Genetic Engineering: Theory& Applications.
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Module IX
Characterization of Isolated Product (Part-1)
Lecture 29
Electrophoresis (Part-1)

Hello everyone, this is Doctor Vishal Trivedi from Department of Biosciences and Bio Engineering IIT Guwahati and what we have discussed so far, we have discussed about the overexpression of the recombinant factors within the host cells, in the previous modules we have also discussed about many properties of the host as well as the plasmates and all other kind of molecular biology protocols.

And now in the previous module we have discussed about how to overexpress these factors into the host cells and then subsequently we had also discussed about how to purify them using the different chromatography techniques. And now in today's lecture we are going to discuss about how to characterize the product which you are going to get from the host cells.

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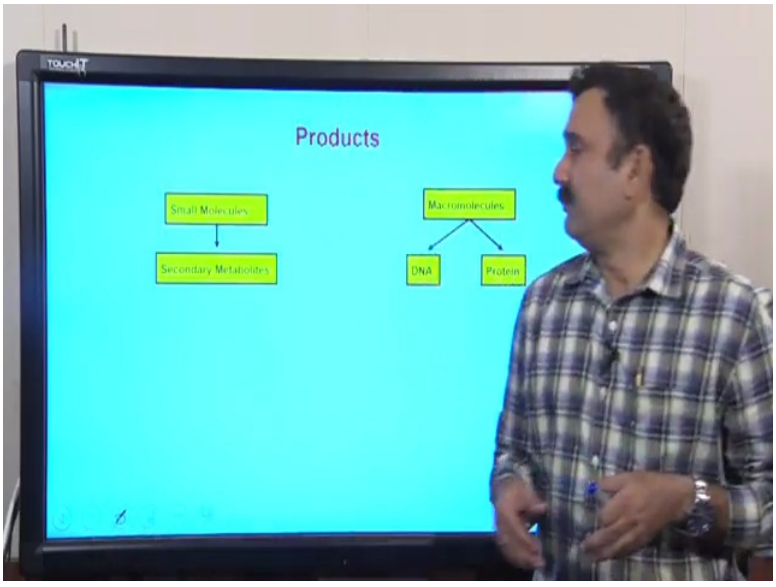

So as far as the product is concerned in the biotechnology you can have the 2 different types of products. The products which are nonbiological in nature or the products which are of small molecules and the products which are of biological in nature such as the protein or the DNA.

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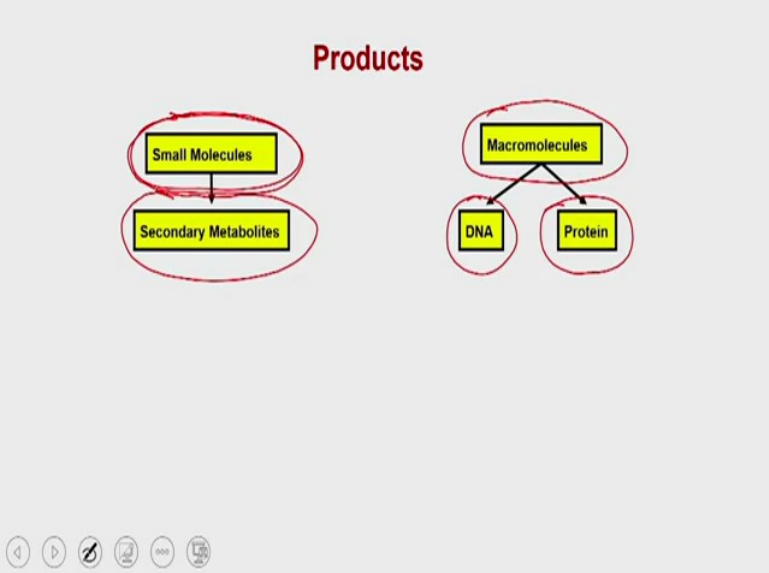

So when you do this kind of overexpression and purification and then suppose you employed the biotechnology related principle. You could have either the small molecules or you could have the macromolecules. When I say the macromolecules we are only going to focus about the DNA as well as the proteins. So you can have the different types of DNA which you are going to overexpress or which you are going to produce in the host cells, simply you can have the different types of proteins which you can produce for different applications.

Either it is for the therapeutic applications or some other kinds of applications. As well as the small molecule is concerned. A small molecule could be for that you are actually producing the secondary metabolites or you can actually also asking the host cells to modulate its metabolism in such a way, so that the host cell is start producing some of the metabolites in a higher concentrations and so on.

So when the product comes from the host cells you have the either the small molecules. When I say small molecules which means I am talking about the molecule which are of the organic component or the small molecule means which are of very-very small molecular weight, when I say about the macromolecules I am talking about the big molecules like DNA or the protein which you are over expressing in these cells.

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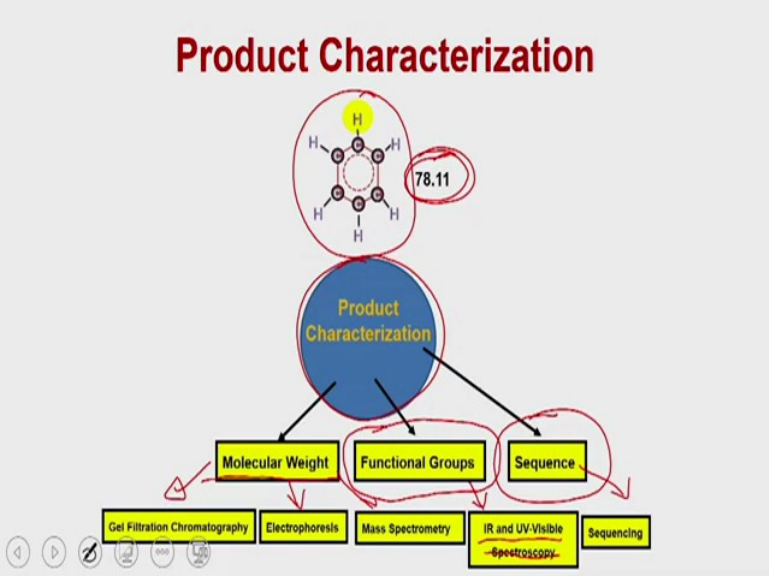

Now, suppose whether you take an example of benzene which is of a molecular weight of 78.11. The product characterization is a very-very critical as well as the important component of the development of a product using the biotechnology related principles and when you talk about the characterization. The characterization can be done on the preset guidelines. For example, you cannot use any random parameter or random criteria to characterize your products.

So the product is always been characterized by a specific set of guidelines and when you say about the product whether it is the small molecule or the macromolecules you have the different properties which you can be used for characterization as well as which can be used very confidently to say that the product what you are developing or whatever you have purified from the host cells are of the good quality.

So when I say about a product, you have the 3 or 4 different properties which can be very surely can be used for characterization. One of the first most property is that you can use the molecular weight of that particular product. For example, in this case of benzene we are saying that the benzene molecular weight is 78.11, but along with that you also have to characterize the functional group of this particular small molecules.

And at the end the third parameter which is also present in the sequence. For example you can have the sequencing of that particular product. If it is a polymeric product than the sequencing will tell you that how the monomeric subunits are being arranged this is more relevant in the case of the polymeric substances.

Such as the DNA or the protein but for the small molecules mostly people are using the molecular weight as well as the presence of functional group on these small molecules to characterize them.

For the molecular weight, we are going to discuss 2 techniques. One is called as the gel filtration chromatography as well as electrophoresis and the third technique what we are going to use is the mass spectrometry whereas for the functional group you can use the techniques such as the IR or the UV visible spectroscopy and for deducing the sequence you can use the sequencing technologies what have been developed for the protein or the DNA.

