Bioengineering: An Interface with Biology and Medicine
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Lecture – 33
Techniques to Study Protein & Proteome-I

Today we are going to talk about basic electrophoretic techniques to separate proteins and steady proteins. So what is electrophoresis?

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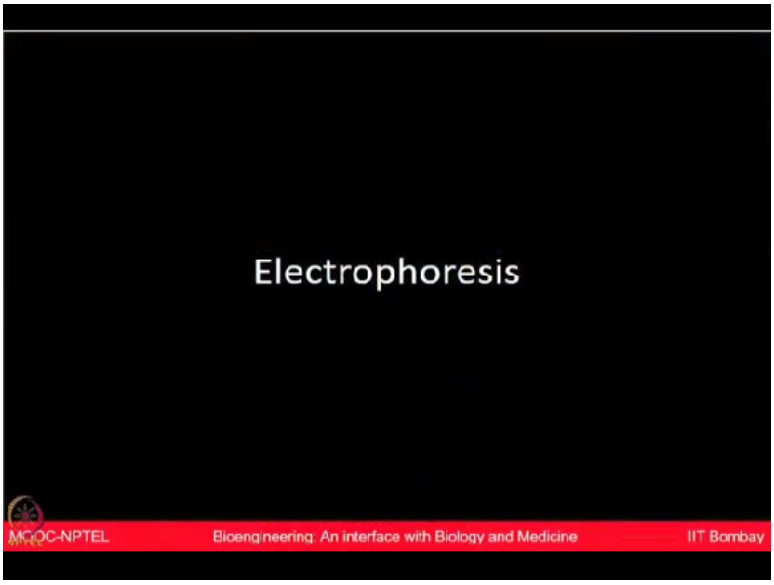

It is one of the powerful techniques for protein separation and the separated proteins can be visualized after subsequent staining steps.

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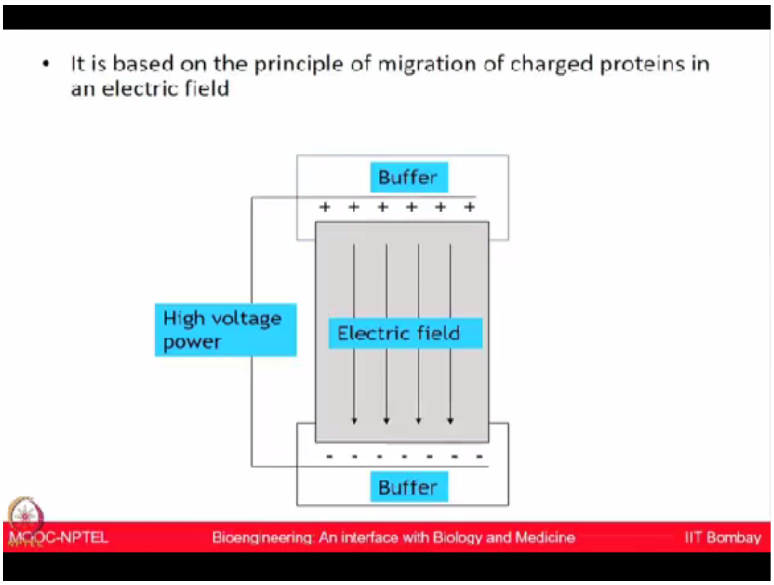

It is based on the principle of migration of the charged proteins in the electrical field. As you have studied in the last 2 lectures some basics of amino acids and proteins and then we moved on to study the proteins and proteomics. You have seen that, you know, now there is emphasis on looking at thousands of proteins simultaneously. That is very relevant especially if you are looking at the complex biological problems because the proteins work in interaction.

They work as a part of signalling pathways. So studying proteins together in totality becomes very crucial. So there are many techniques which are evolving which aims to study proteins in very high-throughput manner and they try to utilize protein properties to separate them well. One of such technique is known as 2-dimensional gel electrophoresis or 2-DE.

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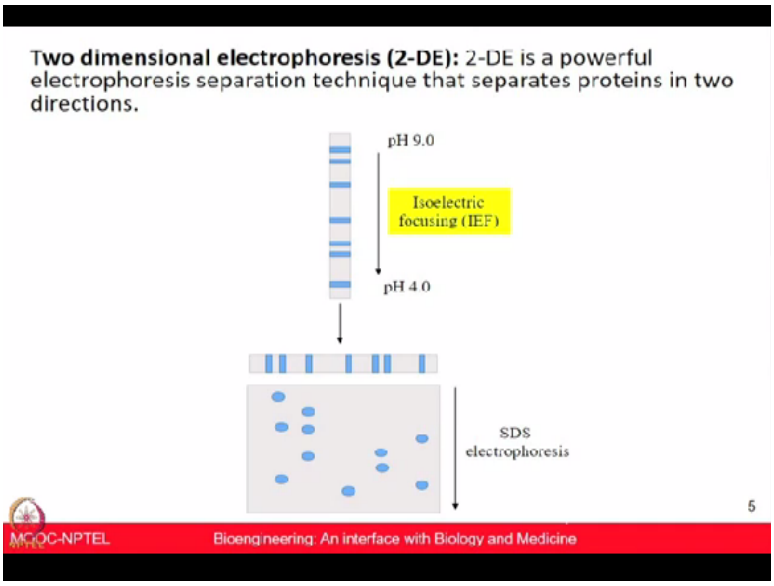

2-DE is a powerful electrophoretic separation technique that separates proteins in 2 directions. The isoelectric focusing which carries out in the first dimension and separate proteins on the basis of their unique isoelectric points.

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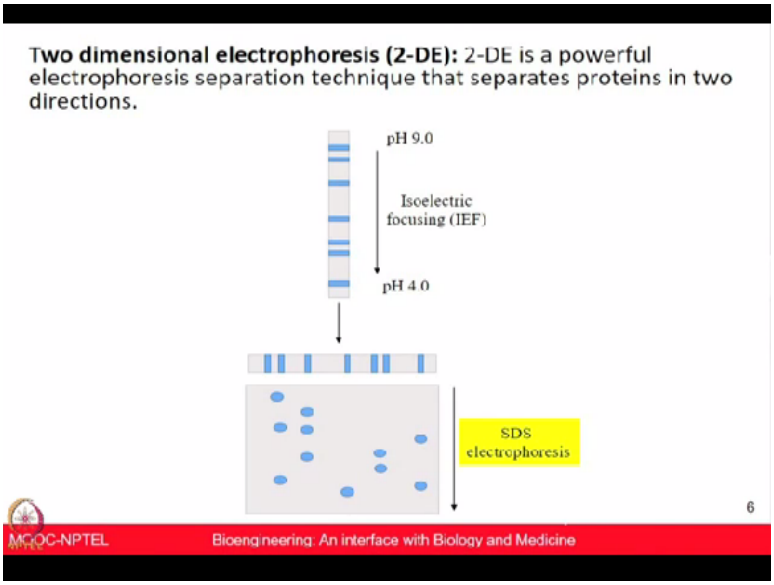

And then the SDS page which separates protein on the basis of their molecular weight and that is known as the second dimensional separation. So because we are utilizing 2 different properties of proteins in the first dimension, isoelectric point and in the second dimension, the SDS page, this technique is known as 2-dimensional electrophoresis. So if you want to do an experiment based on 2-dimensional electrophoresis, this kind of work flow can be followed.

