Experimental Biotechnology
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Module-I
Electrophoresis (Part-1)

Lecture-07
Basics of Electrophoresis (Part-1)

(Video Starts: 00:23) (Video Ends: 00:59) Hello everybody, this is Dr. Vishal Trivedi from department of biosciences and bioengineering IIT Guwahati. And let us start our new topic and the new topic is the electrophoresis. So, electrophoresis is a technique which is been used to analyze the biomolecules such as the protein or the DNA. And to separate them based on the charge or the molecular weight or all other biochemical properties. So, let us start discussing about the electrophoresis.

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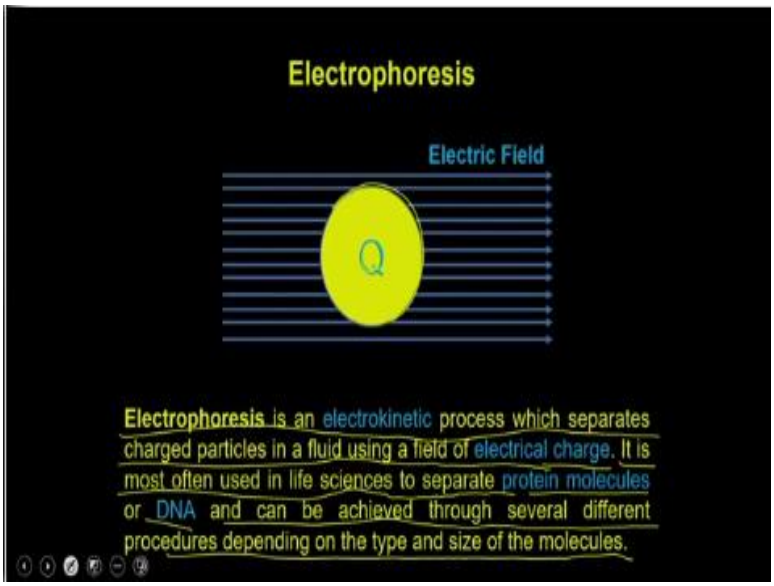

So, as the name suggests, the electrophoresis means the study of or the separation of the molecule when they are running into the electric field. So, you can imagine that you have a biomolecule which is or you have a charged molecule containing Q charge and it is running into a electric field. So, electrophoresis is an electro kinetic process which separates the charge particle in a fluid using the field of the electric charge.

It is most often used in life sciences to separate the protein molecules or the DNA molecule and can be achieved through the several different processes, procedures depending on the type and size of the molecules. So, before getting into the detail of the electrophoresis and how to perform the electrophoresis, let us first discuss what is the basic principle of the electrophoresis.

So, that you will be able to understand each and every factor which is influencing the electrophoretic movement of or the movement of the molecule within the electric field. So, that you will be able to utilize those factors to bring the better separation.

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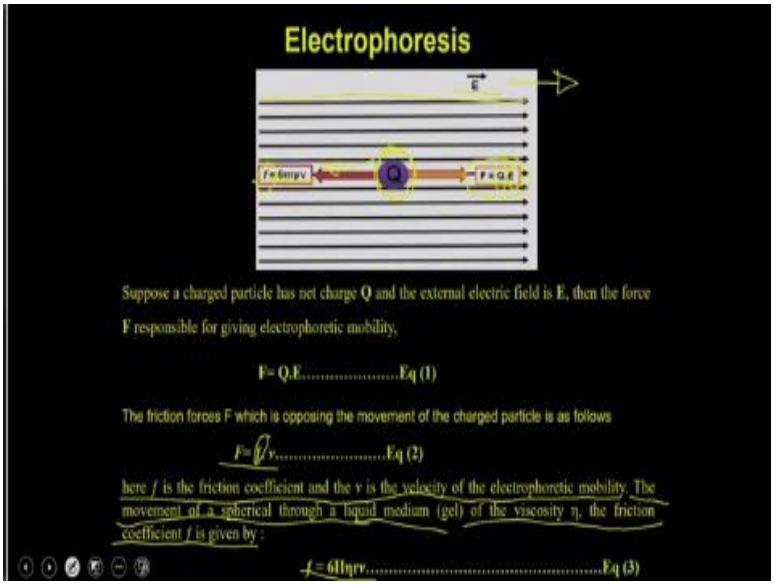

So, you can imagine that a charged particle Q is moving in a electric field E and when a charged particle is moving in electric field it is actually going to experience a electrophoretic force which is going to be in the direction of the electric field. And the quantity is going to be the F is equal to Q E which is where the E is the strength of the electric field and q is the charge of this particular particle.

But when a molecule is moving in an electric field, and if the material through which this molecule is moving is actually going to experience the frictional forces. The frictional forces are going to be oppose the movement of this molecule into the electric field. And frictional forces are going to be equivalent to the f v where the f is the frictional constant. So, f is the frictional coefficient and the v is the velocity of the electrophoretic mobility.

This movement of a spherical object through a liquid media of the viscosity n, the friction of coefficient f is given by the formula 6 pi eta rv. Which means if a molecule is moving through a liquid media which is suppose the gel. Then the viscosity and the friction are going to take place. And as a result the frictional forces are going to be equivalent to the 6 pi eta r and v.