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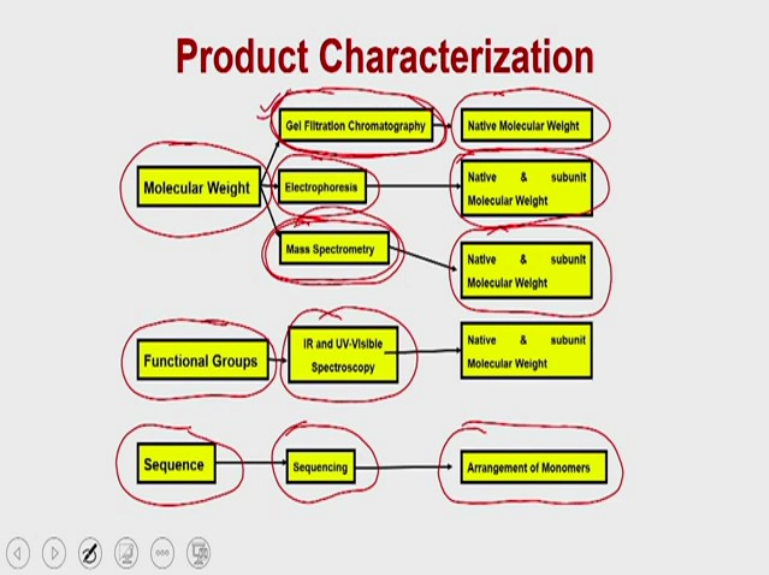

So for the molecular weight you can have the 3 options either you use the gel filtration or you can use the electrophoresis or you can use the mass spectrometry, all these 3 techniques what we are going to discuss in this course are going to give you the different types of molecular weight. So for example in the case of gel filtration you are going to get the native molecular weight, whereas in the case of electrophoresis you are going to get the native as well as the subunit molecular weight.

For example, if you are working with a protein which is of a dimeric protein. So the dimeric could be the homo dimer or hetero dimers, in those cases you can actually calculate the molecular weight under the native condition as well as under the denaturating conditions and the molecular weight what you are going to get under that the denaturating conditions will give you the individual monomer what is the molecular weight of that particular monomer.

Similarly for the mass spectrometry, mass spectrometry is going to give you the native as well as the subunit molecular weight of that particular small molecule or the proteinaceous substances. Functional groups. Functional groups are always been characterized by the IR or the UV visible spectroscopy and whereas the sequence, the sequence will be done by the different types of sequencing technology what have been developed for the protein or the DNA and that actually will give you the information about the arrangements of the monomer in this particular polymeric substances.

So let us start with the first technique that is the gel filtration chromatography. If you remember we have already discussed in detail about the gel filtration chromatography. How the gel filtration chromatography is allowing you to purify the substances and what is the basic principle of chromatography. So we are not going to discuss all those details now, but we are going to tell you that how the gel filtration chromatography can be used to determine the molecular weight of the purified substances.

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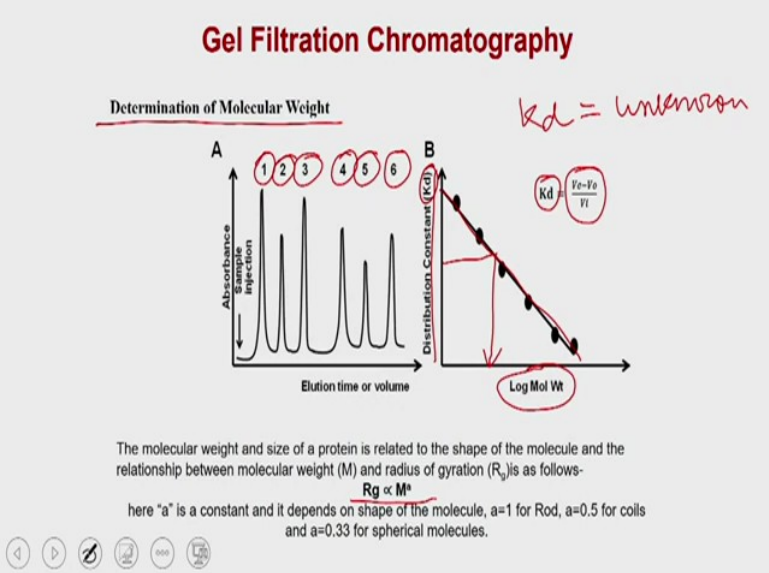

So the determination of the molecular weight by the gel filtration chromatography, if you remember we have discussed this that for determining the molecular weight of an unknown substance or the product what you have produced from the host, what you are supposed to do is, you have to run the different proteins or the different substances with the different molecular weight into the gel filtration chromatography and all these molecules are going to be eluted at a different illusion time or the illusion volume and then what you can do is, you can calculate the distribution coefficient or the distribution constant Kd which is actually being developed by the Kd is equal to Ve minus Vo by Vi.

And that Kd you can plot along with the log molecular weight of this particular molecules and that will actually give you a calibration curve. Now you can use this calibration curve to calculate the molecular weight of the unknown substance. So what you can do is, you can run the unknown substance also along with this under the identical conditions and you can calculate the distribution coefficient or the Kd for the unknown sample or the sample which you are interested to know the molecular weight and then you can use the discalibration curve to calculate the molecular weight.

As it says that the molecular weight and the radius of gyration is directly proportional. So if you remember that the gel filtration chromatography works on the principle of radius of gyration instead of molecular weight or the equation between the distribution constant versus the log molecular weight can be used to calculate the molecular weight of that particular substance.

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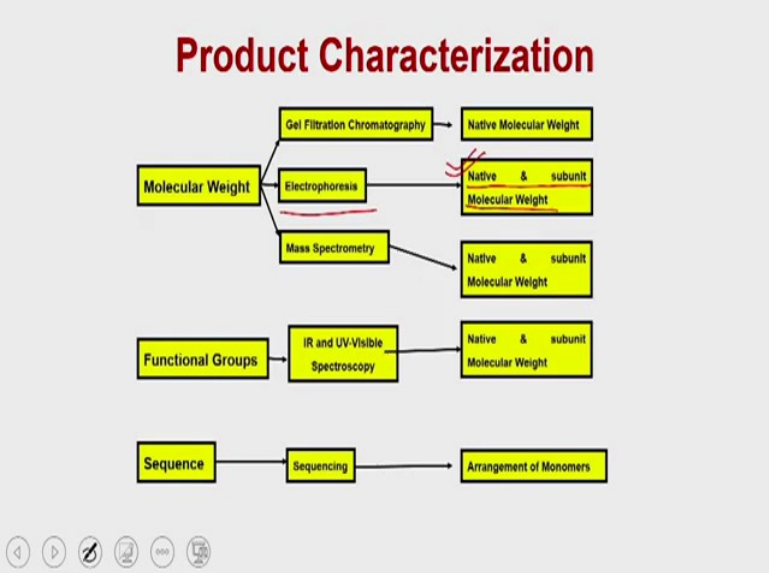

Now we are going to start the discussion about the electrophoresis. Electrophoresis is going to be used to calculate the native as well as the subunit molecular weight. Before getting into the detail of the electrophoresis, let me explain you what is the electrophoresis and how the electrophoresis as a technique is being evolved.