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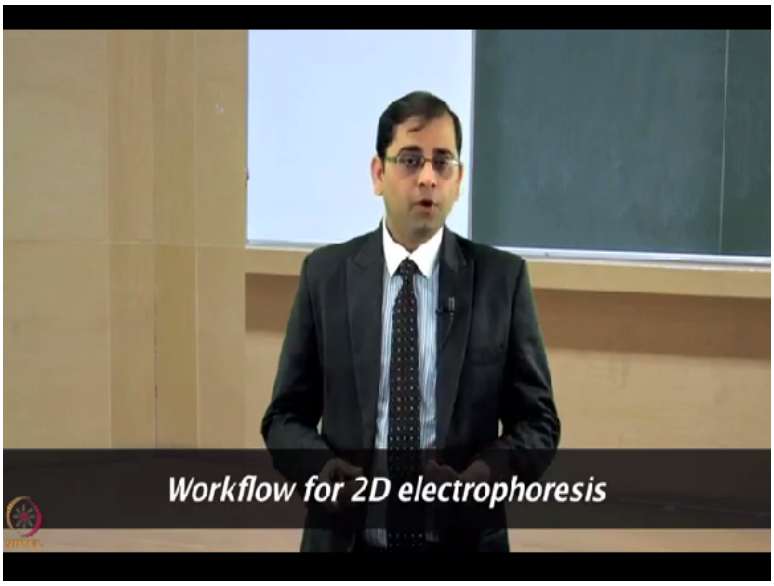

You have stacked the protein.

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You have done all kind of quality control checks to ensure that proteins do not have any contamination like you do not have nucleic acid contamination, carbohydrate, or lipids as a part of the protein and the protein does not have even salt which may even interfere in your assays.

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Then you have to quantify the protein and once, you know, that now you have got a descent clean protein with good concentration, that protein is now ready for the further complex analysis.

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And now your intention is to separate those thousands of proteins present in that mixture using 2-dimensional electrophoresis.

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So to do that the very first step you will do isoelectric focusing in the first dimension. Then you want to prepare the strips to separate them in the SDS page.

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And to that you are doing an equilibration of IPG strips.

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Then the next step comes for the second dimension separation using SDS page.

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Then you want to visualize the gels using staining techniques.

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And then you want to do the image analysis using various softwares. And then those proteins which looks interesting to you, you want to excise those and then identify them using mass spectrometry. So this kind of a typical workflow, so very first thing that you want to look into which kind of immobilized pH gradient steps you can use for doing the protein separation. These are the acrylamide based, you know, plastic strips on which the different pH buffers are coated.

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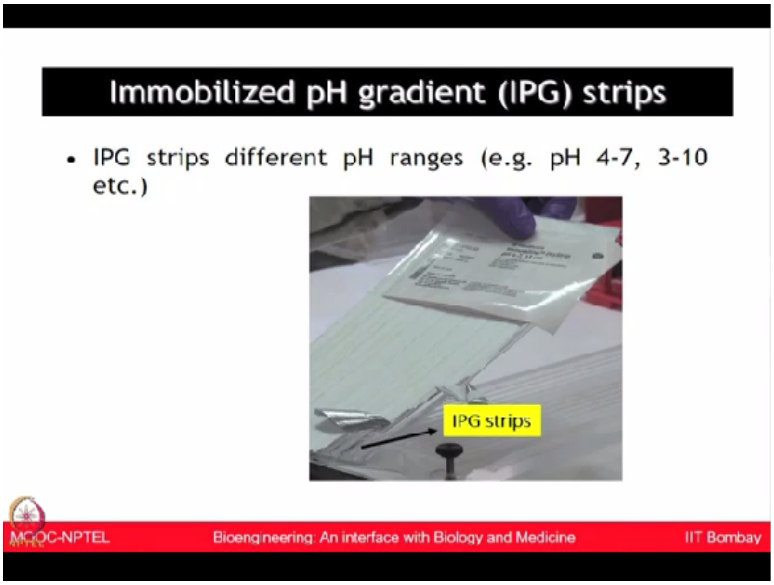

And then they comes in different pH range for example, it can be pH 3 to 10, it can be even 4 to 7 physiological range.

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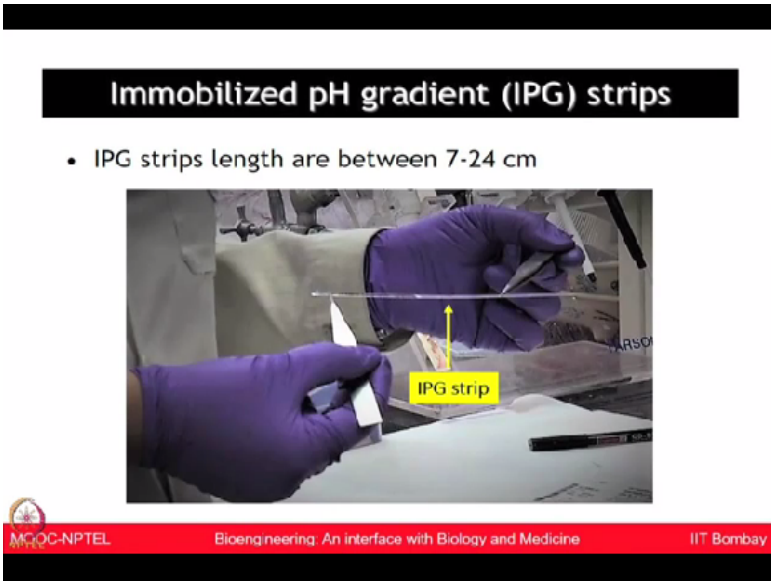

And they also come in different length for example, you can always start with the smaller length of 7 cm but for the actual experiment you can choose the large length of 24 cm.

