**Experimental Biotechnology**
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**Module No # 02**
**Lecture No # 10**
**Different Variants of Gel Electrophoresis**

**(Video Starts: 00:24)**
**(Video Ends: 01:00)**

Hello everybody this is Doctor Vishal Trivedi from department of biosciences and bioengineering IIT, Guwahati. And what we were discussing about the horizontal gel electrophoresis in our previous lecture. We have discuss about the experimental condition and how to perform the gel electrophoresis. We have also shown you a small demo clips how to do it in the lab and I hope that these are these 2 things would have been explained you the horizontal gel electrophoresis in a more detail and you will be able to perform the agarose gel electrophoresis in your own laboratory.

So let us move on to discuss the different variants of the gel electrophoresis which you can do or which have been available.

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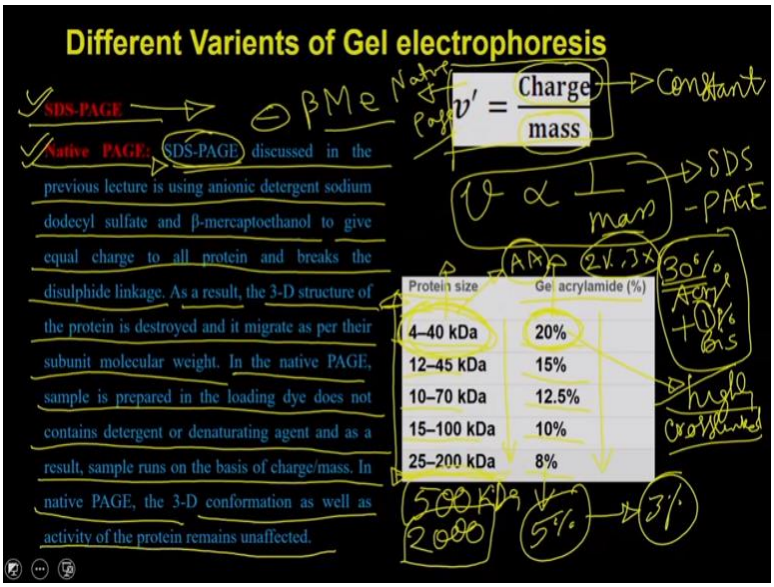


So there are the SDS page which is actually a de-naturating page so as you know that the electrophoretic mobility is actually been governed by charge by mass ratio. So if I keep the

charge by mass ratio so if I keep the charge constant then the electrophoretic mobility is going to be inversely proportional to the mass and that is what the condition for SDS page. So if I add the SDS into the gel electrophoresis the SDS is going to bind to the protein molecule.

And as a result is actually going to give the negative charge and since the charge is imparted by the SDS is going to be uniform for every protein. The charge component is going to be nullified and as a result the electrophoretic mobility is going to be inversely proportional to the mass. In addition to that since the SDS is a detergent it also destroys the 3 dimensional structures of that particular protein.

And if suppose the protein is present in the oligomeric status which means if the protein is present in a dimer, trimer, tetramer or any other higher molecular higher oligomeric status. Those higher oligomers are also going to be denatured with the help of the SDS. So in the SDS what it will happen is the proteins are going to be run as the monomer or the monomer. And since they are actually going to have the equal charges they are also going to beresolved as per the inversely relationship to their molecule.

Which, means the high molecular weight proteins, are going to be slower and the lower molecular weight proteins are going to run faster. Apart from the SDS page you also can have the native page. Native page means where you are not going to have the SDS into the reaction or you also not going to have the any kind of denaturating agent such as the beta-Mercaptoethanol. So SDS page is discussed in the previous lecture and it is using an ionic detergent sodium dodecyl sulfate and beta-mercaptoethanol to give equal charges to all protein and breaks the disulphide linkage.

As a result the 3-D structure of the protein is destroyed and it migrates as per their subunit molecular weight. Whereas, in the native page the sample is prepared in the loading dye does not contains the detergent or the denaturating agent. And as a result the sample runs on the basis of the charge by mass. So this relationship what you see is the electrophoretic mobility is directly proportional to the charge and inversely proportional to the mass or in general the electrophoretic mobility is going to be effected by the charge by mass ratio is actually the condition for the native page.

So in the native page the proteins are going to maintain the 3 dimensional structures they are also going to maintain their intrinsic charge, which means if the protein is positively charged it is going to remain as positively charged if it is negatively charged which is going to remain as negatively charged. And in native page the 3 dimensional confirmations as well as the activity of the protein remains unaffected.

So the native protein and the native page is always been used to ask the questions whether the protein is a monomeric protein or the oligomeric protein and so on. Because if you run the same protein on to the SDS page and if you run the same protein on to the native page and if you calculate their electrophoretic mobility and if you use that information you can be able to answer the question of the oligomeric status of the particular protein because the electrophoretic mobility in the SDS is going to be as per the molecular weight.

Whereas, the electrophoretic mobility; in the native page is going to be as per the charge by mass ratio. Because if the protein is dimeric the mass if going to be of corresponding to the dimer whereas in the SDS page it is going to be monomeric. So the combination of the SDS page and the native page is going to give you the answer about the oligomeric status of the protein. Number 1 number 2 with the help of this native page you can be able to because the native page is going to maintain the 3 dimensional confirmations.

It is also going to maintain the activity of the protein so you can be able to do the functional activities you can be able to lot of activity assays when the protein is present in the gel and you can answer you can resolve many questions related to the bio chemical activity of that particular enzyme or biochemical activity of that particular protein. Number 3 the native proteins because the native page is also going to allow you the 3 dimensional confirmations the native page can be used event for the studying the interaction between the 2 proteins.

For example protein A and protein B is their protein A and B if they interact with each other their resultant charge resultant molecular mass is going to be very high. So that is how you can be able to ask that particular question if you load protein A, protein B and protein AB complex then the electrophoretic mobility is going to be different for the complex. Apart from that if you

see what the protein size and if you see the what kind of gel electrophoresis or what kind of the gel you have to use for seeing the better resolution and separation.

But you see is that if you have the very small molecular weight for example the 4 to 40 kDa protein then you can be able to use the 20% gel electrophoresis which means you have a if you have prepared a 30% acrylamide solution actually contains 1% cross linking agent the (()) (08:36) then from their you can be able to use the 20% gel, if you have if you are working with the protein which are in the range of 4 to 40 kDa.