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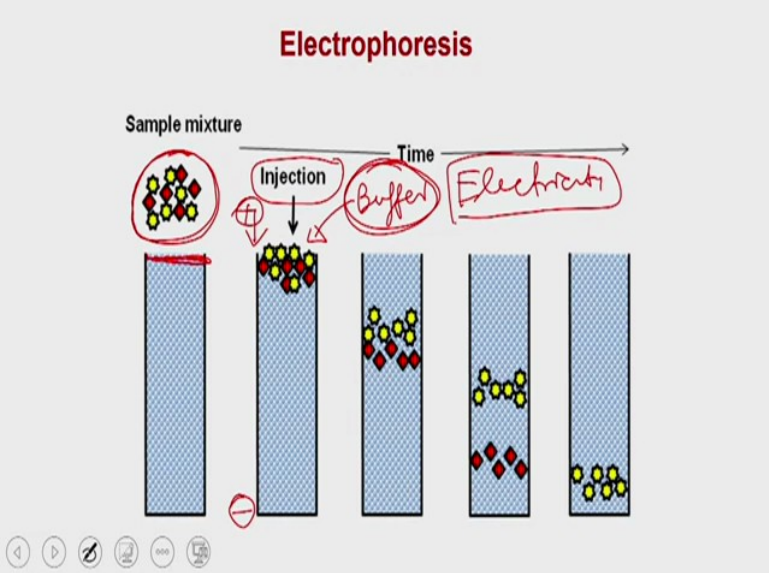

So you can imagine that you are running a chromatography column and what you have done is you have loaded the different types of molecule onto this call and what will happen is, when you inject these molecules they will first run it on to the column and then what you do is, you flow the buffer and because of the buffer these molecules are started distributing between the different planes or different substances and that is how they get separated and then they get immobilized onto the different planes within this particular column and that is how you are actually going to get the purification done.

Whereas, now what is electrophoresis is, that in the case of electrophoresis what you are going to do is, you are going to take the mixture of sample, what you are going to do is, you can elute, you can inject them onto the solid support and then instead of running the buffer what you are going to do is, you are going to put the current and because you put the current with the help of the electrodes what will happen is, these molecules which are already being charged they will going to start migrating to their opposite electrodes.

And as a result instead of buffer you are using the electricity or the current as the force to run the sample within this particular matrix and at the end what will happen is, the molecules are going to be immobilized where they are going to have the net charge and ultimately it is going to be resolved just like as we are doing in the chromatography. The only difference between the chromatography and electrophoresis is that.

In the chromatography you are running a buffer to run the samples along with the column, whereas in the case of electrophoresis you are running the samples in the presence of the field.

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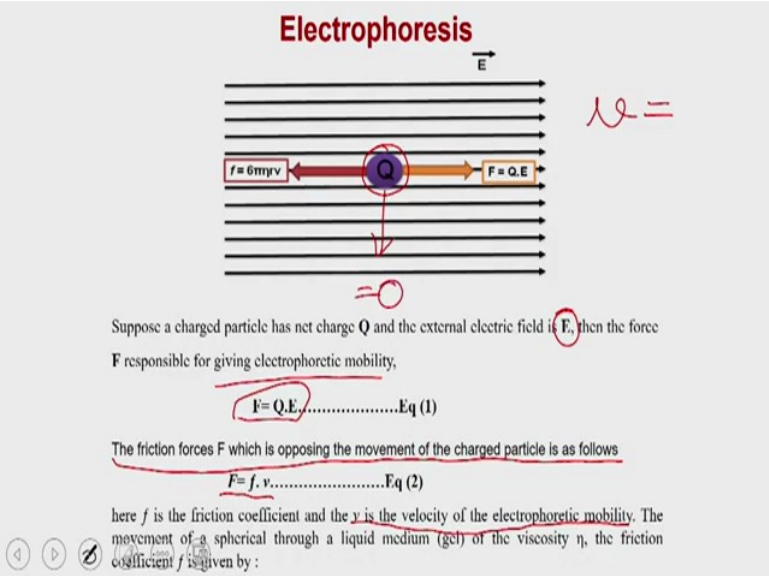

Now, you can imagine that you are having a molecule which is of the Q charge and the external electricity field is E then the force what is a molecule is going to experience means the electrophoretic the force to which will give them the electrophoretic mobility is going to be F equal to Q E.

And you know that if molecule is running along with electricity field it is also going to experience some frictional forces because the molecule is going to experience the friction. So, if the friction force is F is going to oppose the moment of the charge particles which is going to be F is equal to fv where f is a frictional coefficient and the v is the velocity of the electrophoretic mobility.

So, if the molecule is running with a velocity of v the friction is going to be fv where f is the frictional coefficient and the v is going to be the electrophoretic mobility. So where this molecule is going to be immobilized, the molecule is going to be stop running where the net force on this particular molecule is going to be 0.

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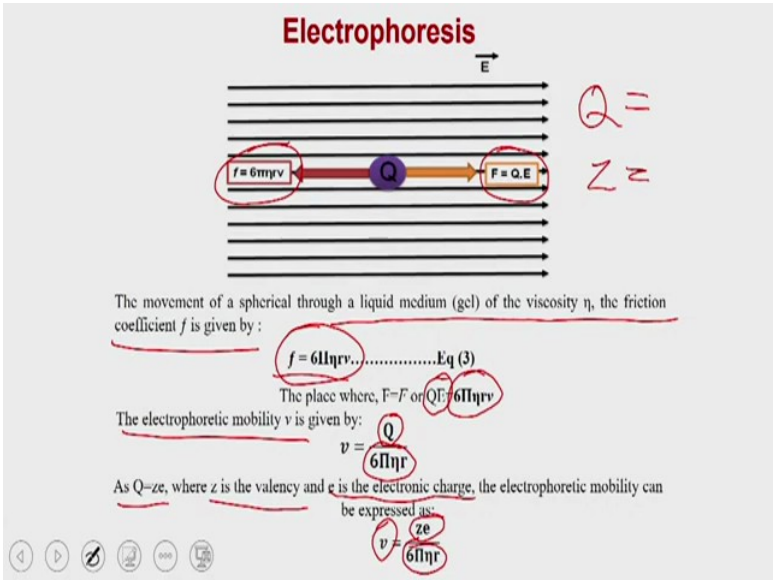

Which means the moment the moment of a spherical throughout a liquid media with the viscosity n, the frictional coefficient f is going to be 6 pi eta rv. So if you replace this f from the frictional forces what you are going to get the QE, so the place where the molecule is going to be immobilized where the electrophoretic forces are going to be equalized by the frictional forces which means the QE is equivalent to 6 pi eta rv which means the electrophoretic mobility is equivalent to Q divided by 6 pi eta r.

As you know that the charge is equivalent to ze which means where the z is the valency of that particular molecule and e is the electronic charge. Then what you can do is, you can write the electrophoretic mobility is equivalent to ze divided by 6 pi eta r.

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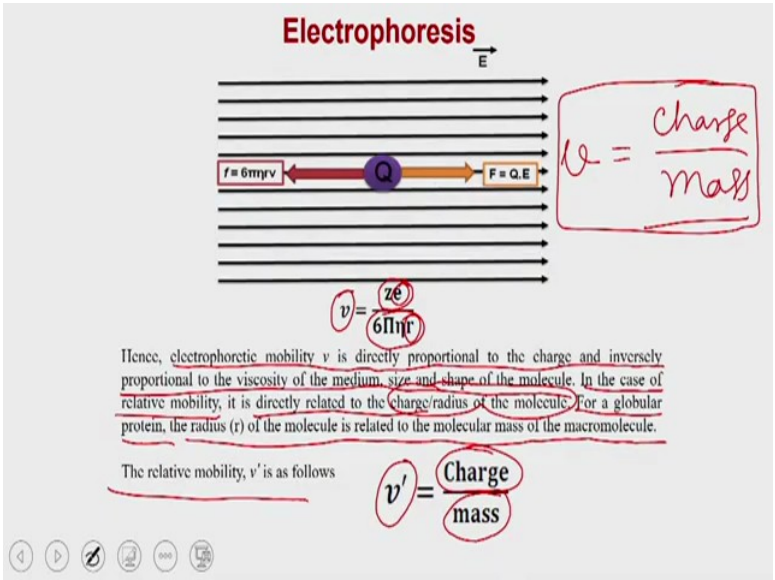

Now, so the electrophoretic mobility is equivalent to ze divided by 6 pi eta r. Hence the electrophoretic mobility v is directly proportional to the charge and the inversely proportional to the viscosity of the media, size, and the shape of the molecule. In the case of the relative mobility it is directly related to the charge by the radius of the molecule. Which means the charge divided by the radius of the particular molecule.