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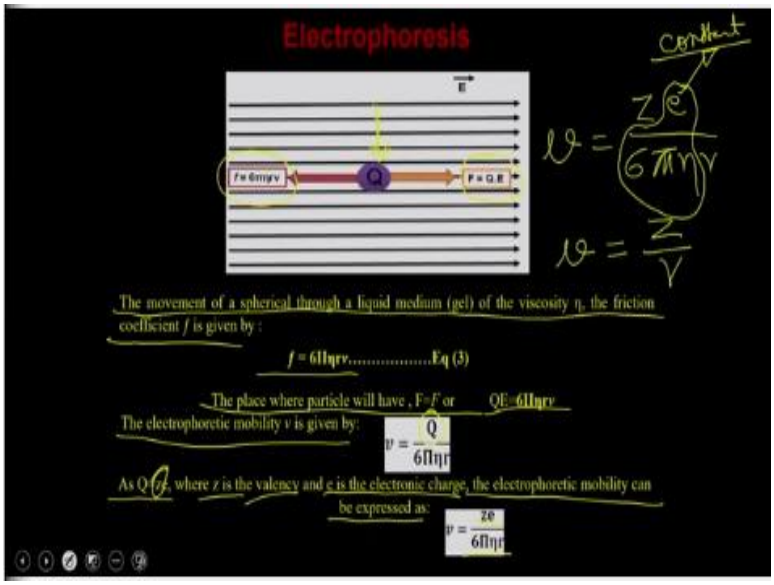

And now, let us imagine that this molecule will stop making the any kind of movement. So, the place where the movement of a spherical molecule of the viscosity and the friction coefficient f is given by the 6 pi eta rv. And the place where particle will have both the forces equivalent or both of these forces are equivalent. That is the place where the molecule is going to stop the movement.

And that place the QE which means the electrophoretic forces as well as the frictional forces are going to be equivalent. So, at this place the electrophoretic mobility will be given by the formula v is equivalent to Q by 6 pi eta r. Whereas, the Q is equivalent to the ze which means if the molecule is made up of the biomolecules, you can calculate the charge simply by having the valency where the z is the valency and the E is the electronic charge.

So, if you multiply the both the thing you are going to get the charge**,** so the electrophoretic mobility can be expressed as the ze by the 6 pi eta rv. So, what you see is the ze by the 6 pi eta r.

And what you see here is that this component is actually a constant which means, it is not going to be dependent onto the molecule which means the v is directly proportional to the z by the r.

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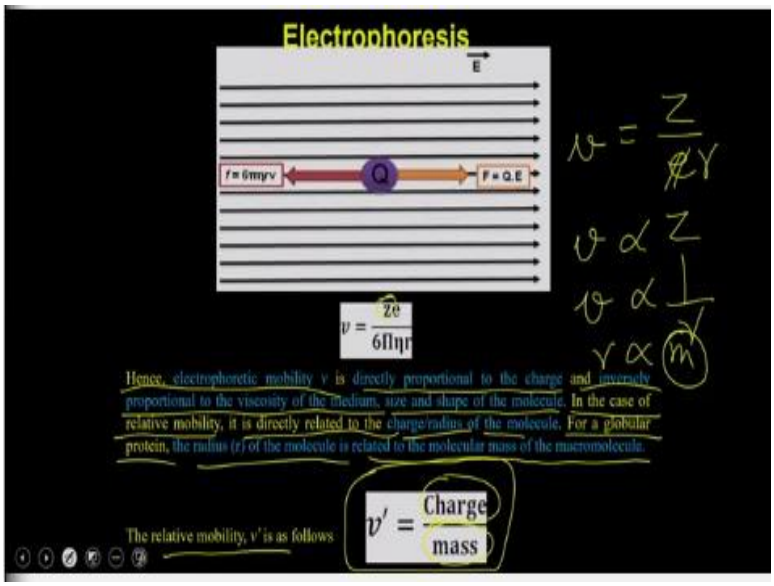

So, hence the electrophoretic mobility is directly proportional to the charge which is this, and the inversely proportional to the viscosity of the media, size and shape of the molecule. In the case of the relative mobility, it is directly related to the charge by a radius of the molecule. That is what we are said right v is equivalent to z by e which means v is directly proportional to the z and v is inversely proportional to the 1 by r.

So, this is r actually, which means and r is directly proportional to the mass of the molecule. So, for a globular protein, the radius of the molecule is directly related to the molecular mass of the macro molecule, which means the relative mobility v is directly proportional to the charge and inversely proportional to the mass. So, this is the relationship between the electrophoretic mobility and the charge of the mass can be exploited to separate the different types of molecule.

Because the different molecules are going to have the different charges and they also going to have the different masses. And this is the way that people have thought that if we resolve the molecules in the electric field we can be able to separate the molecule utilizing this particular property. So, for resolving the molecules they have designed different types of the electrophoretic operators.

Because what you suppose to understand is that you are interested to do the electrophoresis but you are not interested to do the electrolysis. Electrolysis is a process where the molecule will run through the electric field and then they will reach to their respective electrodes and there the molecule is going to be broken down into the individual atoms and that is how the molecule is going to be broken down with the help of the electric field.

So, that is called as the electrolysis whereas in the case of electrophoresis the molecule is always remained intact. And it runs through the electric field, so that it will be get separated from the other molecule based on the charge by mass.

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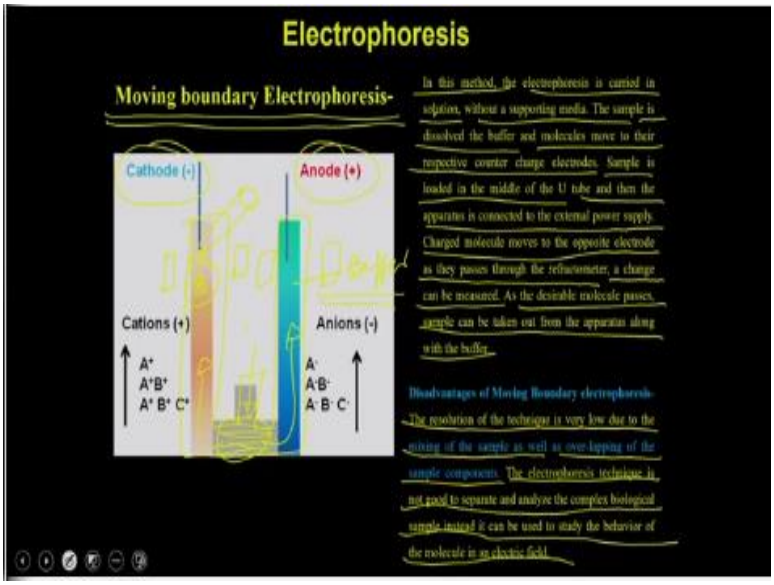

So, the first apparatus what people have designed is called as the moving boundary electrophoresis. So, in this method the electrophoresis is carried out in solution without a supporting media. The sample is dissolved in buffer and the molecule moves to their respective counter charged electrode. Sample is loaded in the middle of the U tube and then the apparatus is connected to the external power supply.