But if you are working in the range of 12 to 45 kDa you can use 15% if you go to 10 to 70 you can use the 12.5. If you use 15 to 100 you can use 10% and if you are working in the range of 25 to 200 you can be able to use the 8% which means as the molecular weight is increasing you are actually decreasing the acrylamide. Because I think if you remember I have shown you that when the polyacrylamide is when the acrylamide is getting cross link by the bisacrylamidand that actually creates a pore within the because of fibers are getting connected by the bisacrylamid

It actually creates a pore or the mess and from that mess the molecule as to pass through so if you take the very high concentrated acrylamide the high molecular weight proteins are not going to enter. So for the practical references point of you this values are been utilized so that is by simply by people has done the different types of experiment and that is how they come up with this value.

But the condition comes when you have to resolve a protein of 500 kDa for example or even 2000 kDa protein. For example if you have such a large proteins or if you have the multi-meric protein complex is how you are going to resolve that because if you drop this consultation to suppose 5% okay or even to like 3% this lower consultation of the acrylamide is so less that it will not go into give you the gel like structures it actually going to make the it is not actually enough to give you a gel which can be manipulated in a very simple way.

So to solve this problem where you have a very large protein and protein complexes the people have develop the new gel electrophoresis technique here they are using the multiple gels and that is how they are been able to utilize and resolve this high molecular weight proteins. Apart from that suppose you have the protein of lower to this consideration. For example if you have the

amino acids which are you are interested to resolve on to the acrylamide gel then you cannot go beyond the 20%. Because that is the maximum what you can prepare from the 30% acrylamide solutions.

But what you can see is that the 30% acrylamide solution as only the 1% bisacrylamide so if you have a proteins of a very small size then what you can do is you can still be able to run the 20% gel but you can increase the percentage of the bisacrylamide. These kind of the gel are called as the highly cross linked gels okay. So if you have a very small molecule weight you can use the highly cross link gels where you can actually use the 2% or 3% bisacrylamide.

Whereas if you have a very high molecule weight protein then you can you have to use a combination of different gels which we are going to discuss in a subsequent slide.

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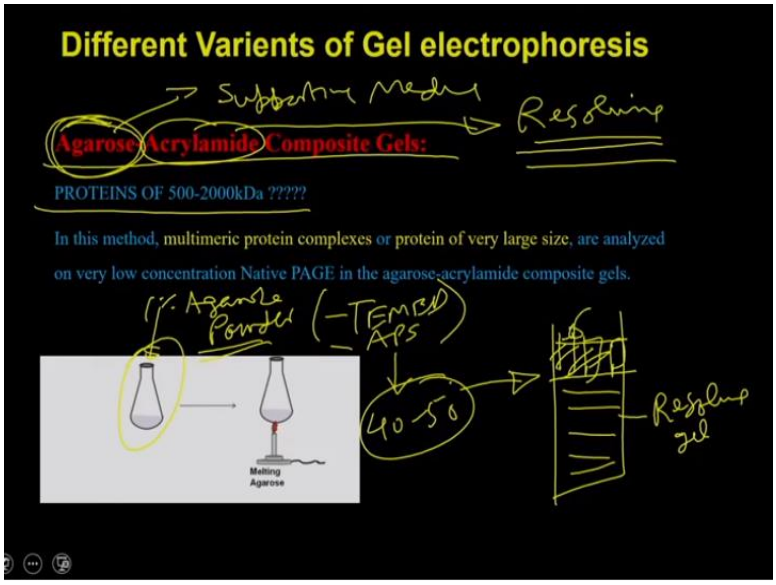


So if you have a protein of 500 to 2000 kDa you can able to use a agarose acrylamide composite gels where you are actually going to use the agarose for providing the supporting media or supporting stuff supporting media whereas the acrylamide is also going to use for resolving the sample. So what you have done you have used the 2 different gels and utilize their properties. So the agarose is been used for providing the solid support so that you will be able to manipulate this gels.

Because if you use the 3% or 4% acrylamide gels they will be very flimsy and they will not be able to so the user you cannot be able to handle them. Because before you stand them with the (()) (13:10) blue they will get broken them into the pieces. So to avoid that and to provide the strength to the system what you do is you just simply add the small quantity of agarose and that actually is going to give you strength so that it actually helps in providing the overall strength so that it actually post operations are going to be easy.

Whereas the acrylamide is still be the material which actually be going to be use for resolving. So how to prepare these gels what you have to do is you have to first prepare the complete acrylamide solutions then what you do is you add the 1% agarose powder and then you boil this as we are actually discuss in the when we were discussing about the horizontal gel electrophoresis.

Now you boil and let the acrylamide to be get dissolved the only thing is that you should not add the temed and you should not add the APS at the system. So you prepare the complete acrylamide solution which means you can take the acrylamide you can add the SDS you can add the trace you can add everything. And then you add the 1% agarose powder as per the calculation of that particular volume you boil it so that the agarose get melted.

And then when this solution get cool down to 40 to 50 degree then what you do is you take this solution and pour it into the glass slides which you are using for the vertical gel electrophoresis and then let this to be resolved and give you the resolving gel okay. So you are going to prepare or resolving gel where the acrylamide percentage is going to be 3 to 4% and the agarose is going to be 1% and then what you do is.

You again cast the staking gel and then you put your com and prepare the wells and then you can load the protein samples and resolve as we discussed when we were discussing about the vertical gel electrophoresis and that actually is going to give you the options to resolve the proteins of 500 to 2000 kilo Dalton a molecular weight. If you require you can run the SDS page or if you require you can run the native pages and that actually is going to be good enough to resolve the single protein of such high molecular weight or the multi-meric protein complexes.