Whereas for our globular protein the radius of the molecule is related to the molecular mass of the macromolecule which means the radius of that molecule is directly proportional to the molecular weight of that particular substance especially for the globular protein because the globular protein, the molecule is always being distributed along with the center. So, if the molecule is of 10 Kda it is maybe having the some diameter and then if the molecule will be 50 Kda it may have the diameter which is in proportion to the molecular weight.

Which means the relative mobility is charged by the mass. So the relative mobility is equivalent to be charged by mass and that is a basic principle of the electrophoresis which means if you are running a substance in the electrophoretic field, the electric forces are going to be opposed by the frictional forces because you are running the sample through the metrics or through a gel like substances and this gel like substance are going to have the viscosity and this viscous material is going to oppose the moment of this molecule through them.

And as a result the frictional forces are going to oppose the moment of this particular molecule and the place where this molecule is going to immobilized, the place where these 2 forces are going to be same. And after that it is not going to move or it is not going to have any kind of absolute migration, after that it is going to have the relative migration and if you go by these equations. The relative mobility is directly proportional to the charge by mass.

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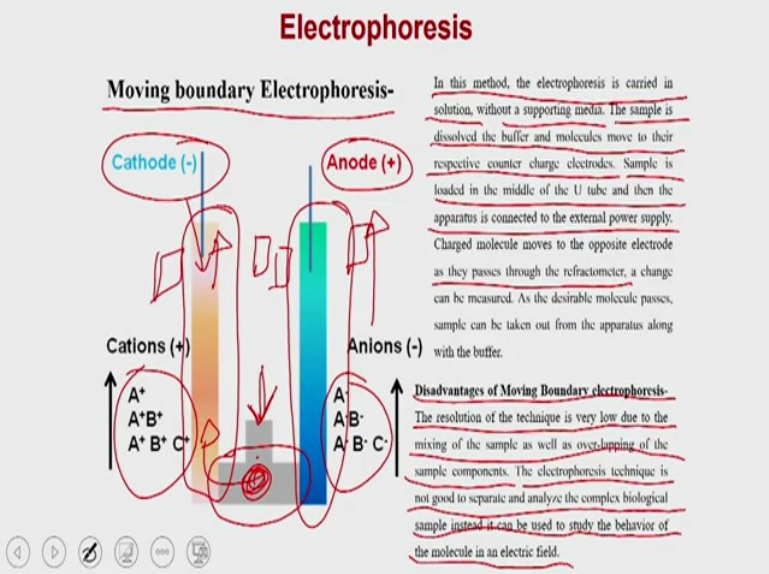

Now, considering these people have started developing the electrophoretic apparatuses and they started doing experiments how to develop a method so that you could be able to see that the separation of the molecules simply by applying the electricity. So what they have done is, they have started making the apparatus which is called as the, and performing electrophoresis in moving boundary electrophoresis.

In this method the electrophoresis is carried in solution without a supporting media. The sample is dissolved in the buffer and molecule move to their respective counter charge electrodes. So in this particular substances what you have is, you have the 2 rods, one is the cathode rod or the cathode electrodes.

The other one is the anode electrodes, so this is a positively charged, this is negatively charged and these 2 electrodes are being joined together by a central tube and the middle of this central tube you have the place from where you can actually apply the sample. The sample is loaded in the middle of the U tube and then the apparatus is connected to the external power supply and then you connect these electrodes for the power supply.

As soon as you connect these electrodes for the power supply. The molecule of the opposite charges will start migrating. So you can load the sample here and then they will start migrating. So the positively charged cations will start migrating towards the cathode. Whereas, the negatively charged anions will start migrating towards the anode. And how you can monitor the moment of these molecules?

You can actually monitor simply by refractometer. So what you can do is, you can put a refractometer on these U tubes on both the sides and as soon as the molecule will pass through this refractometer it is going to actually make the change in the refractive index of this particular liquid which is present in this tube and that is how you can be able to know that a particular molecule is coming out, okay.

But, and once you see that a particular molecule is coming out you could be able to put the Pipette and you can actually take out this particular molecule. But what is the disadvantage of the moving boundary electrophoresis? The resolution of this technique was very low as due to the mixing of the sample as well as the overlapping of the sample component what happen is, while the molecules are moving through the refractometer and you know that your sample is coming out.

The moment you take out, since it is being done in a liquid media there is no supporting system, there is no support system, so because of that the mixing of these molecules happens very frequently and as soon as you turn off the lights or turn off the power, the molecule start mixing them together. And on the other hand when you try to take out the molecule 1, some more molecules are also going to mix up.

The electrophoretic technique is not good to separate and analyze the complex biological sample. Instead it can be used to study the behavior of a molecule in an electric field. So this moving boundary electrophoresis was good simply for studying the behavior of the molecules which means how these molecules are going to behave in the electric field that you can only study, but you cannot analyze or you cannot analyze the complex biological sample.

Because the complex biological sample are going to have the substances of different molecular weight and they cannot be separated by this moving boundary electrophoresis system.

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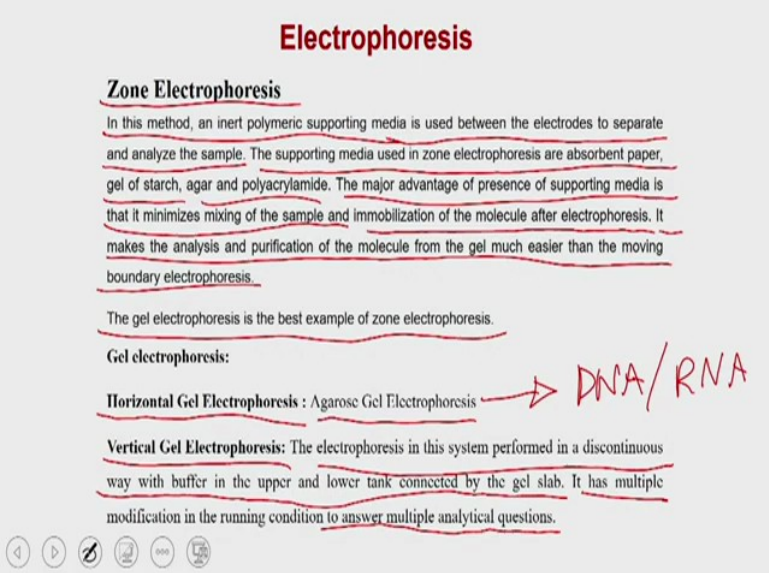

So to overcome this people have developed the zone electrophoresis. In a zone electrophoresis what you have in this method, an inert polymeric support media is being used between the electrode to separate and analyze the sample. The supporting media used in zone electrophoresis are absorbent paper, gel 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 molecules after the electrophoresis.