The charge molecule move to the opposite electrode as they pass through the refractometer, a change can be measured. As the desirable molecule passes sample can be taken out from the apparatus along with the buffer. So, in a moving boundary electrophoresis what they have done

is they have made a U color tube ok. And in this U color tube they have put the cathode on one side and the anode on the other side and this is connected through a sample loading chamber.

So, from this chamber you can actually load the samples then the sample is going to be filled into this middle tube. And then they are going to connect the cathode and anode to the external power supply. So, what will happen is the positively charged molecules are going to run towards the cathode whereas the negatively charged molecules are going to be move towards the anode. And on the terminal end of these tubings they have put the refractometers on both the sides.

So, what will happen is refractometer is a apparatus which actually measures the change in the refractive index of a particular solution. So, what will happen is as soon as the molecule will pass through these refractometers there will be change in the refractive index of this particular buffer. And as a result they will know that when a particular molecule is coming out but that is very, very ambiguous, that is not very accurate.

And on the other hand there are multiple problems when they were going through with the moving boundary electrophoresis, what are these problems. The resolution of the technique is very low due to the mixing of the sample as well as the overlapping of the sample component. For example, if you load the sample in the middle because all these is what you are doing in a buffer which is actually not having any supporting media.

So, as soon as you turn on the electric field, the ions are moving towards the counter electrodes but as soon as you turn off the power they all start mixing together ok. And because it is happening in the free media, there will be a mixing of the samples, there will be no separations and there will be no way that you would know which molecule is moving or passing through a refractometer.

So, because of that it always have a problem of the ambiguity because you have to standardize very nicely. So, that you will know when your molecule of interest is going through the refractometer. So, that you can actually put the pipette and could be able to take out a sample

from the moving boundary electrophoresis apparatus. On the other hand the electrophoresis technique is not good to separate and analyze the complex biological sample.

Instead it can be use to study the behavior of the molecule in a electric field, which means this apparatus was good enough to study the behavior of the molecule, which means, it will say whether the molecule will move towards the cathode or molecule will move towards the anode. But it was never been the good enough to separate the complex biological mixture, for example if you load the E. Coli lysate or mammalian cells lysate, it will not be able to resolve the samples.

Because for resolving you do not have anything were supporting media, so that it will actually going to experience any kind of frictions or any kind of you know separation of the molecules. So, because of that the moving boundary electrophoresis was not very popular and then people have started developing the new techniques.

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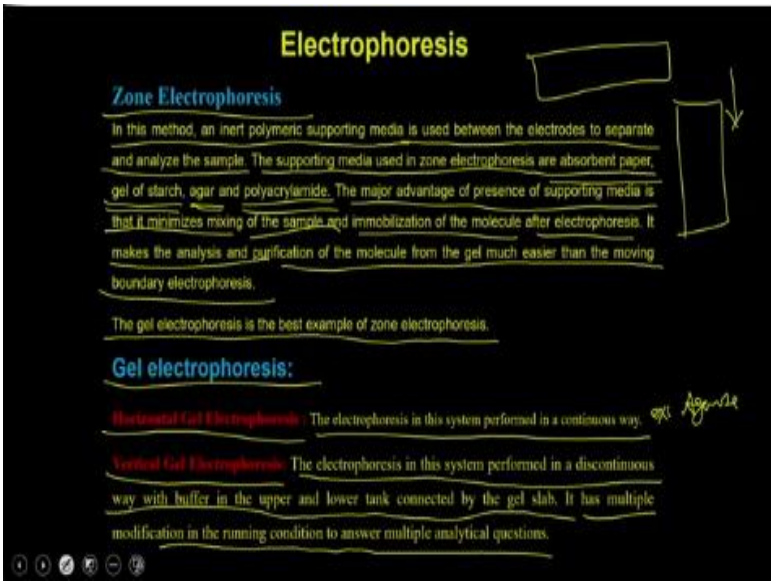

Then they have developed the zone electrophoresis. So, in this method an inert polymeric support media is being used between the electrode to separate and analyze the sample. The supporting media used in zone electrophoresis are absorbent paper, gel of starch, agar and polyacrylamide. The major advantage of the presence of supporting media is that it minimizes mixing of the sample and immobilization of the molecule after the electrophoresis.

It makes the analysis and the purification of the molecule from the gel easier than the moving boundary electrophoresis. So, the major change what people have done when they were moving towards the zone electrophoresis is, that they have started putting the some kind of supporting media, which means they have putting some polymeric substances or some kind of gel. So, that the molecule will be immobilized onto those.

So, because of that the molecule will get separated and you can be able to visualize them because they are immobilized onto the supporting substance. And then you can stain and destain those supporting substances to know what will be the pattern. And then accordingly you can optimized the suppression techniques and accordingly you can be able to achieve the better purifications.

The substances what they were using for the supporting media are the paper, the starch, agar and the polyacrylamide. Accordingly the moving boundary gel electrophoresis is also been called as the gel electrophoresis. And within the gel electrophoresis you have the 2 mode in which you can be able to perform the gel electrophoresis when is called as the horizontal gel electrophoresis, which means you are performing the gel electrophoresis in the horizontal direction.

So, the gel electrophoresis in this system performed in a continuous way, the classical example of this is the agarose gel electrophoresis, then you have the vertical gel electrophoresis. So, in a vertical gel electrophoresis you are performing the electrophoresis from top to bottom. The electrophoresis in this system performed in a discontinuous way with the buffer in the upper and lower tank connected by the gel slab.