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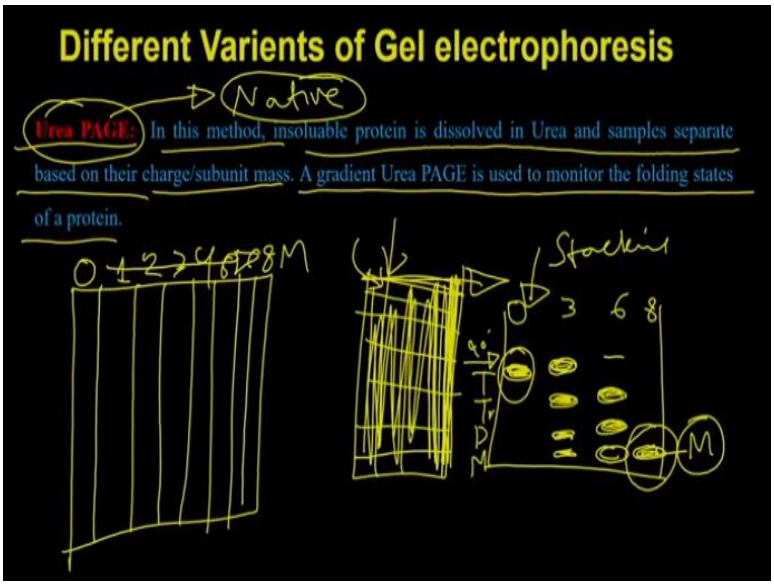


Apart from that we have also the urea page so in this method insoluble protein is dissolved in urea and the samples separates based on the charge by the mass ratio. So, urea page similar to the native page except that it is actually going to destroy the 3 dimensional structures because the urea is denaturated. So it is actually going destroy the 3 dimensional structure. If you use a gradient Urea page you can be able to study the folding and unfolding kinetics of the study.

So what you have to do when you are doing a urea a gradient Urea page is that you have to prepare a vertical gel where you are going to have urea from 0 to 8 molar in different lanes. For example if you are going to have a lane of 0 lane then you are going to have a lane for 1 then you are going to have a lane for 2, 3, 4, 5, 6 and 8. And these are going to be the gradient towards in a horizontal direction.

So the first question comes is how to prepare this kind of gradient urea page? So if because if you remember or you are always pouring from the top and it is this liquid is getting filled into this chamber like this. So because of that you cannot be able to generate a gradient in this directly. So for this purpose what you have to do is you have to rotate this cassette in this direction first okay. So you can imagine that we have just rotated this cassette by the 90 degree and now you started filling from this side okay.

So first you fill this one then you fill this one so when you rotate you have to block from the top as well and then you start pouring from the top and first you fill this one like that okay. So then

once you are done with the casting of all the up to the end then you can rotate it back again and then you can start pouring the stacking gel, and you can be able to cast the you can put the comb and you can cast the different wells, and then you just load the proteins which are already been incubated with the different amount of urea.

And as a result what you are going to see in the observation you are going to see it like for example you have a 0 molar, 3 molar, 6 molar and 8 molar. So in the 0 molar you are going to see a single band which is actually going to be corresponding to the tetramer. So I assume that we are dissolving a tetrameric protein. Now once it reaches to the 3 your tetramer consideration of the tetramer is going to be broken down.

And then you are actually going to see some amount of trimer some amount of dimer and some amount of monomer which means this is actually going to be trimer this is going to be dimer and this is going to be monomer. If you go further up then you band and concentration of the tetramer is going to be reduced whereas the all the other proteins are going to be increased. But once you reach to the 8 molar all these will get reach to the mole-meric proteins.

So all will get broken down in to the monomer so if you can actually be able to follow these kind of study and if you do this kind of a experiments that actually will be going to tell you the stability of these individual oligomers towards the urea. So it will actually tell you that which monomer is going to be broken down and at what step the protein is losing its 3 dimensional structures and at what step it is actually losing its interaction with the neighbors.

So, that the tetrameric protein is getting converted into trimeric and the dimeric and monomeric. And ultimately everything will get converted into the monomeric so at this stage all your protein got unfolded whereas this stage all your protein is under the folding stage. So by doing this you can be able to study the different intermediates what the protein is going to go through to becomes completely folded protein into completely unfolded protein.

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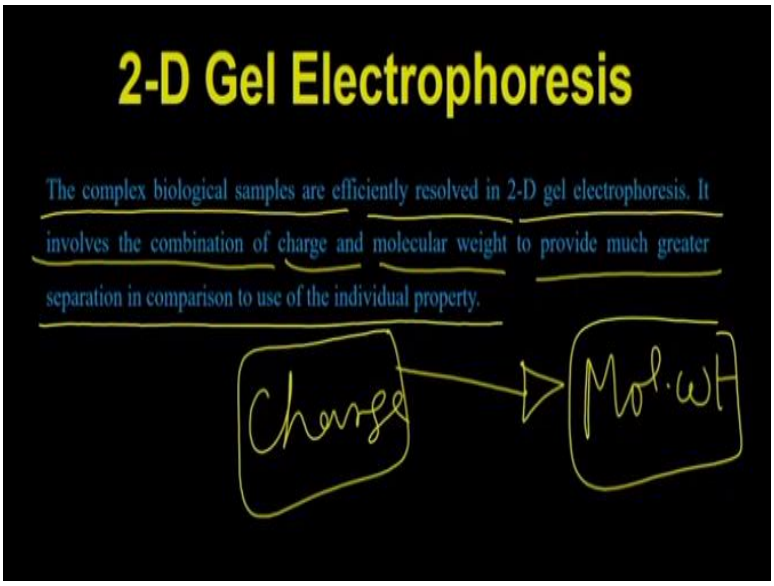


Now let us move on to the next topic and that is the 2 dimensional gel electrophoresis because so far we were discussing only the gel electrophoresis. But the gel electrophoresis is always utilizing the 1 property either it is the charge or the mass or the combination of the charge by mass. But it was not utilizing the 2 properties to resolve the samples. So if you; utilize the properties and to resolve the samples then it is called as the 2-dimensional gel electrophoresis.

For the complex biological samples are efficiently resolved in 2-D gel electrophoresis it involves the combination of charge and the molecular weight to provide the much better separation in comparison to use of the individual property. Which; means you are first going to resolve the molecule based on the charge. And then you are also going to resolve them as per the molecular weight so because of that it is actually going to give you the better separation.