So compare to the moving boundary electrophoresis people have started developing the zone electrophoresis where what they have done is, they have put a supporting media and this supporting media is either the paper or agar or polyacrylamide, so this supporting media was the inert media and how the supporting media has helped, that it started immobilizing the substances on this inert media. And as a result the molecules can be analyzed in a better way.

It makes the analyses and the purification of the molecules from the gel much easier than the moving boundary electrophoresis. The gel electrophoresis is the best example of the zone electrophoresis. So gel electrophoresis could be of 2 types, the horizontal gel electrophoresis. The classical example is the Agarose gel electrophoresis which mostly people use for analyzing the DNA or the RNA.

Whereas the vertical gel electrophoresis, so vertical gel electrophoresis in this system, 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 we are going to start with the vertical gel electrophoresis.

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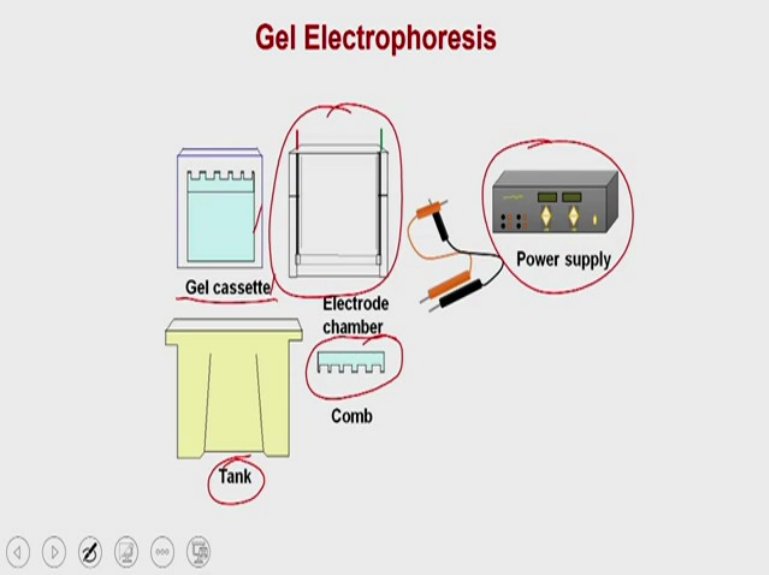

Vertical gel electrophoresis has multiple components such as the gel cassette. You have the electrode chamber, you have the power pack or the power supply unit. The purpose of the power supply unit is that it will allow to run the supply current of the positive or the negative charges, then you have the tank and then you have the comb to prepare the wells and you also have the gel cassette, so that you can be able to cast the gel of your choice.

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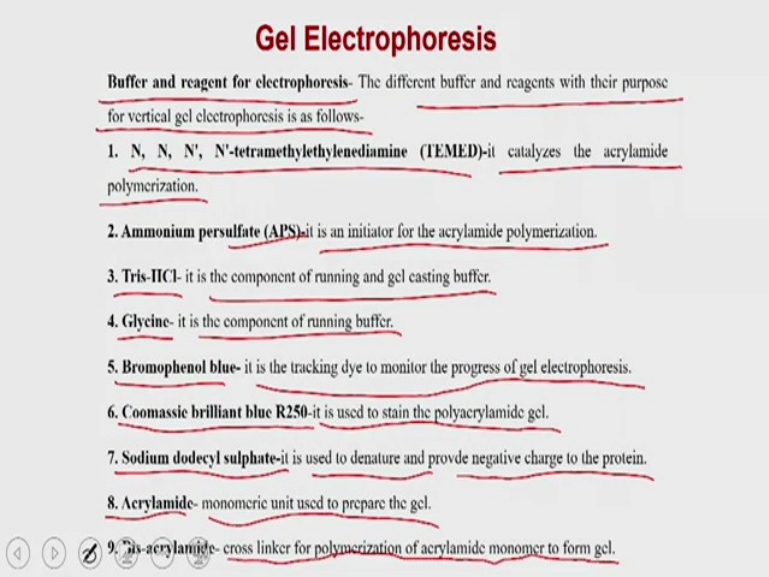

The buffer and the reagent for the electrophoresis, the different buffer and reagent for their purpose for vertical gel electrophoresis is that you have the TEMED. The TEMED it catalyses the acrylamide polymerization. Then you have the APS, it is the initiator for the acrylamide polymerization. Then you have the TrisHcl, it is the component of the running as well as gel casting buffer.

Then you have that Glycine, it is the component of the running buffer. Then you have the Bromophenol blue, it is the tracking dye to monitor the progress of the gel electrophoresis. Then you have the Coomassic brilliant blue R250, it is used to stay in the polyacrylamide gel. Then you have the Sds, it is used to denature and provide negative charge to the protein. And then you have the acrylamide, the monomeric unit used to prepare the gel.

And then you have the Bis acrylamide. Bis acrylamide is across linker for polymerization of the acrylamide monomer to form the gel. So these are the different buffer as well as the reagent what you need to perform the vertical gel electrophoresis.

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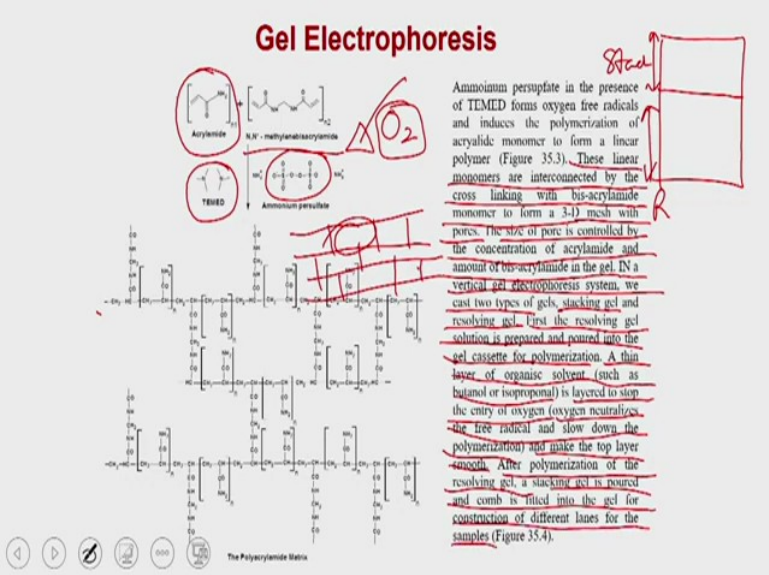

How these molecules are helping to prepare the gel is that you have the acrylamide which is actually the monomeric substances and then you have the Bis acrylamide which is a cross-linking agent. When you put them together in the presence of TEMED which is actually and the ammonium per sulphate what happened is that these linear monomers are interconnected by the cross-linking with a Bis acrylamide monomer to form a 3-D mesh with varying pores.

The size of the pore is controlled by concentration of the acrylamide and the amount of Bis acrylamide which is present in the gel. So what happen is, when you have the acrylamide as

well as the Bis acrylamide in the presence of TEMED and APS the Bis acrylamide monomers are actually connecting these acrylamide monomers and that is how you are actually making a mesh of fibres which means like.

These are the acrylamide monomers which are actually being corrected by the Bis acrylamide and that is how you are actually making the three-dimensional gel. And that three-dimensional gel is actually forming a pores and the size of these pores are going to be controlled by the amount of acrylamide as well as the amount of Bis acrylamide which you are going to provide.

In a vertical gel electrophoresis we cast 2 types of gel which means you are going to cast the stacking gel as well as the resolving gel. So in a vertical gel electrophoresis you are actually casting 2 types of them. One is called the stacking gel and the other one is called as the resolving gel. First the resolving gel solution is prepared and poured into the gel cassette for a polymerization.