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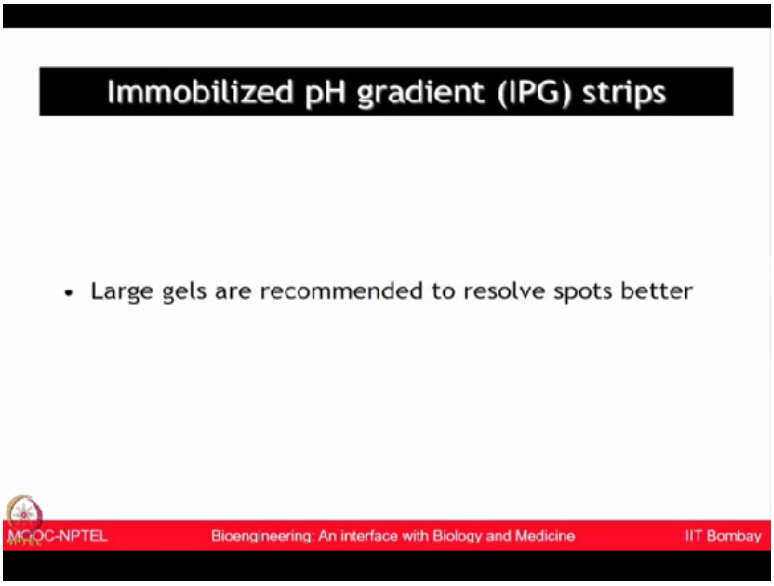

And then your intention is to resolve the proteins on the larger gels so that you have a good separation of various protein spots. So these immobilized pH gradient strips, they are very stable. They are much more durable.

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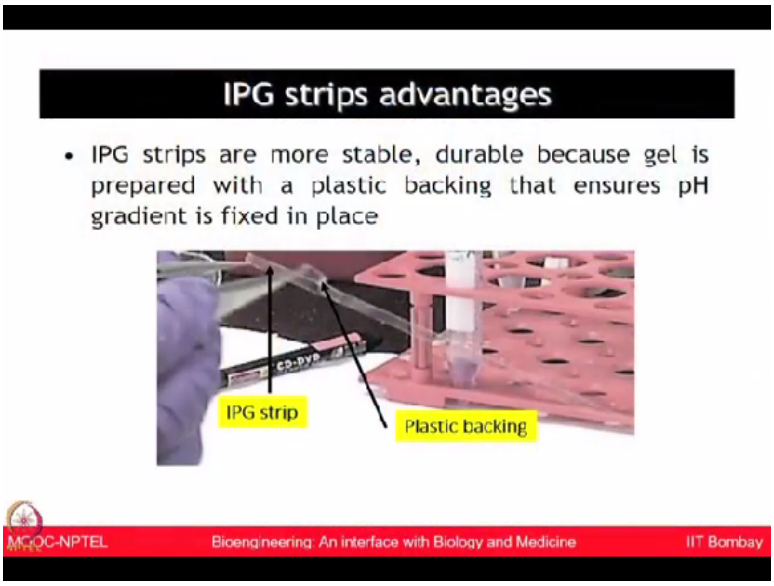

And because the gel is prepared with the plastic backbone, that actually ensure that the pH gradient is fixed in the place. It provides high resolution and it has also improved lot of reproducibility in different laboratory comparison which was one of the drawbacks in the earlier 2D technologies which were based on the tube gels.

These IPG strips provide you, you know, good capacity for loading lot of proteins and therefore, now you are ready to separate proteins in the first dimension. But before, you know, you want to, when you are doing an experiment, the very first step you want to do, you want to take your protein in the solution form and you want to get it immobilized in the IPG strip or immobilized pH gradient strips. To do that, your very first step is rehydration step.

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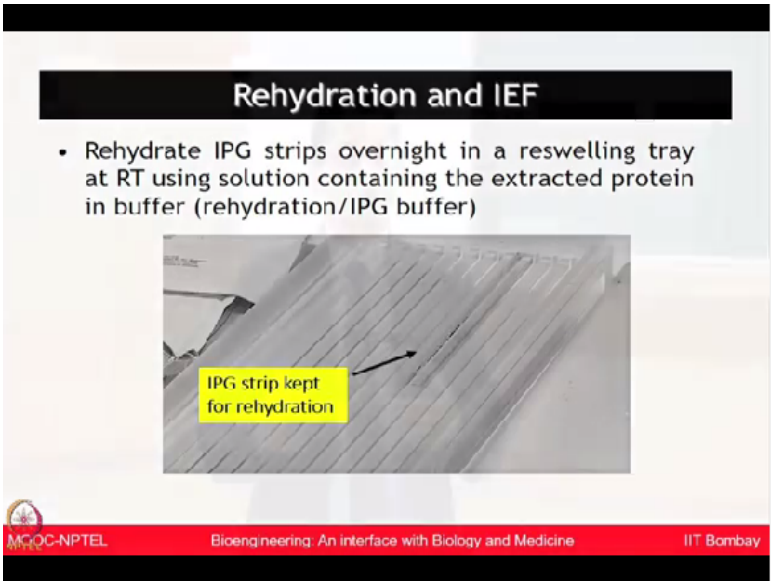

So you want to rehydrate the protein solution on to IPG strip in the overnight in reswelling tray and that you can do either, you know, using a passive rehydration method or you can do using an active rehydration method. So in passive rehydration, there is no voltage is applied. Whereas in case of active rehydration, you can apply the low voltage.

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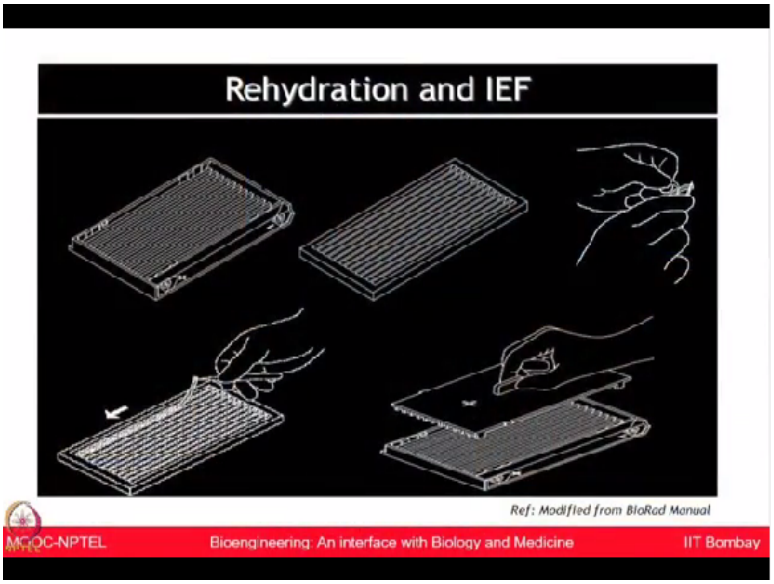

Then after, you know, you have put your protein solution, you have added now the IPG strip and then you have covered it with some mineral oils so that, you know, the proteins are not solidifying on the edges and it is not getting evaporated, so then overnight you are leaving it so that proteins slowly get absorbed in the IPG strip. And then the next day when you want to start, your first dimension separation, then you are ready to do the isoelectric focusing.