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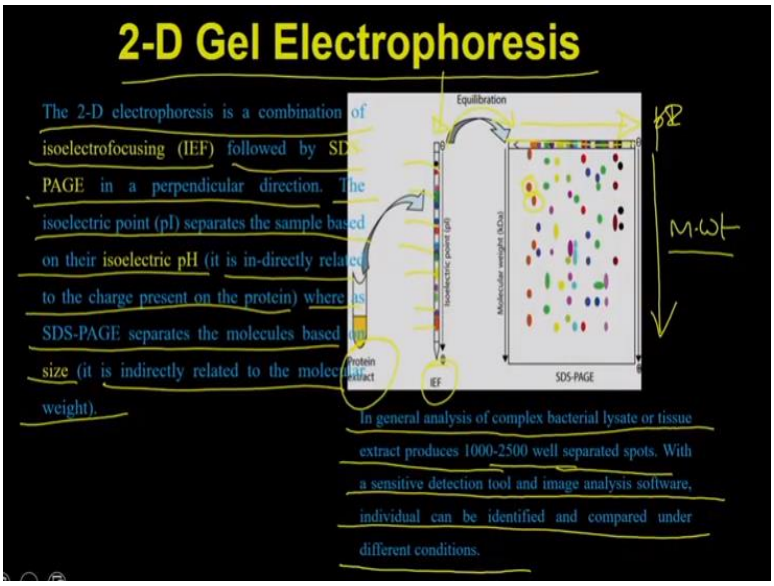


The 2 dimensional gels electrophoresis is a combination of isoelectric focusing followed by the SDS page in a perpendicular direction. Isoelectric points, separates the samples based on their isoelectric pH it is indirectly related to the charge which is present on the proteins. Whereas the SDS page; separates the molecule based on the size which is indirectly related to the molecular weight.

So what you can see is suppose you have a complex protein mixture and if you take this protein mixture in the first dimension what you can do is you can load it on to the isoelectric focusing strips and that actually is going to resolve these molecules based on the isoelectric point. Then what you can do is you can just rotate to the 90 degree and run it as per the molecular weight. So now in this direction it is been resolved as per the PI and in this direction it is going to be resolved as per the molecular weight.

And because of these combinations you are going to see the individual parts of which where the 2 in 2 proteins of the similar properties are going to be resolved. Because the 2 proteins which are actually have the same molecular weight may not have the same isoelectric point. So first they will be get separated based on the isoelectric point and then they will be get separated based on the molecular weight.

So even if the 2 proteins and the same is true for the isoelectric point so if the 2 proteins are of identical isoelectric point but they have the different molecular weight then also it is also going

to give you the 2 parts. You can imagine if I put and resolve the same example either using the isoelectric point or to the molecular weight then the samples will not get resolved properly or will not get resolved completely.

In general the analysis of complex bacterial lysate or tissue extract can produce even to 1000 to 2500 well separated spots. With a sensitivity detection tool and image analysis software individual of these spots can be identified under the different conditions. So the 2D gel electrophoresis is very popular in terms of looking for the changes in the pattern of the proteins when you are treating a particular organism or bacteria with something which is actually changing the protieum of that particular organism.

And because these changes are very subtle and these changes are very difficult to map simply by using the property either the isoelectric point or to the molecular weight that is why people are using the 2 dimensional gel electrophoresis to resolve them. And in general you are going to produce 1000 to 2500 parts only and that actually is going to give you the enough separation to see each and every spot.

And once you got this spots you can be able to extract the protein from those parts and you can be able to identify those spots and you can be able to identify the proteins. How to perform the 2 dimensional; electrophoresis?

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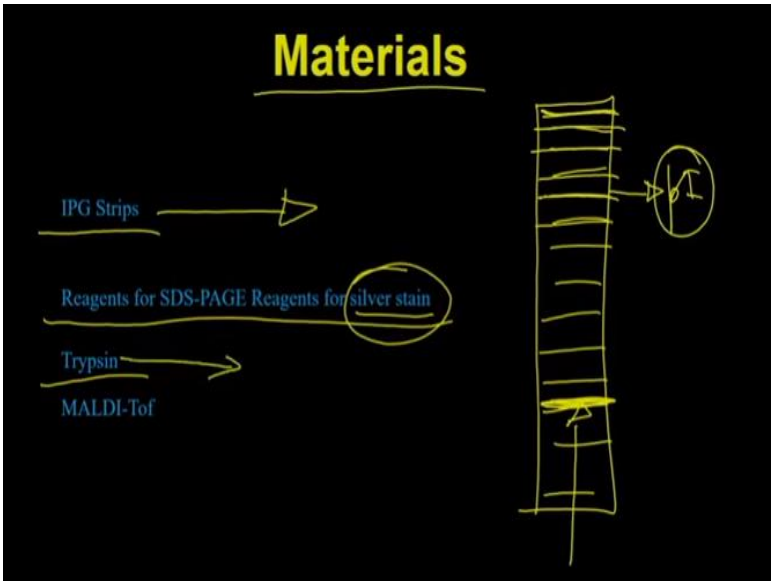

The material what you require is for the 2 dimensional gel electrophoresis you require the isoelectric focusing strips. These isoelectric focusing strips are nothing but these strips of the cellulous where you have the ampholytes are arranged on to the strips of different charges. So this empollights are nothing but the amphoteric molecules and these strips are coated with these molecules so this region is actually going to be corresponding to a particular type of PI.

So because of that when the proteins are going to run and when they reach to their isoelectric point they will get immobilized to that particular region. And because of that it they will get separated based on the isoelectric point. Then you need a reagent for the SDS page and then you also required the reagent because you want to do a sensitive detection so you can also require a reagent for the silver staining.

Then you require a trypsin because once you got the (()) (26:15) you have to (()) (26:16) them so that the proteins are going to produce the peptides and then these peptides can be analyze in the mass spectrometric to know what is the mass of this peptides and then you will be able to identify the protein.