A thin layer of organic solvents such as butanol or isopropanol is layered to stop the entry of oxygen. So when you polymerize the resolving gel you actually overlay a small solution of the butanol or the isopropanol that is actually to stop the entry of oxygen because the polymerization by using the acrylamide as well as the Bis acrylamide is a free radical mediated reaction.

So, when the TEMED and the APS are mixed together they formed the free radicals and that is how they activate the cross-linking of the acrylamide monomer by the Bis acrylamide cross-linking agents. But, if you have the oxygen, these free radicals are going to be neutralized and that is how it is actually going to inhibit the polymerizations. Oxygen neutralizes the free radicals and slowdown the polymerization and make the top layer smooth. After polymerization of the resolving gel, stacking gel is poured and comb is fitted into the gel for construction of different lanes for the sample.

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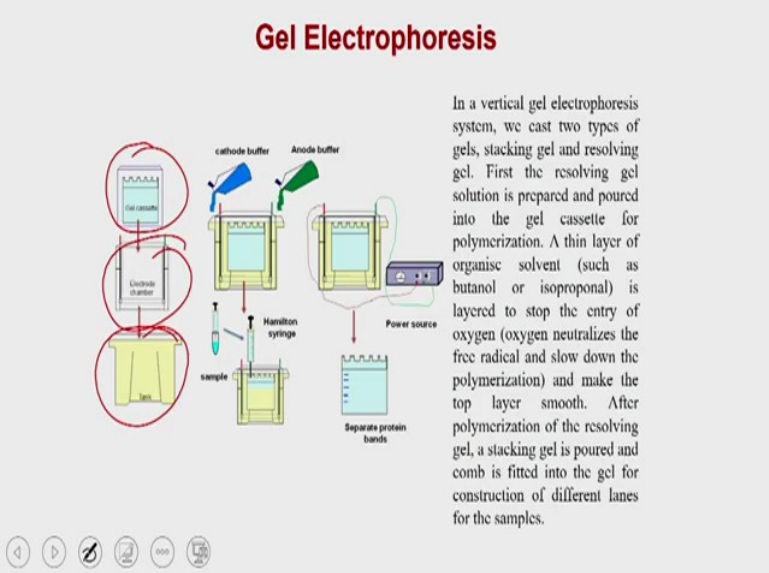

So this is what, you have the gel cassette, you have the electrode chamber and then you have the tank where you are going to place this. Initially what you do is, you first cast the resolving gel, then you cast the stacking gel, the only difference between the resolving and stacking gel is that the composition of the resolving as well as stacking gel is very different. And then once this casting is over then you use, then you are actually going to place this particular cassette into the apparatus and then you are going to apply the sample and you are going to resolve them.

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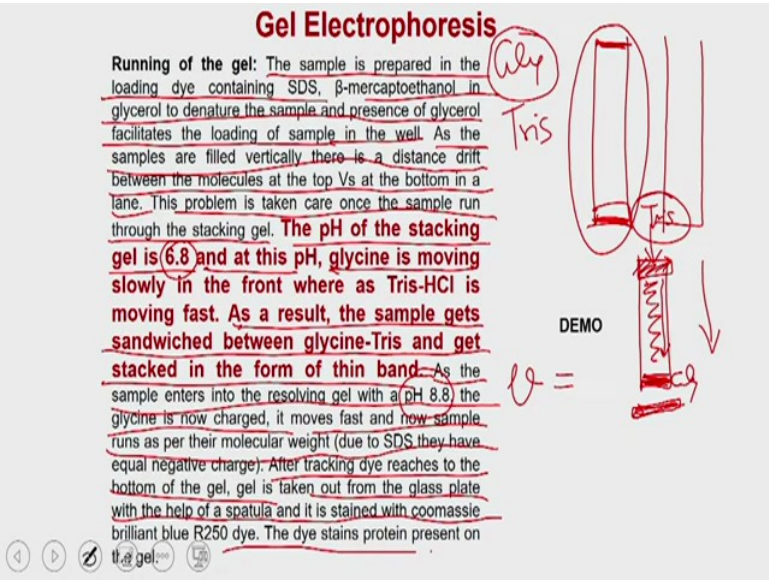

How to run the gel? The sample is prepared in the loading dye containing SDS. Beta-mercaptoethanol in glycerol to denature the sample and the presence of glycerol facilitate the

loading of the sample in the well. As the samples are filled in a vertical there is a distance drift between the molecules at the top versus a molecule at the bottom. So you can see that in a vertical gel electrophoresis when you have the lanes which are actually in the vertical direction.

So what happen is, the molecule which is present here and the molecule which is present here is actually having the distance and because of this if you analyze them and if you do not do anything, so that they will come together. They are actually going to have the separation between them which is not okay. So this problem is taken care once the sample run through the stacking gel.

The pH of the stacking gel is 6.8 and at this pH, the glycine, so when you run these samples, you run them in a buffer which contains the glycine as well as the Tris. And glycine is an amino acid because, so glycine at the pH 6.8 is actually having very little charge because its pK is close to this particular pH. So what happen is, that glycine is not moving, so you can imagine that you have a well where the glycine is sitting at the bottom.

So glycine is not moving and whereas from the bottom you have the Tris. And Tris is actually very charged molecule. So Tris is actually on the backside of this and in between you have the sample. So what happened is, the Tris which is actually moving very fast from the backside, whereas the glycine which is not moving, so the Tris is pushing this sample while they are running into the stacking gel and as a result their substance comes very close to each other.

And what happen is they form a small band while they are running in a stacking gel and this process of stacking the sample, so that they will form a thin line is called as the stacking of the sample and that is why this gel is also called as the stacking gel. And the importance of stacking gel is that it reduces the distances between the 2 molecules because of the well which is present in the vertical direction.

As a result the sample gets sandwiched between the glycine-Tris and get stacked in the form of a thin band. As the sample enters into the resolving gel which is actually having a pH of 8.8 the glycine is now charged it moved fast and now sample run as per their molecular weight due to the SDS they have the equal negative charge, so they run as per their molecular weight.

If you remember the v is directly proportional to the charge by mass. So, if the charge is same they are going to run by according to their mass. So after tracking dye reaches to the bottom of the gel. The gel is taken out from the glass plate with the help of the spatula and it is stained with the coomassic brilliant blue and that stained protein present on the gel. So this is all about the theoretical description of how to assemble the gels and how to run them.

But I would like to take you to my lab and I would like to show you a small demo, so that you will be able to see how to cast the gels, how to prepare the samples on how to resolve them onto the polyacrylamide gels and how to analyze these protein samples.

[Video Presentation Starts]

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In this video we will demonstrate you how to draw a SDS page gel and how to prepare various reagents required for the running of SDS Page gel. And what are the different instruments we can use.

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So here this is the gel casting stand.

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So where we can use these glass plates to prepare the gel, in between there is space where we can pour our gel, gel solution. Then we will keep on some time at least 20 to 30 minutes, let it solidify then we will prepare stacking gel then we will load the 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 repair acrylamide 30 percentage.

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30 percentage means 29 grams of acrylamide.

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And 1 gram of this acrylamide, these both we can use 29 is to 1 ratio in 100 ML of water to get 30 percentage 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 pH8.8. In addition to that we also need 10 percentage SDS prepared in W distilled water and also 10 percentage ammonium per sulphate and also TEMED, the role of ammonium per sulphate and TEMED we can see during preparation of gel they act as a catalyst.