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Let us also see some of these details of doing rehydration and IEF in the animation form. (Video Starts 05:50 - Video Ends 08:22)

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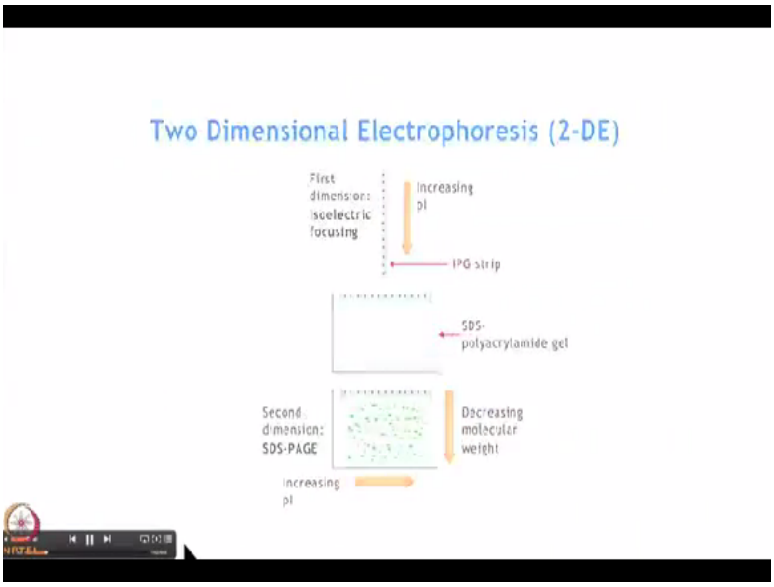

Prior to isoelectric focussing in 2DE, the commercially available IPG strips must be rehydrated. This can be done either by passive rehydration or active rehydration. In passive rehydration, the IPG strip is placed with its gel side downwards in a well containing the protein sample reconstituted with the suitable buffered solution. This is then covered with mineral oil to prevent the gel from drying up and left overnight for 10 to 20 hours depending on the length of the strip.

In active rehydration, the protein sample is added to the strip via a sample cup followed by cover fluid to prevent the gel from drying up. This is then placed in the isoelectric focusing instrument and low voltage is applied which allows the strip to take up the protein sample. Active rehydration is also performed for 10 to 20 hours depending upon the length of IPG strip being used.

These loaded strips are then focused on an isoelectric focusing unit by passing current. The various proteins of the sample mixture migrate in the electric field and comes to rest when the pH is equal to their pI, that is they become neutral and are no longer affected by the electric field. Progress of electrophoresis is monitored by means of a tracking dye like Bromophenol Blue, BPB which is a small molecule and therefore, migrates ahead of all other proteins.

So let us talk about isoelectric focusing. So as we have studied in the one of the earlier lectures that, you know, these amino acids have different side chains, provides the positive and negative charge. So proteins have overall net negative or positive charges and the pH determines the ionization states of these amino acids and therefore, the charge on the protein. So isoelectric point is the pH at which the net charge on the protein is 0.

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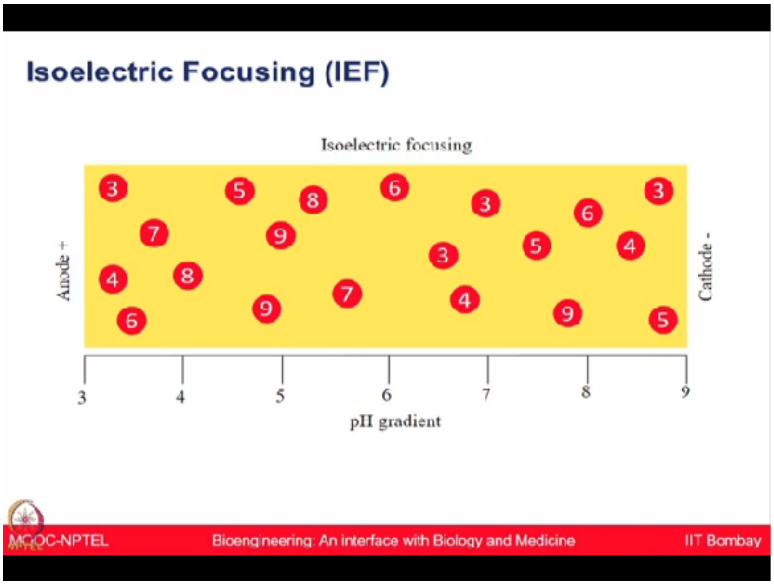

So when pH=pI, there is no mobility of these proteins and isoelectric focusing or the IEF uses this particular fundamental property to separate the proteins and depending on what kind of pH

strip you are using, you can separate either in the pH range of 3 to 10 or 4 to 7. So when you are running proteins on SDS page gel when you want to separate only based on the molecular weight, you want to, you know, boil the protein, you want to denature the protein, you want to separate the denatured protein on SDS page gel.

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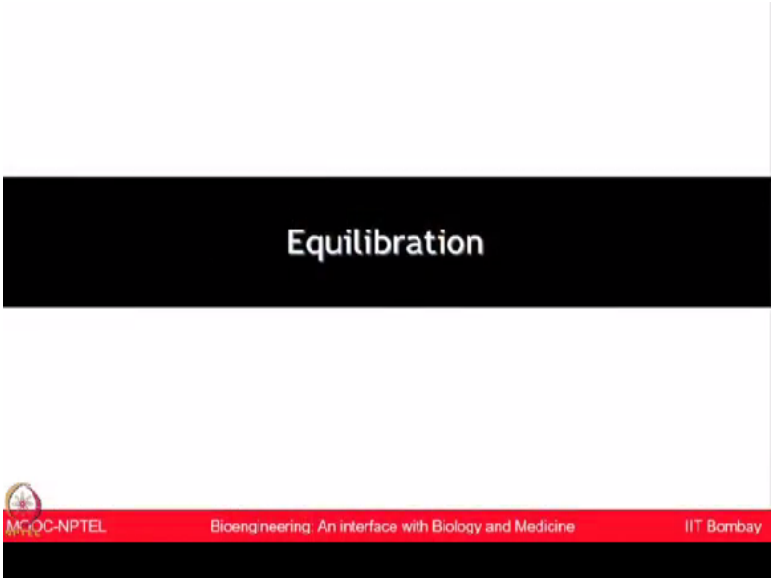

However, in case of 2-dimensional electrophoresis, because you have started on the IPG strip, so you cannot boil them. So you have to prepare the IPG strip before you load them on to the SDS page gel and that is done in a process known as equilibration. So this is one of the conditioning step which you apply so that the proteins which are separated by the IEF, they are now prepared to be separated in the second dimension based on the molecular weight.