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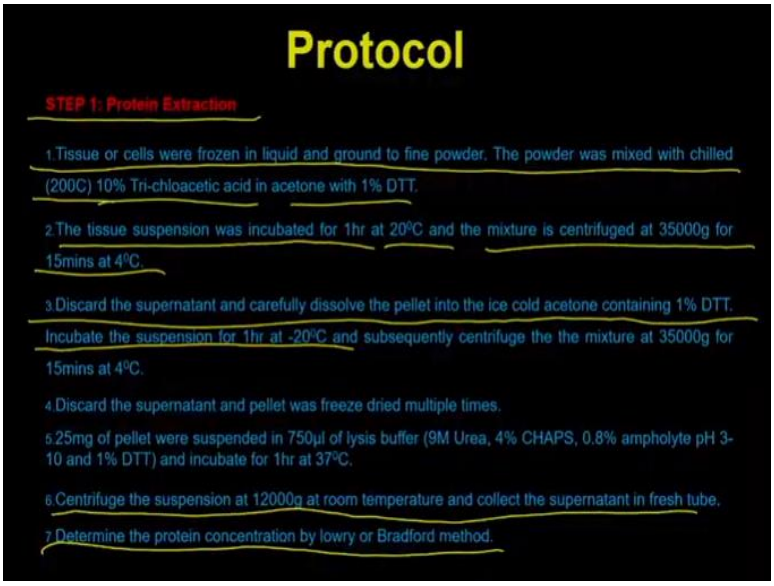


This is as the multiple steps for example the step 1 you are going to do the protein extractions. In the protein extraction the tissue or the cell wall frozen into and will make a find powder then this same powder is mixed with the chilled 10% Tca in acetone with 1% DTT. The tissue suspension

was incubated with 1 hour's at 20 degree and the mixture is centrifuged at 35000g for 15 minutes at 4 degree.

You can discard the supernatant and carefully dissolve the palate into the ice and acetone containing 1% DTT. Incubate the suspension and by going through this procedure ultimately what you are going to get the material and before you load these material into the isoelectric focusing strip you have to estimate the protein with the help of the either Lowry or the Bradford method.

So that it should not that to be you load very high quantity of the protein or very less quantity of protein. Because if you load very high quantity of the protein then the protein is going to overlap with each other and operation is going to be compromise if you load very little then you are may miss some of those protein which was present in the sample. But since the level was so low it may not be it may not be up to the level of detections.

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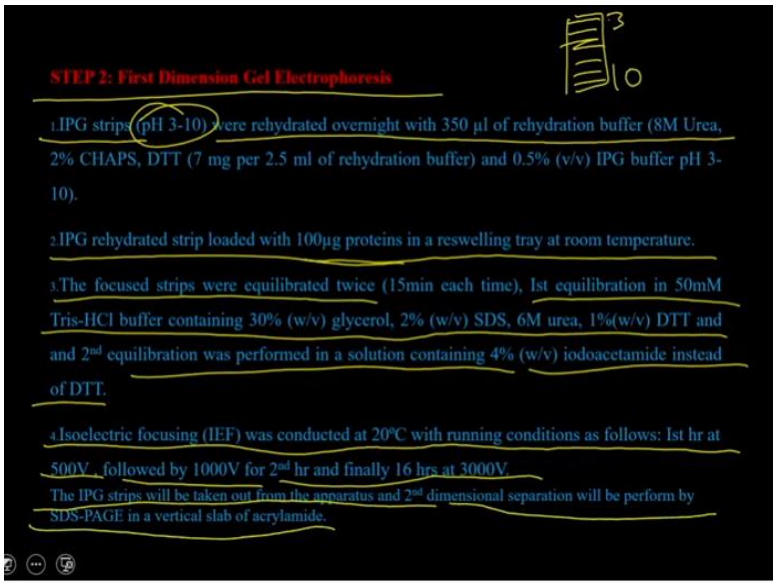


Then the step 2 you are going to do the first dimensions so the IPG strips in this case we have I am taking an example pH 3 to 10. So IPG strips either said you know are actually having the empollights which are coated and you can have a IPG strip of any range like 2 to 5, 2 to 10, 3 to 10 is rehydrated overnight with 350 micro liter of 3 rehydration bar offers. And once it get rehydrated you can load the IPG strip with 1000 micro gram in a reswelling tray at room temperature.

This focused strips were equilibrated twice first equilibration in 50 milli meter trips we have containing strips and the second was perform solution containing 4% iodoacetamide instead of DTT. So you are going to do the 2 focusing round in one of them it is going to have the DTT the other one you are going to have the iodoacetomide. Once the isoelectric focusing was conducted at 20 degree for running condition first hour at 500 volts followed by the 1000 volts for 2 hours and finally the 16 hours at 3000 volts.

So when you run these for the 16 hours at 300 volts the proteins are going to migrate throughout the isoelectric focusing strip and then it will get immobilized to its individual spots. The IPG strips will be taken out from the operators and the second dimensional separation will be performed in the SDS page in a vertical slab of the acrylamide.

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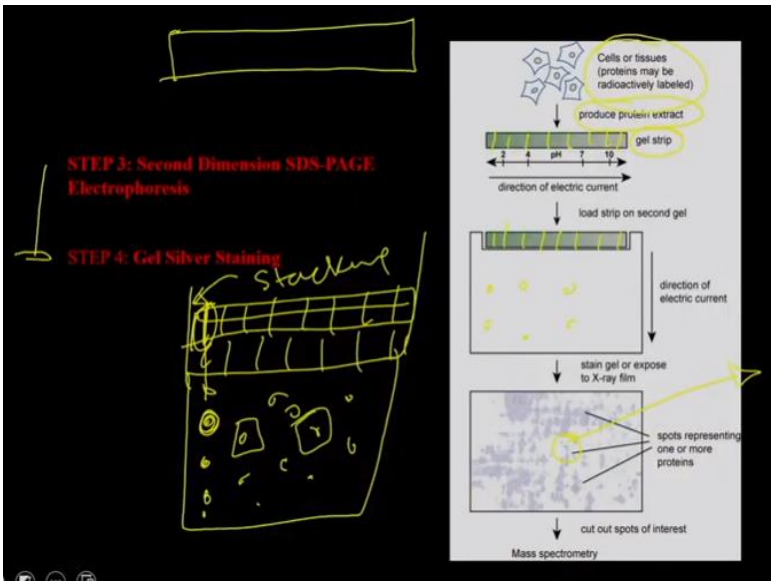


Now once you are done with this first dimensions then what you are going to have is you are going to have the IPG strips okay. And then what you have to do is you take the IPG strips and put it into the acrylamide solutions so what you are going to do? You are going to do first caste the resolving gels okay but you do not need to cast the stacking gels and so first you cast the resolving gel and you put the your strip okay and then you actually pour the stacking gel and because of that you are actually going to seal the differences between the IPG strip as well as the SDS page.

And then it becomes the continuous 1 gel and then you are going to perform the second dimension SDS page as we were discussing about the vertical gel electrophoresis. The proteins which are actually going to be migrated like this are now going to run in these direction okay which means all these protein is going to be concentrate and that is how you are actually going to get a spot from this protein. So you might have multiple proteins which are been present in this particular spots and that is why you are going to see the multiple spots from a single IPG strip focusing area.