It has the multiple modification in the running condition to answer the multiple analytical questions. So, this zone electrophoresis is also been called as the gel electrophoresis and the gel electrophoresis can be performed in 2 different mode. Either the horizontal gel electrophoresis where the system is going to be continuous and the second is the vertical gel electrophoresis where you are going to have the discontinuous system, where the gel is been placed between the upper and the lower tanks.

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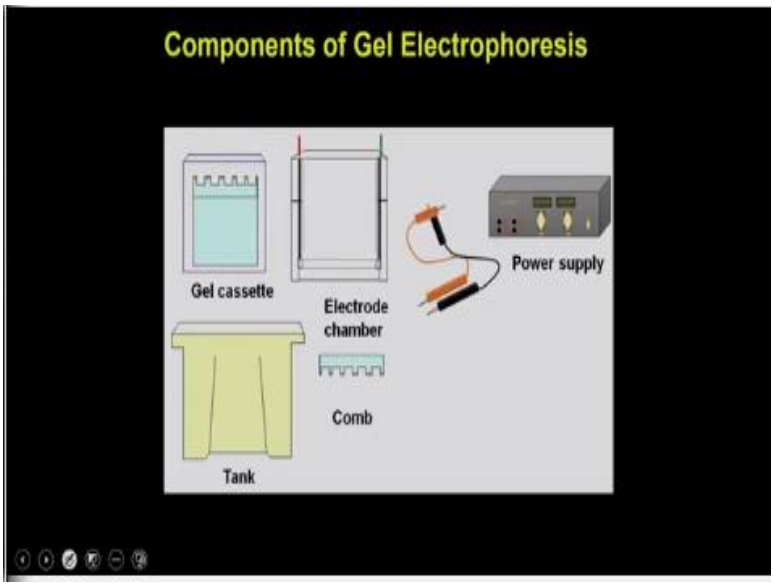

So, these are the components of the vertical gel electrophoresis where you have the gel cassettes, electrode chambers. Then you have the tank where you are going to place this, then you have to have the comb, then you have this electrodes or the power cord. And then you have the power supply unit which is actually going to supply the desirable power between the electrodes. So, these are the electrodes what is being present into the electrode chamber.

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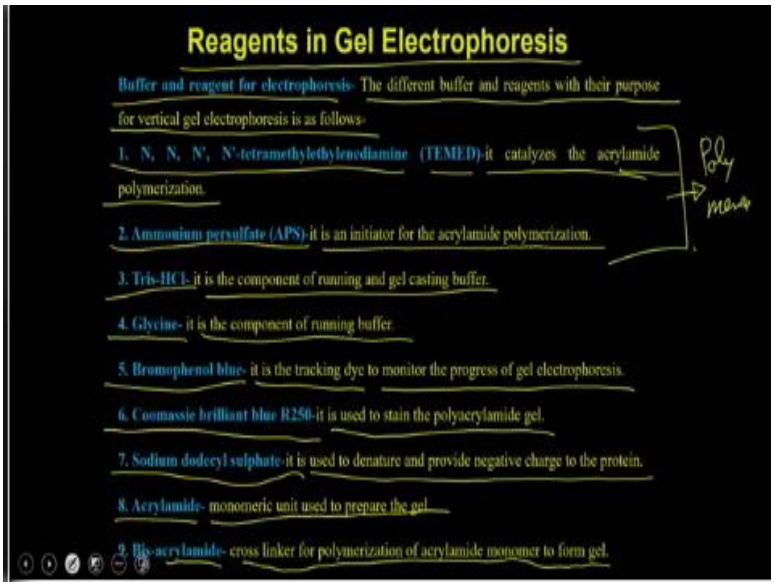

The reagents what you are looking for the vertical gel electrophoresis. So, the buffer and the reagent for the electrophoresis, the different buffers and reagent with their purpose for vertical electrophoresis is as follows. The first thing what you need is the TEMED or the N, N, N, N-

tetramethylethylenediamine. And the TEMED is a catalyst, it catalyses the acrylamide polymerizations.

Then you require the ammonium per sulfate or APS, it is the initiator of the acrylamide polymerization together these two components are required to polymerize the acrylamide. Then you require the Tris HCL, so Tris HCL is required to prepare the running as well as the gel casting buffers. Then you have the glycine which is a component of the running buffer, then you have the bromophenol blue.

So, bromophenol blue is a tracking dye and it is required to monitor the progress of the gel electrophoresis. Then you require the coomassie brilliant blue R250 which is a staining dye which is actually going to stain the polyacrylamide gel. Then you require the sodium dodecyl sulphate, sodium dodecyl sulphate or the SDS. It is used to denatured and provide the negative charge to the proteins.

And then you require the acrylamide, so acrylamide is the monomeric unit use to prepare the gel. And then you require the bis-acrylamide which is the cross linker for polymerization of the acrylamide monomer to form the gel.

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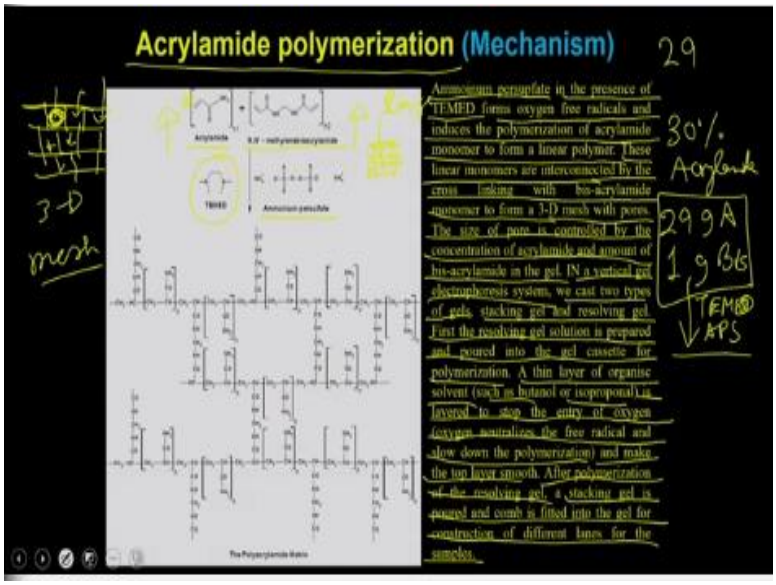

Now let us see how the acrylamide is going to be polymerize, so what you do actually is you are actually always making a gel solution which is actually the 30% acrylamide solution. So, in the 30% acrylamide solution what you do is, you add the 29 grams of acrylamide and then 1 grams of the Bis-acrylamide. And keeping these together when you are adding the TEMED and APS that actually is catalyzing the cross linking of the acrylamide monomer with the bis-acrylamide.