After solidifying we have to use, we have to prepare stacking gel. So stacking gel is nothing but, composition is same but we can say it is diluted. It contains pH 6.8 Tris-HCL and remaining components same, but in less quantities. So after preparing the gel we will load the marker in the protein which is denatured at 100 degrees Celsius for 3 minutes.

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After that we will fix this gel into this one. We will keep, keeping this reservoir then we will connect to the power pack and run the gel. So this is the overall introduction of how to prepare SDS Page gel. So let us start preparing gel. We will learn more things while preparing the gel.

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Before preparing the reserving gel we have to prepare set up the casting gel.

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So this is the glass plate. This is very thin one, so this is the main glass plate this is 1.5 mm glass plate. It is available in 1 mm glass plates also. If your loading solution is less like you want to load only 20 micro-litre, 30 micro-litre then 1 mm gel is good enough, but if you have extended volumes like 70 micro-litre you can use 1.5.

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You have to arrange like this shot plates on this.

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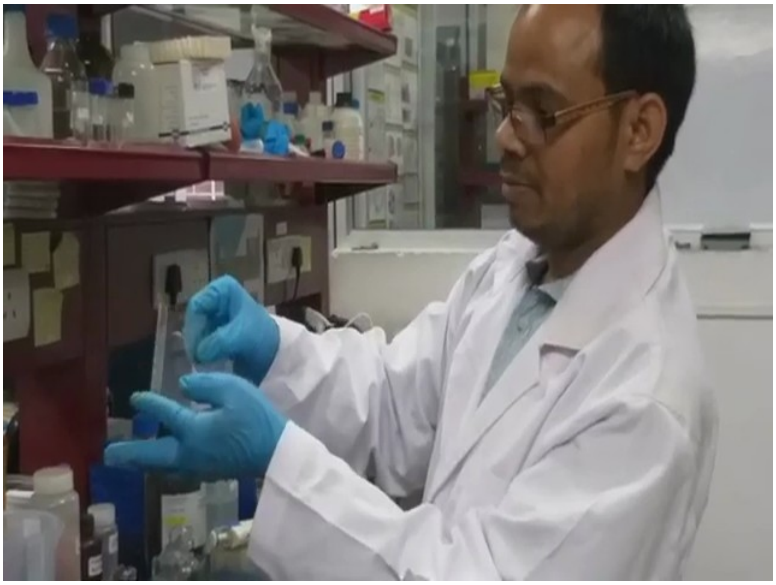

And the bottoms should be equal.

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Then we have to put in this one, this tray. Then you have to keep it like this. So we have to check, if we perfectly set up this one then there should not be any leakage, but if there is any leakage your reserving 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 okay or not. So I am going to use milky water to just.

After checking the gel if there any leakage is not, so we moved forward for preparing reserving gel. So the composition 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 I am going to add 3.3 ml of already prepared 30 percent 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 of acrylamide solution 30 percentage. So I have to adjust 300 micro-meter. The next component is 1.5 molar Tris pH 8.8 we have to add 2.5 ml. Next component is SDS. Here SDS functions as, plays a dual role like one thing is that, it gives negative charge, across negative charge on the polypeptide chain. The next component we have to add is SDS. 10 percentage SDS. We have to add 100 micro (())(43:32) SDS to preserving gel.

It plays very crucial role in polyacrylamide gel electrophoresis like it embarks negative charge and the polypeptide chain, so that despite of their charge they remove based on the molecular weight. So I am going to add SDS. The other important the thing is that 10 percentage ammonium per sulphate. Ammonium per sulphate which is catalysed by the TEMED provides free radical species which accelerate the forming mesh like shape in acrylamide gel like it will catalyze forming the mesh.

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So this is the 10 percentage APS I just had 100 microlitre of 10 percentage APS to reserving gel. In final step we have to add TEMED. TEMED (after finishing) 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 (())(45:25).

So I am going to add 5 microlitre of this TEMED which catalysis the ammonium per sulphate. Ammonium per sulphate in turn provides free radical species, and free radical species accelerate the polymerization, this is the overall principle of this reserving gel. So I will add TEMED. We have to mix properly then add slowly (())(46:45). So after this we have to overlay with, on the top layer we have to overlay with some solvent like 2 butanol or isopropanol or with water.

So, why we are doing this? Because if the gel is exposed to air in the oxygen from the air they will interfere in the polymerization of the gel. So we have to add either 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 overlay 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 milky 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, 30 microlitre of acrylamide. 630 microlitre of Tris-HCL pH 6.8. 50 microlitre of TEMED and 50 microlitre of SDS we have to add. At the end we have to add 5 microlitre 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 and we will shift to the power tank and then we will run the gel. While the stacking gel is solidifying we have to prepare sample for loading the SDS Page gel. So further we have to prepare loading type 10x or 6x loading type. It mainly contains 250 millimole of, millimolar Tris pH 6.8, 30 percentage glycerol, 10 percentage SDS and 0505 percentage of (())(53:03).

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So here you can add 10 millimole of DTT also as a reducing agent. SDS mainly works as imparting negative charge and the polypeptide chain and DTT breaks down the disulphide parts. If you have dimer which you can see as a monomeric in SDS page gel. 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 DTT breaks down the disulphide part and you can see only single part. If you want to see actual molecular weight you have to run it on native place where there is no reducing agent but no data checked.

The other thing is glycerol. While loading the gel since the protein solution 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 percentage glycerol is sufficient for keeping the protein solution intact at 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 10 percentage 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 microlitre of loading dye to this protein solution. You can tap down or (())(55:34) this protein solution then we have to heat it for 3 minutes at 100 degrees Celsius. So that all the polypeptide chains, I mean dimers are, if any multimers are present they will break out and we can see nice band.

So I am going to keep this at 100 degrees 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, this one and we have to keep inside the tank. So just take out the gel. So inside this tank we only have this side (())(56:43), you have to cover other side also. So for that we use a dummy plate, just hold it tight and fold this thing. After that gradually adjust the gel. So just we have to fix like this.

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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 litres of solution, 1x solution. So this is 1x I 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.

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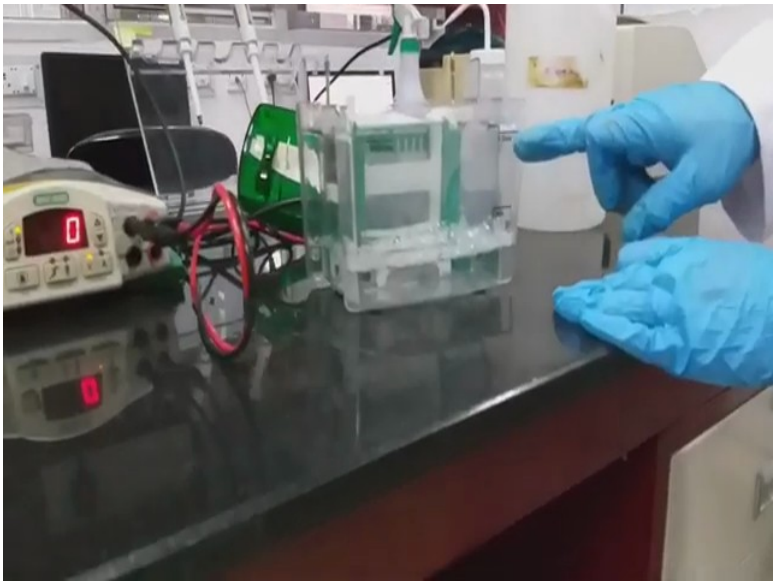

So for difference you can see here, for 4 gels we have to add till here the buffer, we have to load outside this gel, so for 2 gels here, for 1 gel we can add like this.