Which means at a PI of 3.1 you might have 5 protein and all these 5 proteins may have a different molecular weight. So that is why you are going to see the proteins of the spots of the different positions and same will be true for multiple places. Once you got the spots you can be able to you know analyze these pot pattern on the second dimension gel and you can be able to compare it with the untreated samples or the different treatment samples and that is how it actually going to tell you that what are the spots are differentially been expressed.

Which means these are the spots which are additionally been present and that is the spots you can be able to extract out from the gel and that you can be able to use the downstream 2 dimensional gel electrophoresis approaches. And (()) (32:03) for example you can do the trypsmization and then you do the multi mass and all that and you can be able to identify the proteins.

There are couple of good MOOC courses are available from the IIT Bombay if you are interested to study the 2 dimensional gel electrophoresis as well as the proteomic. So if you are interested you can actually go to those IIT Bombay's and MOOC courses and you can study that in more in details. So here we are not going to discuss each and every those steps I am just trying to tell you that these are the actually obstance which are available for you to utilize gel electrophoresis to answer many critical questions.

So in typical what you have what we have done so what we have discussed so far that you are first going to take the cell or the tissue, you are going to prepare the extract the first you are going to run this extract on to the IPG strips. And that will be going to resolve them as in the

form of the bands on to these strips, and then what you are going to do is you are going to load these strip on to the SDS page.

So each strip, each spot is or each band it now going to be resolve into the individual spots and that is how you are going to get the 1000 to 2500 different spots and these spots can be cut out from the gel and can be done for the downstream applications like the proteous treatment the mass spectrometry.

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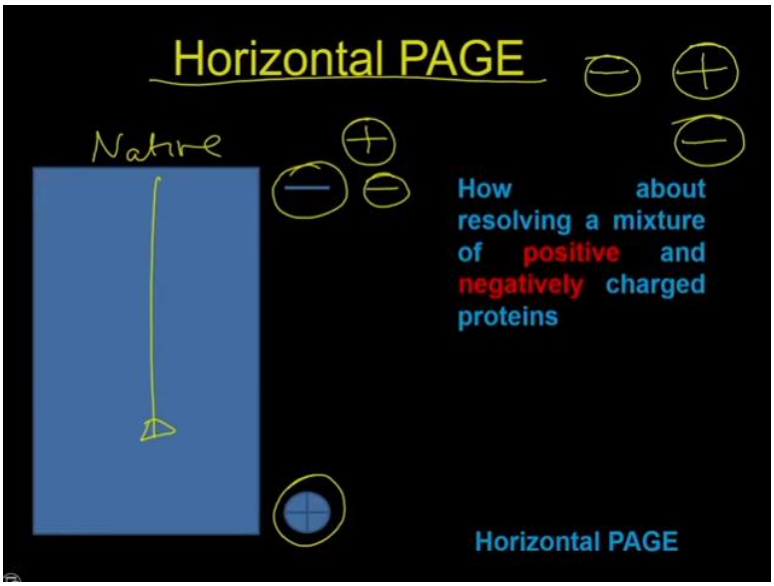


Now we have discuss about the native page we have discuss about the SDS page we have discuss about the agarose and acrylamide gels and we have also discuss about the 2 dimensional gel electrophoresis. What is the limitation of the native page is that it always governed by the intrinsic charge of the particular protein which means if the protein is negatively charge it is actually going to run as per the negative charge.

But you know that the electrophoresis operates has a negative electrode on to the top and positive electrode at the bottom. But what will be the condition if you have a mixture where you are going to have the positively charged and as well as the negatively charged protein. In those kind of complex mixture you will not be able to utilize the native page because either the positively charged protein will run or the natively charged protein runs.

Irrespective of whether so if you have a complex mixture where you have the positively charged or the negatively charged proteins you have to run the horizontal page. So horizontal page is similar to the agarose gel but you are using the acrylamide instead of the Agarose.

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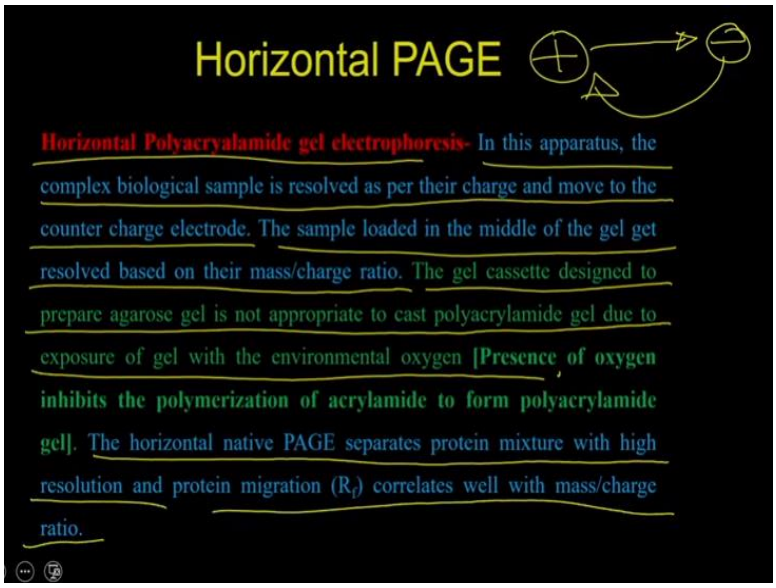


So, horizontal gel electrophoresis in this apparatus the complex biological sample is resolved as per their charge and move to the counter charge electrode. Which means the positive will move towards the negative and the negative will move towards the positive. The sample; loaded in the middle of the gel get resolved based on their mass by charge ratio. The gel cassette is designed to prepare agarose gel is not appropriate to cast the polyacrylamide gel due to the exposer of gel with the environmental oxygen.

So the gel cassette what we use for the agarose gel electrophoresis is not good enough to cast the polyacrylamide gels. Because, it is been open from the top so because of that it is actually going to get the direct entry of oxygen; and you know that the oxygen is a exhibitor of the acrylamide polymerization. So if the oxygen is present the polyacrylamide is not going to get polymerized to give you the page.

So because of that you require a specialized native page operator which actually can resolve the sample based on the charge by mass ratio. The horizontal native page separates the protein mixture with the high resolution and the protein migration is corresponding well with the mass by charge ratio.