So, how the acrylamide is going to be polymerize is, that you have the acrylamide and then you have the Bis-acrylamide. And when you are incubating these with the TEMED and the APS, what happened is that the APS which is ammonium per sulphate in the presence of TEMED forms the oxygen free radicals and induces the polymerization of acrylamide monomer to form a linear Polymer.

These linear polymers are interconnected by the cross linking with the bis-acrylamide monomer to form a 3-dimensional mesh with the pore. So, what happened is, this acrylamide monomers are being polymerized, so they will form the fibers like this. So, because these 2 molecules are forming the radicals and suppose they form the radicals on one molecule. These radical-radical is actually it interacting with each other and that is how they are actually making the polymeric fibers.

And then these polymeric fibers are also been connected with the help of the bis-acrylamide because the bis-acrylamide has the cross linking groups on the both side. And that is how you are actually making a 3-D mesh which is actually containing the pores in between. And these pores are always been used for the biomolecule to the pass through. So, because of this mesh you can be able to experience or you can be able to produce the friction for the different types of molecule.

And that is how the friction is going to play in separation of the molecule. So, because this mesh has the sizes of different sizes these mesh is going to play a kind of a separation filter or something like that. So, that it will actually going to help in getting the separation of the biomolecules. The size of the pore is controlled by the concentration of the acrylamide and the amount of bis-acrylamide which means.

If you increase the bis-acrylamide concentration or if you increase the acrylamide concentration, you are actually going to decrease the pores. Because you will be keep putting more and more fiber and as a result the pore size is going to be smaller and smaller, which means you cannot be able to use a very, very highly crosslinked gel with a large protein. Because if you use the large protein, the large protein will not be able to enter into these pores and as a result it will be get excluded from the gel.

In a vertical gel electrophoresis system we cast 2 different types of gel stacking gel and the resolving gel. First the resolving gel solution is prepared and poured into the gel cassette, for polymerization a thin layer of organic solvents, such as butanol or the isopropanol is layered to on the top to stop the entry of oxygen, why it is so. Because the oxygen neutralizes the free radicals and slow down the polymerization and mix the top layer smooth.

After polymerization of the resolving gel, a stacking gel is pour and the comb is fitted into gel for construction of the different lanes for the sample.

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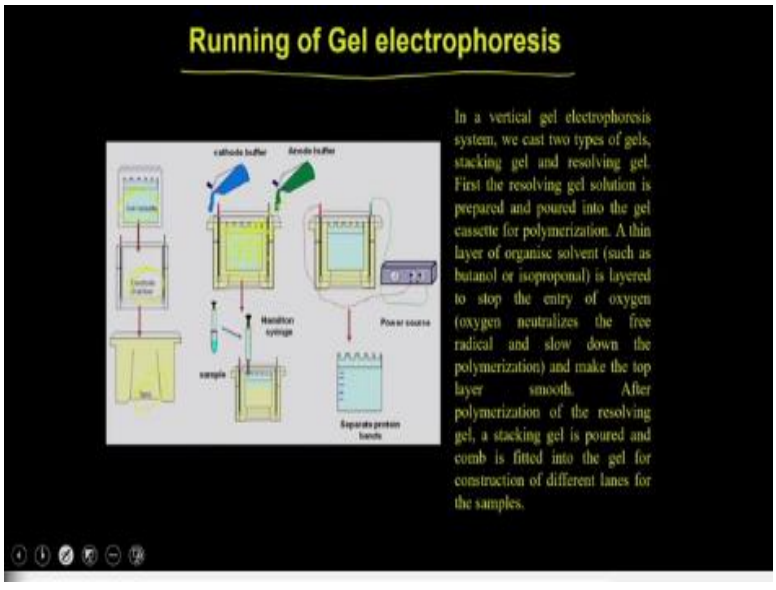

So, the running of a vertical gel electrophoresis as I think discuss the you need a gel cassette then you need a electrode chamber, then you need a tank. And what you do actually is that you first pour the resolving gel because these are the gel which is going to use for separation of the

molecule. And then on top of that you are actually going to put the stacking gel the difference between the resolving and the stacking gel is in terms of decomposition of the acrylamide.

As well as the pH of the buffer what you use for these two gels are also different. And then when you cast the resolving gel, you actually put the layer of the organic solvent. So, that it actually helps in terms of the polymerization of the acrylamide.