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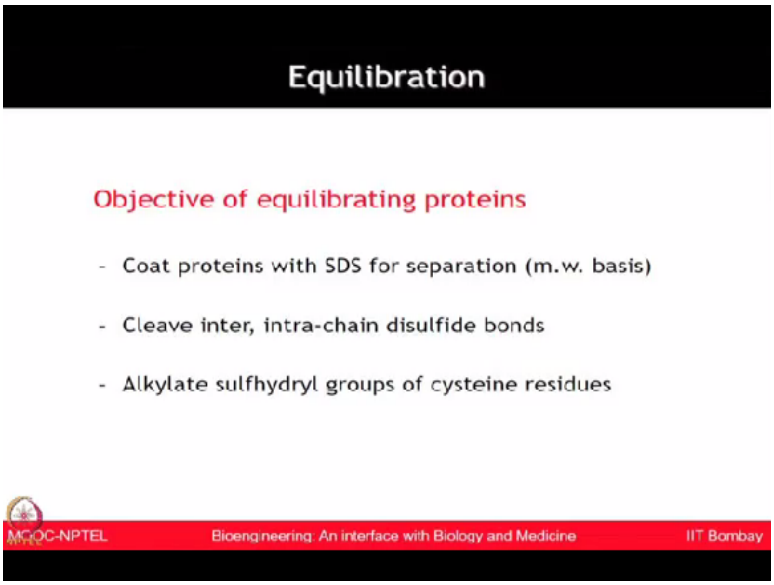

So the objective of equilibrating these proteins on to the IPG strip, are to coat the proteins with SDS for the separation which you use for the molecular weight basis. Then you want to also clean the disulfide bonds which is inter or intra-chain disulfide bonds and you want to also alkylate the sulfhydryl groups of the cysteine residues. So let us look at some of the recipe which is used for doing the equilibration.

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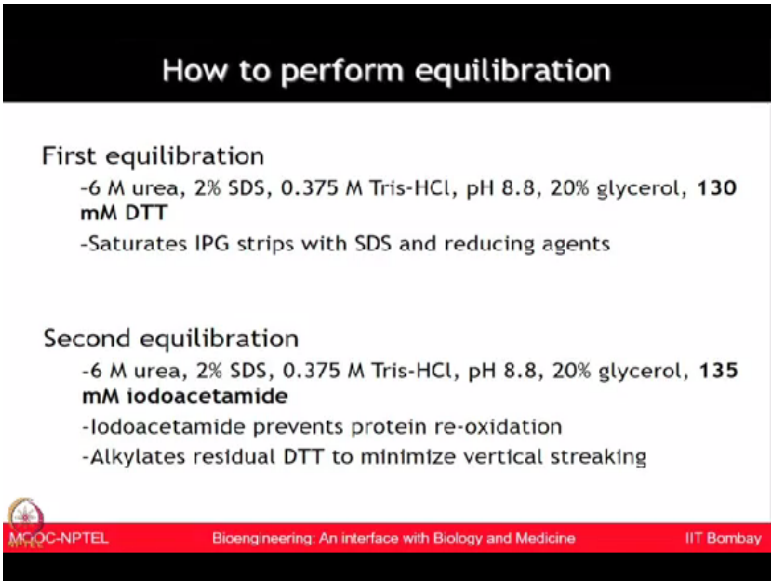

The first equilibration step involves addition of DTT and that DTT along with, you know, you are adding the SDS, you are adding the Tris-HCl and glycerol and in the second dimension, you are adding the iodoacetamide. This iodoacetamide is going to prevent the protein re-oxidation. So whatever you have denatured and those bonds should not be reformed again and these

alkylates, the residual DTT to minimize the vertical streaking as well.

So you have to perform both first and the second equilibration steps and ideally you can do it, you know, a repeat process of that to ensure that, you know, your reduction and alkylation has happened properly. So once the IPG strip you have already focused the protein based on the first dimension property and now you have also prepared the strip to be separated further based on the molecular weight.

So now you can use the second dimension protein separation property and that itself is a very, you know, interesting technology which is known as SDS page, SDS polyacrylamide gel electrophoresis. So the proteins, when they exhibit different molecular weights depending on the amino acid composition, this property can be utilized to separate the proteins using SDS page gel. And this electrophoretic method which is SDS page is aiming to separate the proteins based on their molecular weight.

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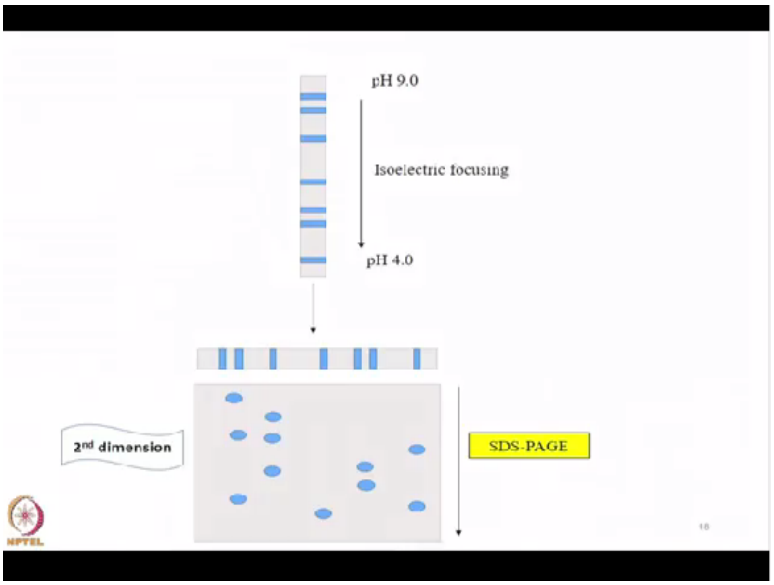

So now whatever we have separate proteins based on the pI values, they are going to get further separated based on the molecular weight. So SDS page is one of the widely used electrophoretic technique. It separate proteins according to their size. The detergent SDS sodium dodecyl sulfate is negatively charged.