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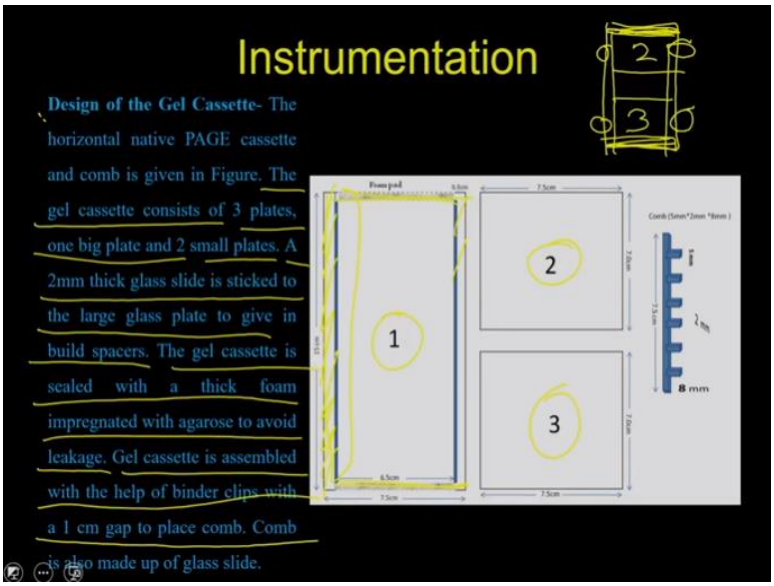


So first discuss about the instrumentation part so the design the gel cassettes. The gel cassette consists of 3 plates the 1 big plate which is the plate number 1 and the 2 small plates which are number 2 and 3 are 2 mm thick glass slide is stick to the large plate to give the build spaces which means you are actually going to put the 2mm thick glass slide on to the slide. And that actually is going to give you the spacers the gel cassette is sealed with the thick foam on to the both side

Okay on the both side you have a very thick foam which is actually going to use for the sealing of this particular operators which is impregnated with agarose to avoid the leakage. Then the gel cassette is assembled with the help of a binder clips with a 1mm 1 centimeter gap to place the comb. Which means you have this main plate you have a comb you have a foam on this side you have spacer on both the sides.

And then you what you are going to do is you are going to place the number 2 you are going to place the number 3 like this and then you are going to put the clips on both the sides. So that this whole operator is going to be assembled as 1 and then you are going to start casting the polyacrylamide gels.

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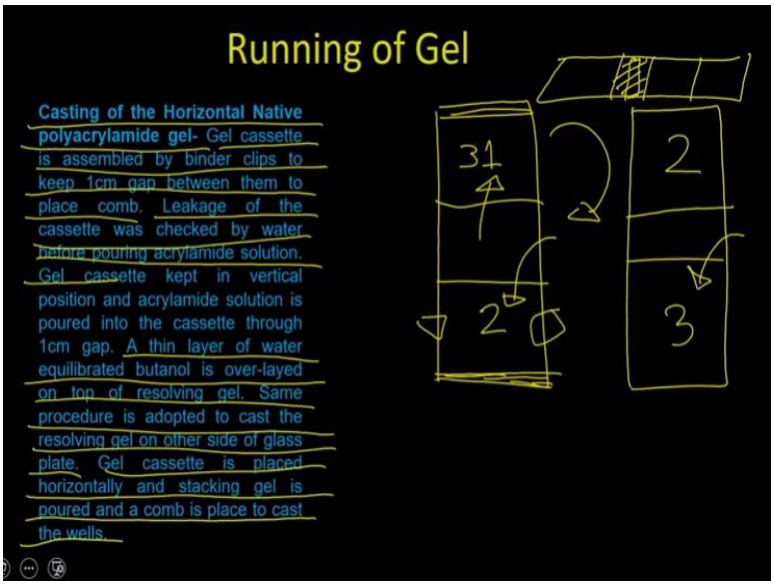


The casting of the horizontal native acrylamide gels the gel cassette is assembled by the binder clips to keep 1 centimeter gap between them to place the combs. The leakage of the cassette was checked by the water before pouring the acrylamide solution. So what you have is you have a main plate where you are going to have the foam at the bottom and the top so first what you do is you are so this is the plate number 1.

The first what you do is put the plate number 2 is okay put the clips on both the sides and then you pour the liquid okay. And once the it is get resolved and it get polymerized then you have to rotate this okay and then you put t number 3 then this side and you bring it down okay and then pour it again. So you can imagine that this is like this if you are going to see for this so number 2 will go on top and number 3 will come at the bottom and that is how you are going to pour into this.

So actually the casting is going to be a 2 step event first you cast for the number 2 then you cast for the number 3 and then the middle portion is going to be empty and the thin layer of water equilibrated brutanol is over-layed on top of resolving gel. Same procedure is adopted to cast the resolving gel on the other side of the glass plate. Gel cassette is placed horizontally and stacking gel is poured and a comb is placed to cast the wells which means once these are things done then you take the plate like this okay.

So this side is casted this side is casted then you pour the stacking gel in between and put a comb. So if you put the comb the well is going to be prepared just like it was prepared for the DNA.

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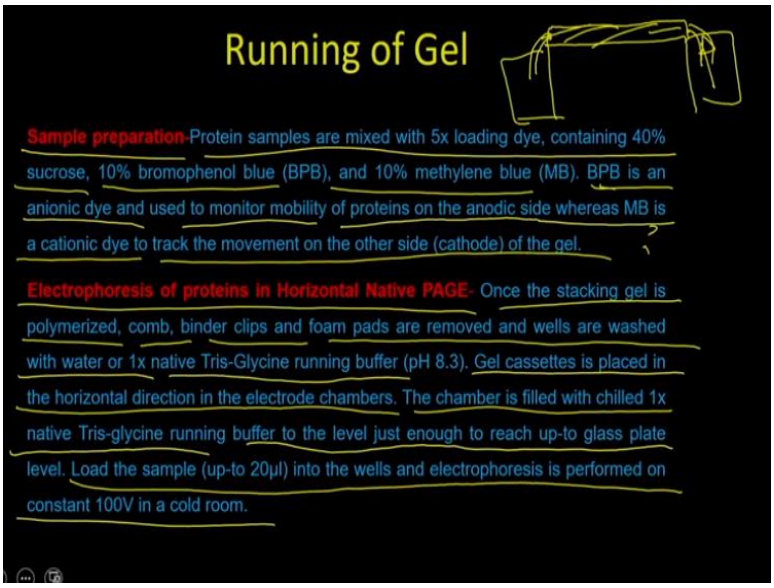


Now running of the gel the sample preparation the protein samples are mixed with the 5x loading dye containing 40% sucrose 10% of bromophenol blue and 10% methylene blue. Bromophenol blue is a anionic dye and used to monitor the mobility of proteins on the anodic side whereas MB is a cationic dye to track the moment of the other side. The electrophoresis once you put that once the stacking gel is polymerized the comb binder clips and foam pads are removed and wells are washed with water and 1x the native Tris-glycine running buffer.