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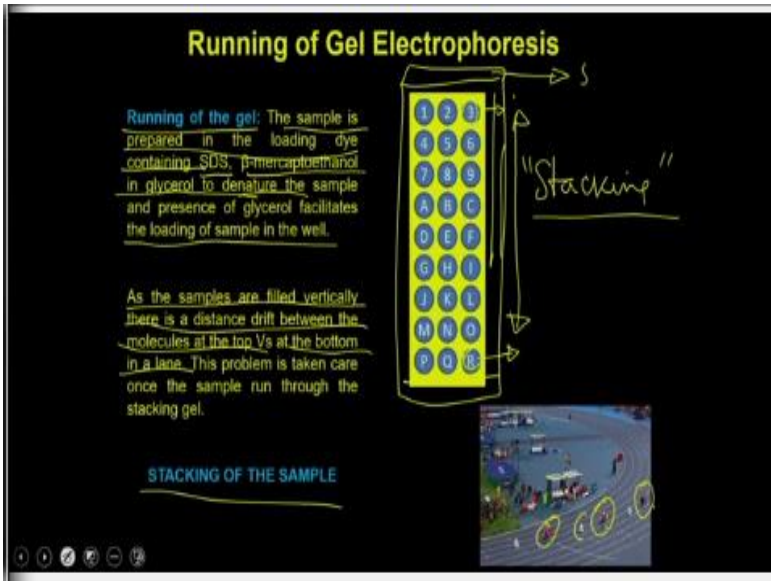

The running of the gel, the sample is prepared in the loading dye which contains the SDS which means the denaturing agents beta mercaptoethanol which is actually going to break the disulfide linkages. And you need and all these you will be presenting the glycerol because the glycerol is required to provide the density in the sample. So, that it facilitate the loading of the sample in the well.

Now, since these wells, so you can imagine that this is a typical well with where you have loaded the different types of samples. Since all the samples are filled once vertically there is a distance drift between the molecule at a top versus the molecule at the bottom in a lane. So, you can imagine that you have this is a lane, where you have different types of molecules and all these molecules are having.

For example a molecule number 3 and the molecule number R, all these molecule, imagine if the 3 and R of a same molecular weight even then they will be a drift of the distances between the 2. Because, so if you resolve them without going through the stacking of the sample or without putting them at the same place for running they will be having a drift, you can understand that simply by looking at the racing track.

So, if you see a racing track what you see is that the players are sitting at a very different location. Because all these different locations the diameter of the circle is different. But in this case, you do not have such options, so what you have to do is by any mean you have to bring the 3 and the R together. And you can do simply if you do a stacking of the samples. Now question is how you can be able to stack the sample.

So, to stacking of the sample, you have to first put a stacking gel. So, that is why the well has to be prepared in the stacking gel and you have to stack the sample.

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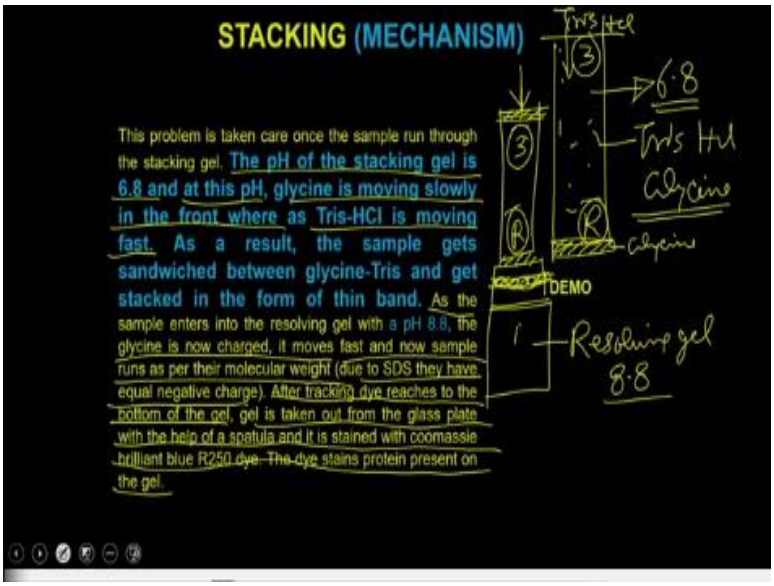

And why the stacking gel is actually doing the stacking because the pH of the stacking gel is 6.8 and at this pH the glycine is moving slowly in the front whereas the Tris HCL is moving very fast. So, you can imagine that you have this well, where this is the sample number 3 and this is the sample number R which is very, very far away. But these samples are present in a buffer which is actually having a composition of Tris HCL and glycine.

So, and the pH of this gel is 6.8 which is actually the pH of the stacking gel. So, at 6.8 the glycine is having the very, very low electrophoretic mobility. Because of that there will be a blockage of glycine in the front. So, you have a glycine which molecule which are sitting in the front and then you have the Tris molecule which are actually present in the from the top.

So, because of that you can imagine that you are putting the molecules the 2 molecules like number 3 molecule and the R molecule between the like where the Tris is going to be used as a plunger. So, what happen is the Tris is pushing the molecules whereas the glycine is not allowing these molecules to move as per their electrophoretic mobility. So, because of that all the molecule will reach to this point and they will be able to get stacked.

But, what happen is and this will happen because they will be keep running into the stacking gel for some time. And during this period only the 3 and R are going to come together and they will form a single band which is actually going to contain 3 and R together and that will happen while they are running into the stacking gel. But as soon as they will enter into the resolving gel, so after this they will enter into the resolving gel.

So, resolving gel has a pH of 8.8 which means at this pH the glycine is now charge and it move fast and now sample run as per their molecular weight because the SDS is going to provide them the equal negative charge. After the tracking dye reaches to the bottom of the gel, gel is taken out from the glass plate with the help of a spatula and it is stained with coomassie brilliant blue R250, the dye stains protein present on the gel.

So, as soon as it enters into the stacking dye, this blockage of the glycine is going to be removed. And as a result the proteins or the molecules are going to be resolved based on the molecular weight, the higher molecular weight are going to run slower and the smaller molecular weight are going to run smaller. And because you want to know how much the molecules are going to or travelling into the resolving gel, you are also using the tracking dye that is the bromophenol blue.

And to so bromophenol blue is a very, very small dye, so it runs in the front and as soon as it reaches to the end of the gel, you will be able to know that ok the electrophoresis is now over. And then I can stop the gel and I can take out the gel and I can do the staining and destaining and to see what is the pattern of the gel.

So, this is all about the theoretical information of the electrophoresis, how the history of the electrophoretic operators, how the operators is been evolved by exploiting the or by developing the different types of electrophoretic operators. So, but this is all the theoretical knowledge. Now, I would like to take you to my lab and I would like to show you a small demo, how to cast the gels and how to perform the electrophoresis.