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This is the power pack where we can adjust the how many (())(58:41) we want to run.

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The protein samples are ready, we heated sufficient time, now we have to load this. So we have to remove the comb carefully.

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Then first I am going to load marker or protein ladder.

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Next I will load (())(59:37). Once the loading was over we have to fix this gas (())(60:06).

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I am going to set it at 70 volts.

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As we can see it is almost over, so we can take out the gel then (())(60:35) stained and (()) (60:37). Generally what we will do is, there are 2 ways of staining the anti-staining process. One is we can do keep staining like we have to keep it with the staining solution which contains coomassie brilliant blue and (())(60:53) methanol 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 stay in cocomassie brilliant blue staining solution then we will destain in methanol water containing solvent.

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So we are going to start the (())(61:32) then I will remove it, I will show you how to remove the gel. Take out the glass plates.

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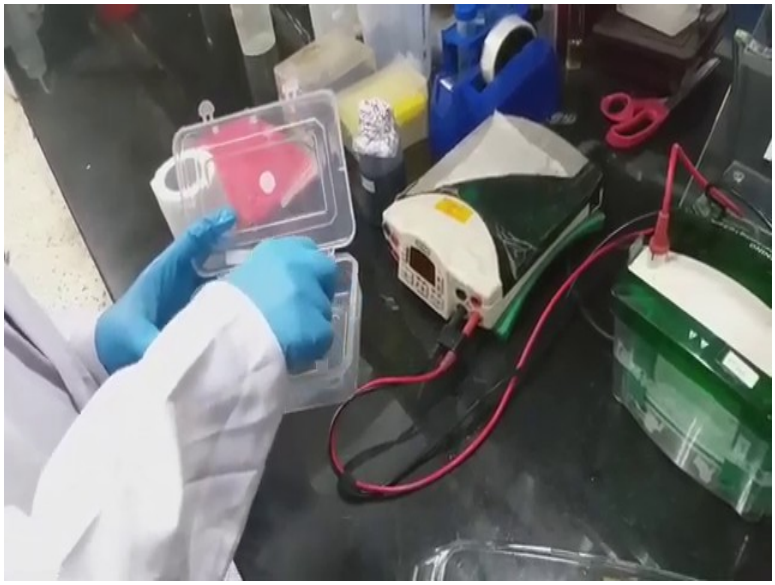

Here we have to be very careful while taking out gel otherwise the short plates may break. On a corner we have to take and lift the gel like this and keep the gel in a staining box which is more or less plastic one but it can sustain the.

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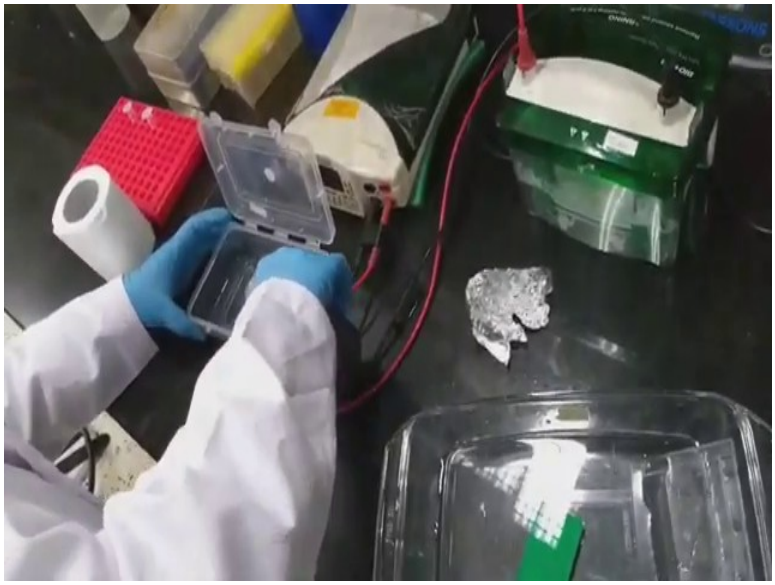

So then I am going to add staining solution. I will keep it for a rotation for (())(63:29) at least 2 hours then we will destain over.

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So once the time is over after 2 hours we will destain this mixture.

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Now we kept 2 hours this staining solution. As we can see the staining is over like we 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 (())(64:44) for 2 hours for destaining. So the composition contains per 100 ml of destaining solution, 50 ML of water, W distilled water and 40 ML of methanol and 10 ML of (())(65:03).

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So I am going to keep this on (())(65:06). We have run the gel and stained and destained.

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Now, we will capture the gel image. 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 (())(65:37). So I will show you how to capture images. So here we will use the white tray. There is another one grey or UV tray is also there.

So there you can see any fluorescent one or stained with the itenium bromide or (() (66:12) you can (())(66:13). But for normal protein imaging we can use this white tray. So I am going to keep the gel on this one. So we 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 you just push it back. So we have to log onto account.

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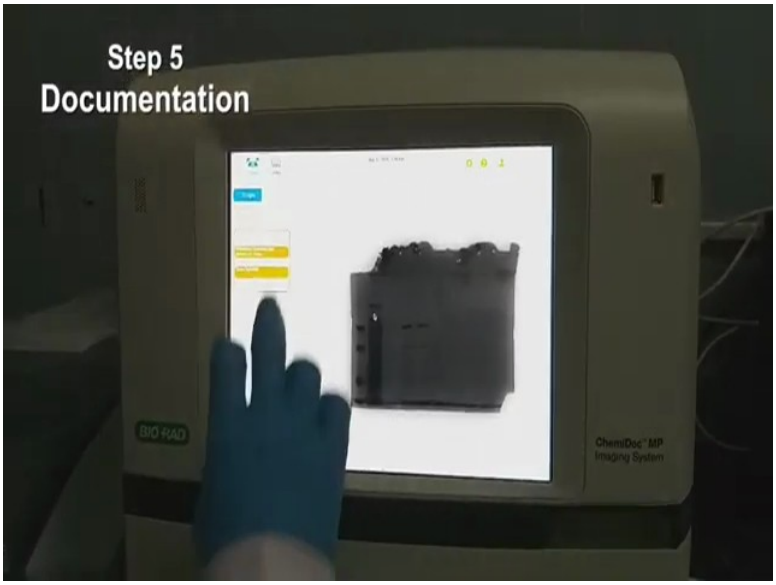

So this is SDS Page gel. You can select the application whatever you want. So here nuclic acids, protein gels, (())(67:26) three different categories are there. So we are observing here protein gels. Protein gels stained with the coomassie blue. Or white tray, we are using white tray, so this is the right tray. You can use coomassie blue stained one grey tray also but 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, suppose you want to decrease or increase the signal intensity. So these kinds of changes you can do.

So, if you want to send this gel you can have sent the (())(68:38). If you have any drive connected to this one you can send directly to that thing. So for image analysis part we will show in the upcoming video how to analyze what this band of interest correspond to which molecular weight. So we already loaded the molecular weight, so we can easily find out using Image Lab software.

In this video we have learned how to prepare SDS Page gel and how to run it, what are the precautions need to be taken while preparing the gel. And how to record the gel using gel documentation system. So I hope this will give you a (())(69:33) of how to prepare and run a SDS page gel and analyze the protein sample.

So in this particular demo, the (())(69:43) and my lab students have discussed about how to run the SDS page, how to prepare the gels, how to cast the gels and how to run the gel and

analyze the samples and at the end they have also discussed what are the different precautions you should take while you are running the protein onto the polyacrylamide gels and I hope these videos or the demo will be beneficial for you to advance your work, thank you.