The gel cassette is placed in the horizontal direction in the electrode chamber. The chamber is filled with the chilled 1x native Tris-glycine running buffer to the level just enough to touch to the glass plate which means if you have this and you have kept the glass plate like this okay. So this side you have a chamber you connect these with a something so that it actually take up the buffer but it is not a continuous mode of running.

It is going to be discontinuous mode so that the current goes through the gel from 1 buffer chamber to the next buffer chamber. So the principle remains the same except that now we are doing the electrophoresis in the horizontal directions. Load the samples up to the 20 mica liter an electrophoresis is performed with the constant 100 volts in a cold room. Because the

electrophoresis is going to be for 18 to 20 hours so it as to be in the cold room so that the sample does not get denatured and destroyed.

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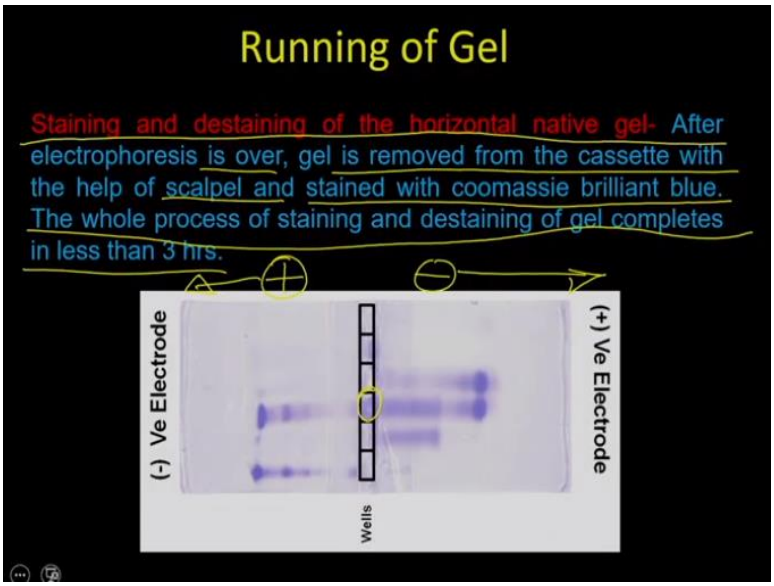


Then once you are done with that you do the staining and de-staining after the electrophoresis is over gel is removed from the cassette with the help of a scalpel and the stained with the coomassie brilliant blue. The whole process of staining and de-staining of the gel complete is less than 3 hours and what you can is that i have loaded a bacterial lycate and what you can see is that the positively charged proteins are going towards the negative electrodes and the negatively charged proteins are migrating towards the positive electrodes and that is how you can be able to.

And all the both of these samples are native in so that is how you can be able to resolve and you can be able to visualize the pattern of the positive as well as the negative protein present in the same mixture using the horizontal gel electrophoresis.

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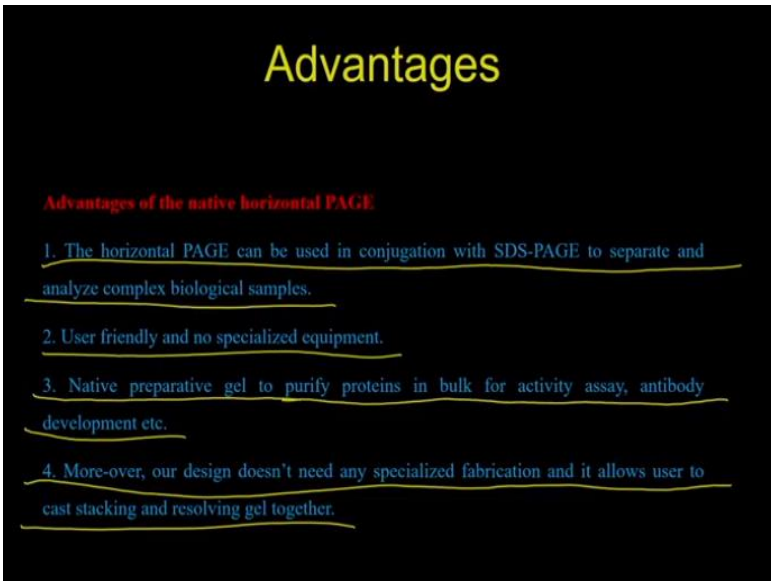


What are the advantages of this gel electrophoresis the horizontal gel electrophoresis can be used in this conjugation with SDS page to separate and analyze the complex biological samples. It is user friendly and no specialized equipment is required the native page native preparative gel is purify in bulk quantity the protein in the bulk quantity for the antibody development as well as for the activity as said.

Moreover this design does not require any specialized fabrication and in allow user to cast stacking and resolving gel together. Which means the horizontal page is giving you the many advantages where you can actually utilize them to resolve the proteins of the positively as well as the negatively charged which are present in the sample together. Number 2 because it is actually resolving the sample based on the charge by mass ratio you can be able to utilize the gel in conjugation with the SDS page, and that actually will allow you to resolve the very complex biological samples.

And there are other advantage that is user friendly compare to the other TDS the horizontal page (()) (43:59) are available and so with this we would like to conclude our lecture here. In our subsequent lecture we are actually going to discuss the experiments as well as the research problems what are been related to the electrophoresis. And we also going to discuss these the most staining techniques which are also available in the which are also been used in the electrophoresis.

So with this I would like to conclude my lecture here in a subsequent lecture we will be going to discuss the more topics related to the electrophoresis thank you.