And in this demo the students have dissolve the samples and then they have also shown you how to stain and destain. **(Video Starts: 29:55)** In this video, we will demonstrate you how to run a SDS-PAGE gel how to prepare various reagents required for the running of SDS gel and what are the different instruments we can use. So, here this is the gel casting stamp, so where we can use this glass plates to prepare the gel. In between there is a space where we can pour our gel, gel solution.

Then we will keep for some time at least 20 to 30 minutes, let it solidify, then we will prepare stacking gel, then we will note the our protein solution. So, here before doing that we need some reagents, so what are those reagents, the first reagent we need for this experiment is acrylamide. So, generally we will prepare acrylamide 30%, 30% means 29 grams of acrylamide and 1 gram of bis-acrylamide.

These both we can use 29:1 ratio in 100 ml of water to get 30% of acrylamide. So, both these are neurotoxic, so we have to wear gloves always, after this we have to prepare resolving gel. For resolving gel, we need 1.5 molar Tris HCL, pH 8.8. In addition to that we also need 10% SDS prepared in double distilled water and also 10% ammonium per sulfate and also TEMED. The role of ammonium per sulphate and TEMED we can see during preparation of gel, they acts as a catalyst. After solidifying we have to use, we how to prepare stacking gel, so stacking gel is nothing but composition is same.

But we can say it is a diluted, it contains pH 6.8. Tris HCL and remaining components same but in less quantities. So, after preparing the gel, we load the marker and the protein which is denatured at 100 degrees Celsius for 3 minutes. After that we will fix this gel into this one, we keep getting this reservoir then we will connect to the power pack and run the gel.

So, this is the overall introduction of how to prepare a SDS-PAGE gel. So let us start to preparing gel, we will learn more things like preparing the gel. Before preparing the resolving gel, we have to prepare setup the casting gel. So, this is the glass plates, this is a very thin one, so this is the main glass plate, this is 1.5mm glass plate , it is available in 1mm glass plates also. If your loading solution is less like you want to load only 20 microliter, 30 micro liter then 1mm gel is good enough.

But if you have extended volumes like 70 micro liter, you can use 1.5mm, you have to arrange like this share plates on this. And the bottoms should be equal, then we have to put in this one this tray. Then we are going to keep this, so we have to check if we perfectly setup this one, then there should not be any leakage. But if there is any leakage you are resolving gel may leak out and you will get nothing.

So, in that case we have to check it prior to pouring the gel. So, whether it is ok or not, so I am going to use milli Q water just after checking the gel if there any leakages or not. So, we have moved forward by preparing resolving gel, so the competition is given in this slide, please go through that slide. This is just water, first I use water I am going to add sequentially 4 ml of water. Now, how to add 3.3 ml of already prepared 30% of acrylamide.

Already in introduction, I explained how much percentage we have to prepare and how much quantities of acrylamide and bis-acrylamide need to take. So, here we have to add 3.3 ml acrylamide solution 30%, so I have to adjust the 300 micro liter. The next component is 1.5 molar Tris pH 8.8, we have to add 2.5 ml, next component is SDS. Here SDS plays as dual role, like one thing is that it gives negative charge, cross negative charge on the polypeptide chain.

The next component we have to add is SDS, 10% SDS we have to add 100 micro liter of SDS to reserving gel. It plays a very crucial role in polyacrylamide gel electrophoresis. Like it imparts negative charge and the polypeptide chain, so that despite of their charge they will move based on the molecular weight. So, I am going to add SDS, the other important thing is that 10% ammonium per sulfate, ammonium per sulfate which is catalyzed by TEMED provides free radical species which accelerate the farming mesh like shape in acrylamide gel like it will catalyze forming the mesh.

So this is the 10% APS, I just add 100 micro liters of 10% APS to resolving gel. In final step we have to add TEMED, TEMED after adding all the components at the end of the gel, we have to add TEMED. Because if you add earlier it will quickly facilitate the polymerization, so you cannot take out with the pipette. So, it completely solidifies, so that is why you have to add at the end of the resolving gel.

So, at I am going to add 5 micro liter of this TEMED which catalyses the ammonium per sulphate, ammonium per sulphate it turn provides free radical species and free radical species accelerate the polymerization, this is the overall principle of (()) (40:53). So, I will add, we have to mix properly then add slowly it one corner, so after this we have to overlay it on the top layer we have to overlay some solvent like 2-butanol or isopropanol or with water.

So, why we are doing this, because if the gel is exposed to air then the oxygen from the air will interfere in the polymerization of the gel, so we have to add wither water or 2-butanol for this purpose. Now we have to check whether it is solidified or not, so it is solidified, now we have to remove the overlying layer like we have used water. So, no need to remove, if you are using isopropanol or butanol you have to remove that and wash with the milli Q water.

So, now we will start preparing the stacking gel, the compositions are given in the video, you have to add 3.4 ml of water first. Next lay 30 micro liter of acrylamide, 630 micro liter of Tris HCL pH 6.8, 50 micro liter of TEMED and 50 micro liter of SDS we have to add. At the end we have to add 5 micro liter of TEMED, we have to mix properly after adding the TEMED and you just add at one corner.

Next we will keep comb, now we will wait until the gel got solidified, then we will shift it to buffer tank and then we will run the gel. Well, the stacking gel is solidifying, we have to prepare sample for loading in SDS-PAGE gel. So, for that we have to prepare loading dye 10X or 6X loading dye, it mainly context 250 millimolar Tris pH 6.8, 30% glycerol, 10% SDS and 0,05% of bromophenol blue.

So, here we can add 10 millimole of DDT also as a reducing agent SDS mainly works as imparting negative charge in the polypeptide chain. And DDT based on the disulfide bond, if you have dimer which you can see as a monomer in SDS-PAGE. Suppose you have 20 kda, 20 kda that means 40 kda protein which is a dimer actually, you can see only 20 kda band corresponding to that protein.