So in case of if you are just directly separating proteins on the SDS page gel, you can simply boil the protein in SDS and beta-mercaptoethanol. In case of 2D electrophoresis, you are going to prepare the IPG strip with the equilibration steps after reduction and alkylation steps. Now those IPG strips are ready to be separated further on the second dimension SDS page.

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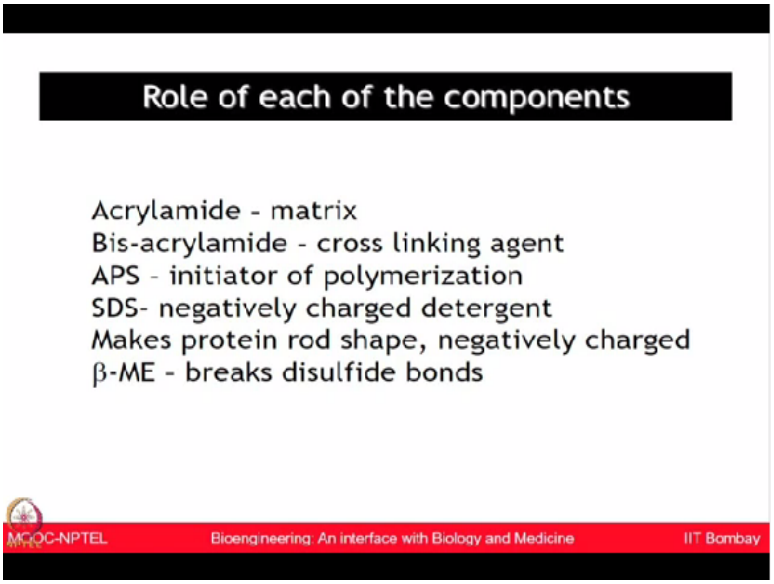

Just kind of, you know, brief about SDS page, some of the chemicals which are used to make the gels, what is the role of each of these components for example acrylamide, it is providing the matrix. Bis-acrylamide is a cross linking agent. Ammonium persulfate or APS, it initiates the polymerization process. The SDS is negatively charged detergent which makes the protein rod shaped and negatively charged.

And then beta-mercaptoethanol, it breaks the disulfide bonds. So some of these chemicals are used for the SDS page process. I think it is a good idea for you to know the role of them and how they work in the, while performing the SDS page gel. So you have started with your protein immobilized in the rehydration process on to IPG strip.

You have done the first dimension separation using isoelectric point, equilibrated the strips to prepare them and now you have separated proteins in the SDS page gel based on their molecular weight properties. So for everything whatever you are doing, it is all on the transparent gel. You have literally no idea that what can be seen on those gels and what you have separated, until

unless you are adding some staining reagents which can visualize the gels.

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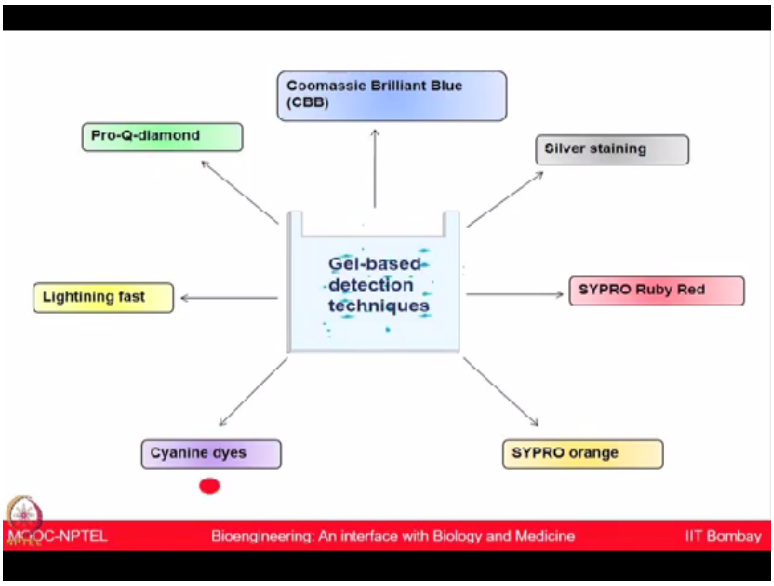

So therefore, different type of staining reagents becomes very important. The most commonly used staining reagent is Coomassie Brilliant Blue but you can also use the silver staining, you can use different sensitive dyes like SYPRO Ruby, even if you are looking at some modification at the PTM level, you can use even Pro-Q diamond or you can look at for very sensitive detection, you can use even cyanine dyes.

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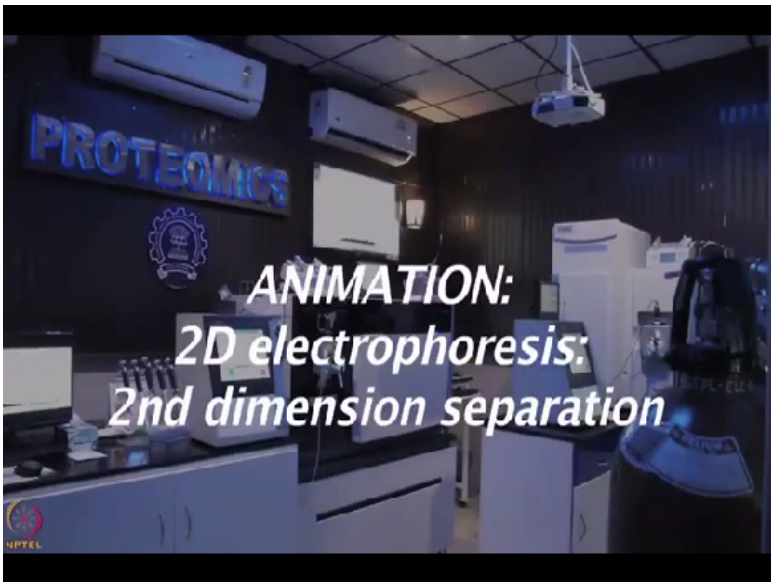

Let us watch the following animation to understand this concept better. (Video Starts 13:59 - Video Ends 15:19)

