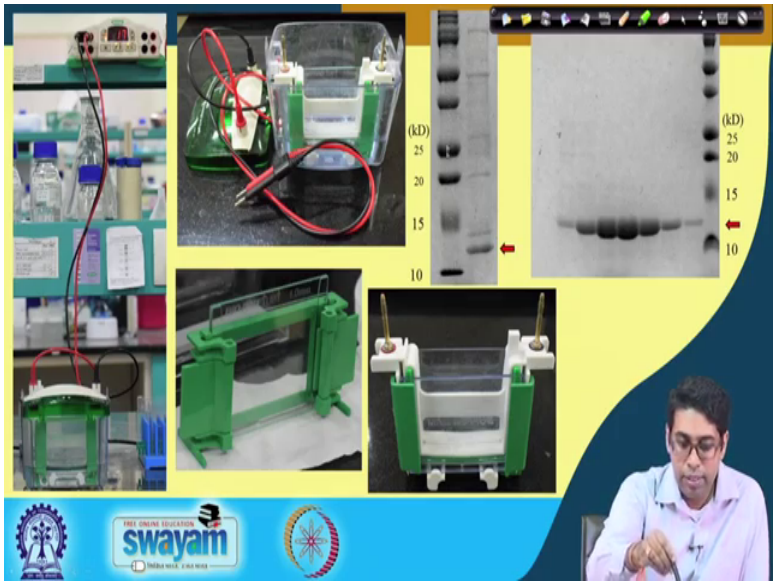

So, here what we see is a setup of a SDS page gel electrophoresis. This gel is run vertically. So, this is where the gel is run and electric current is provided by this power source these are the different components. So, this is the tank where the protein. So, this is the glass plate the gel is formed inside this, this glass plate is packed inside this, and this whole thing goes inside the tank, and the buffer is filled into this tank, and then you set it up and pass electric current through it.

So, once a gel is run and once we stain it would look something like this. So, this is a typical molecular weight marker that is used to where this different bands correspond to different size proteins and from that we can tell that what is the molecular weight of our protein. So, this protein will be somewhere in between 10 kilo Daltons to 15 kilo Daltons. And, sometimes when we purify protein we can tell that our protein is highly pure and there are no other bands in this gel. So, we can determine the purity of a sample and we can also roughly estimate the molecular weight of a sample.

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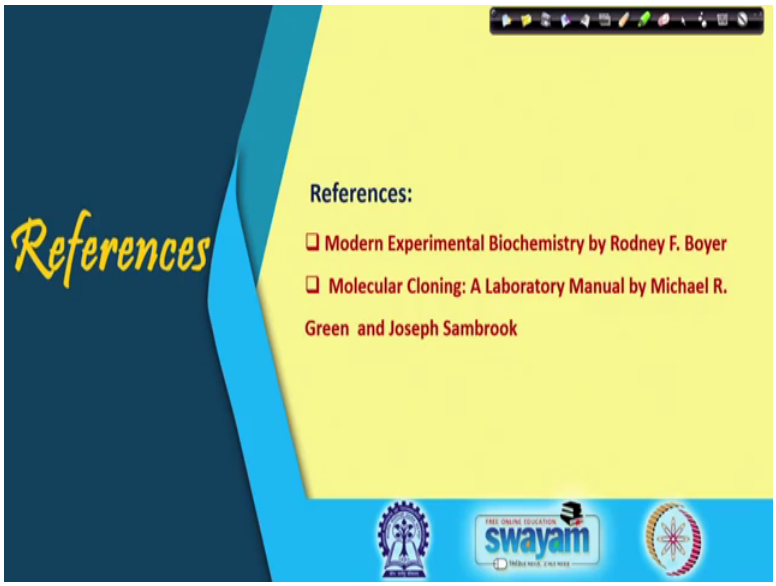

So, that is all for this lecture, in the next lecture I will talk about some more applications of gel electrophoresis and some other types of gel electrophoresis.

Thank you.