Because DDT breaks down the disulfide bond and you can see only single band. If you want to see actual molecular weight, you have to run it on native place where there is no reducing agent or no detergent. The other thing is glycerol, while loading the gel (()) (49:13) is not that much dense, it may come out from well. So, in order to prevent this thing, we have to load with the denser solution like glycerol.

So, 30 to 50% glycerol is substance for keeping the protein solution intact in the bottom of the well. So, other thing bromophenol blue, bromophenol blue we use for just tracking the how much gel completed. So, this is the loading dye, so we have to take the protein solution here we already prepared a 10% of loading dye. So, that means this is 10X loading dye, we have to prepare 1X to mix with the protein solution.

So, this is 100 ml of solution loading solution, we mix 10 micro liter of loading dye to this protein solution. You can tap down or pipette this protein solution then we have to heat it for 3 minutes at 100 degree Celsius. So, that the all the polypeptide chains I mean dimmers are if any multimers are present they will break down and we can see nice band. So, I am going to heat this at 100 degree Celsius for 3 minutes.

This is the remaining of stacking gel solution, so we can see it is solidified, so that means the stacking gel also got solidified. We have to remove that gel and fix it into this one and we have to keep inside the tank. So, just take out the gel, so in inside this tank we only have this side one, you have to cover other side also. So, for that we use the dummy plate, just hold it tight and close this thing after that gradually adjust the gel length.

So, just we have to fix, once fixing here we have to add this running buffer. The running buffer contains 15 grams of Tris, 72 grams of glycine and 5 grams of SDS for 2 liters of solution 1X solution. So, this is 1X I have already prepared, I am going to add, we added in this tank but the main tank surrounding to this one, we have to add up to the mark. So, for difference you can see here for 4 gels we have to add till here the buffer we have to load outside this gel.

So, for 2 gels here , for 1 gel which can add like this, this is the power pack where we can adjust the how many volts we want to run. The protein samples are ready, we heat at sufficient time, now we have to load this. So, we have to remove the comb carefully then first I am going to load marker at protein ladder, next I will load sample. Once the loading was over we have to fix this cascade, I am going to set it 70 volts.

As we can see the it is almost over, so you can take out the gel, then we will do stain and destain it. Generally what we will do is, we will there are 2 ways of staining and destaining process, one is we can do quick staining. Like we have to heat it with the staining solution which contains coomassie brilliant blue and along with ethanol and water. So, then we will try to destain with the water by heating.

But in another way the simplest way is we will just stain the gel for 2 hours then we will destain overnight. So, I am going to show the simplest way, first we will stain in coomassie brilliant blue staining solution, then we will destain in methanol water containing solvent. So, I am going to start the gel run, then I will remove it, I will show you how to remove the gel. Take out the glass plates, here we have to be very careful while taking out gel otherwise the share plates may broke.

On a corner we have to take and lift the gel like this, so keep the gel in a staining box which is more or less a plastic one but it can sustain the, so then I am going to add staining solution, I will keep for a location for on shaker for at least 2 hours, then we will destain overnight. So, once the time is over, after 2 hours we will destain the solution. Now we kept 2 hours in staining solution and we can see the staining is over like you can see the gel completely turned into blue.

So, we remove the solution then I am going to add destaining solution and I keep this on a rocker for 2 hours for destaining. So, the composition contain per 100 ml of destaining solution for 50 ml of water double distilled water and 40 ml of methanol and 10 ml of glacial acetic acid. So, I am going to keep this on a rocker, we have run the gel and stained and destained, now we will capture the gel image also.

So, you can see manually also but for record purpose, we have to capture it through gel doc, so this is the gel doc imaging system from Biorad. So, I will show you how to capture the images. So, here we will use white tray, there is another one grey or UV trays also there. So, there you can see any fluorescent one or stained with the iridium bromide or BLAST, chemiluminescence you can use that. But for normal protein imaging we can use this white tray. So, I am going to keep the gel on this one, so you have to open properly.

This is very important step, you have to align the tray in a proper way, so otherwise it will show error. So, once it is over just push it back, so you have to log onto account, so this is SDS-PAGE gel, you can select the application whatever you want. So, here nucleic acids, protein gels, BLAST 3 different categories are there. So, we are observing here protein gels, protein gels stained with the coomassie blue or white tray, we are using white tray.

So, this is the right tray, you can use coomassie blue stained one grey tray also but we are using as we are using white tray, so we will use coomassie blue. So, auto optimal then I will ask for capture, so it will take 1 to 3 minutes based on the signal intensity. So, as we can see it is optimizing the signal intensity, you can minimize this one also, so that you can see the gel image.

So, now it is over, if you want to do any modifications to images for suppose you want to decrease or increase the signal intensity. So, this kind of changes you can do, so if you want to send this gel, you can have send and save. If you have any drive connected to this one, you can send directly to that one that thing. So, for image analysis part we will show in the upcoming video how to analyze the what this band of interest correspond to which molecular weight.

So, we already loaded the molecular weight 1, so we can easily find out using Imagelab software. In this video before learn that how to prepare a SDS-PAGE gel and how to run it, what are the precautions need to be taken while preparing the gel. And how to observe how to record the gel using gel documentation system. So, I hope this will give you gist of how to prepare and run a SDS-PAGE gel and analyze the protein sample. **(Video Ends: 1:04:46)**

So, with this demo we have shown you the different steps what is required to prepare the acrylamide solutions and then how to pour how to prepare the resolving gel and the stacking gel, how to load the samples and then how to perform the electrophoresis. And then ultimately we have also shown how to do the staining and destaining, I hope that the this demo video could be helpful to understand the electrophoresis the vertical gel electrophoresis.

So, with this we would like to conclude our lecture here, in our subsequent lecture we are going to discuss about the imaging and the staining and destaining. And how to analyze the gel picture what you are going to capture just after the electrophoresis. So, with this I would like to conclude our lecture here, thank you.