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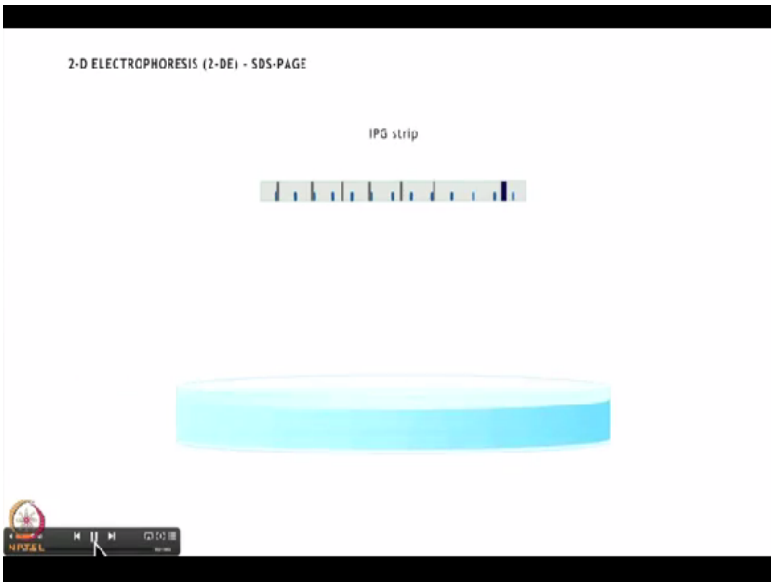

The IPG strip is equilibrated in a reducing agent like DTT, followed by an alkylating agent, iodoacetamide which prevents reformation of the reduced bonds. This strip containing the separated proteins is then placed on the SDS polyacrylamide gel slab and subjected to SDS page by applying a direct current between 100 to 350 volts depending upon the size of the gel.

Any proteins that may have been present as a single band on the IPG strip due to similar isoelectric points can now be separated on the basis of a molecular weight with smaller proteins migrating farthest. View of a sample 2DE gel which has been stained with Coomassie Blue. Each spot provides information about the molecular weight and isoelectric point of the proteins.

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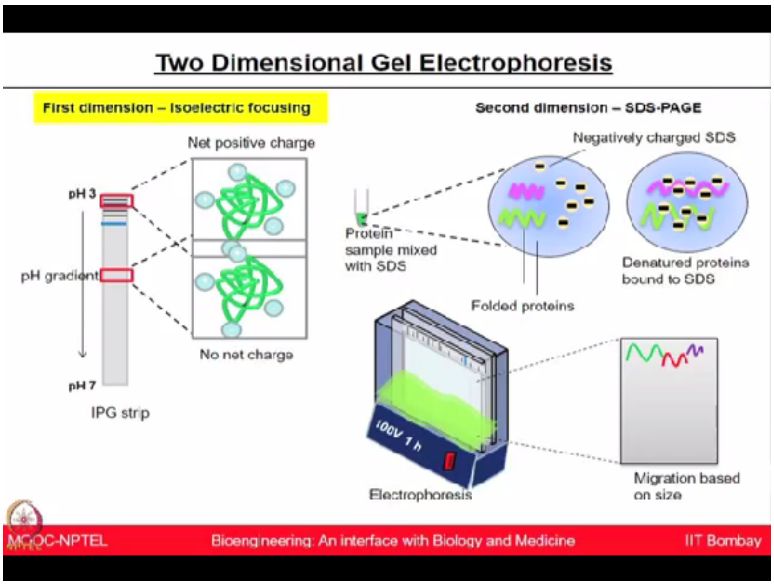

So broadly, you know, talking about 2-dimensional electrophoresis, the very first part you did, the first dimension separation in the isoelectric focusing when you are looking at, you know, there is a pH=pI, then there is no net charge and proteins are separated based on that depending on what IPG strip you have used.

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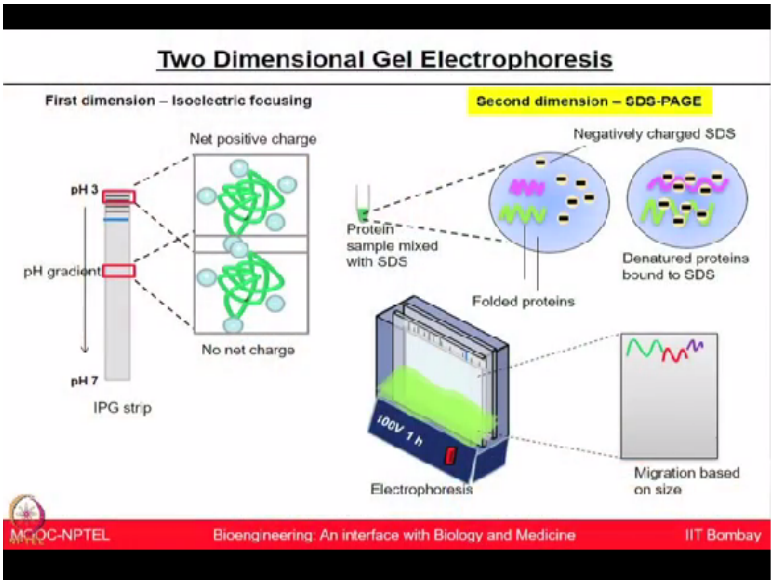

Then in the second dimension, we separated protein further in the SDS page based on their molecular weight property and proteins are migrating based on their size. So this kind, you know, technology of 2-dimensional electrophoresis could be used for studying the differential protein analysis and that is widely used for many applications, you know, a lot of biological applications have used 2-dimensional electrophoresis to separate thousands of proteins on the gel.

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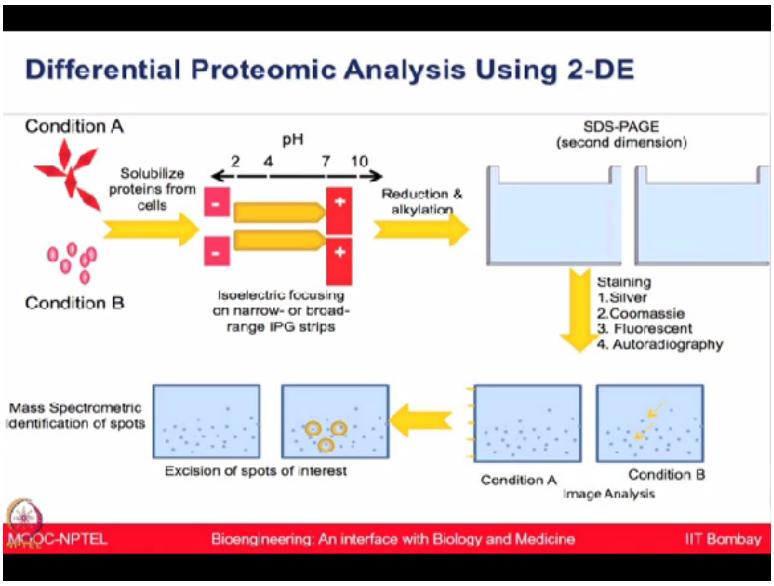

And then they have compared a condition A with condition B for (()) (16:08) healthy individuals with the disease individual. So you have got first one to separate the proteins from these, you know, 2 populations, you want to solubilize the proteins, you want to separate them on the, you know, IPG strips. Then after doing the equilibration step, you want to separate them on the second dimension shown on the schematic on the screen.

Then you are staining them with one of the staining reagents and after staining, then you can start visualizing, you know, thousands of spot on the gel and then the analysis becomes very crucial, the automation in the software and the, you know, our capability for data analysis becomes very critical here because you want to now pick up the differential protein responses or few very unique proteins which might have emerged in a disease condition.

So you want to pick up those interesting proteins and now from the gel, you can simply excise those protein spots which are based on the 2D separation. So once you have excised the proteins of interest which is the spot you are seeing on the gel and then you can further process that, you know, with some enzymes like trypsin which converts the protein to the peptide forms and then we can further analyze them using mass spectrometry to identify the proteins.

So today we have tried to learn about some of the basic electrophoretic techniques for the protein

separation and we have mainly studied the workflow of 2-dimensional electrophoresis which is one of the interesting, simple but very elegant technology which can separate thousands of proteins.

Of course, it becomes, you know, little challenging when you talk about clinical samples because you have, you know, large number of sample to be analyzed from the control versus test conditions and that is where, you know, there are some limitation which people start encountering. So then as a part of 2D electrophoresis, I also tried to brief you about the SDS page gels which is separating proteins on the molecular weight basis.

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So by now, you have got good concept of how 2-dimensional electrophoresis can be performed. Let us start a laboratory demonstration session where my TA will show you the various steps involved in performing 2D electrophoresis starting from rehydration, then doing isoelectric focusing, equilibration steps and performing the second dimensional separation. So let us start the SDS page lab demonstration session now. (Video Starts 18:23 - Video Ends 28:57)

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Hello, I am Shalini. I am a TA of this course. So today we are going to learn how to make SDS gel and how to cast it and then how to run it. So first of all I would like to explain about SDS. So that is a technique using which we can separate all the proteins on the basis of its mass. So these are some buffers which we will require. Like one is 1.5 molar Tris buffer solution or 6.8 pH. Other one is Tris buffer and that is 8.8 pH.

The other is 10% SDS solution, this is 30% acrylamide and bis-acrylamide solution. Here it is TEMED and here it is APS. So all these 3 are required to be stored at 4 degree Celsius because these are very sensitive. So we have already taken aliquotes of it and we will store it. This is glass tile which is having spacers which is called thick plate and this is called thin plate which is separated by these spacers.

And here you can see it is mentioned 1.0 mm which is the space between these 2 plates. This is the comb which allow us to make the wells and where we put the sample, we load the sample on this. So this is the casting unit and this is the casting stand. Here this is the dummy plate which we use if you are running single gel. Now I will tell how to assemble these 2 plates in the casting unit.

So first of all we have to hold these plate like this. Now we have to place it on the floor so that both are on the same level. Now we have to place it inside this unit. As you can see now it is

done. So we have to lock it at its position and we can place it here in the casting unit. Now to check the leakage, we have to pour some water so that we can see whether it is leaking or not. So we can fill it up to half well level and we can leave it for some time to check whether it is leaking or it is okay.

So after ensuring that the setup is not leaking, the water which we have added to it is not leaking, we had removed the water and now we will make the resolving gel which will help us in resolving the bands according to their molecular weight. So this is the composition in which we have to add Tris-chloride pH 8.8, distilled water, 10% SDS, 10% APS,30% acrylamide bis-acrylamide solution and TEMED.

Out of these solutions, 10% APS and TEMED are very sensitive to temperature and light. So they are kept covered with aluminium foil. As you can see, this is APS and TEMED. And it is to be removed from plate just before the experiment to be started, before you want to cast the gel. So here we have added everything except TEMED and APS. So I will add it. First I am adding APS, that is 50 microliter for 1 gel.

Now I am adding TEMED that is 2 microliter. It starts solidifying fast. So we have to mix it properly. And then we have to start loading in the gel setup. You have to fill it up to this level. Here you can see there is a level. So you can fill up to this and then you can add water to remove all water or isopropanol to remove any bubble which is present here up to this green line. Till the time we have left this gel to get solidified, we will start making this stacking gel composition of which is given here as we can see here, stacking gel.

Everything is same except the pH of the Tris-Chloride solution which we are using here in the stacking gel is 6.8 unlike in the resolving gel where it was 8.8. As you can see here, now the gel is casted and the comb is placed. For easier visibility, we have also marked the wells which are formed by using the comb. Now we have to remove it from here and we have to use this gel run unit which is having electrodes in it.

Now we have to place it in such a way that the well are inside, facing inside. So carefully place it

here and now as we are running only 1 gel, we need a dummy plate which will go on the other side of the gel running unit. Now we have to close it, now just to seal. Now we have to transfer this whole unit in the buffer tank which is having 1X SDS running buffer. As you can see here, there is a red marking and here there is a black marking.

So we have to place it in such a way that red comes along the side of red. Now the setup is complete and we have to pour some more buffer inside this running unit. So now this is 1X SDS running buffer. We have to add this in the unit and now it is, you can see it is filled up to the brim. Now we can remove the comb from here. And now you can see the wells are made. Now we have to prepare the samples and for preparing the sample, we have already taken S1 and S2 that is sample 1 and sample 2, 10 microliter each.

Now in this we have to add 5X SDS loading dye which looks like this. It contains SDS and beta-mercaptoethanol mainly. And now we are placing it here at 95 degree Celsius for 5 to 10 minutes for the sample preparation. Now the samples are heated up and now we can keep it for some time for cooling down and after cooling down, we can start loading the samples as they are prepared. So this is sample 1 and now as you can see it is very difficult to see the lanes.

So we can use this for guiding where the wells are. As you can see the dye is getting settled down in the well and the well is clearly visible now. Similarly, we will change the tip now and we will load the second sample. Now the loading is done. We can remove this and now we have to start running the gel. So just remember red colour goes with red and black colour goes with black.

Now we can close it. Now from here we can set it for manual 90 volts till the time it is getting stacked and after that, when it is stacked, we can shift it, we can switch it to 120 volts. So now we will start running the gel. Bubbles can be seen from here, that means the gel has started running. After the gel has run, then we have to stain and destain the gel to see the bands which are separated on the basis of mass using SDS gel.

For staining, we have placed it in a plastic plate. As you can see here, the gel is there and we

have added Coomassie Blue and we will keep it for approximately 2 to 3 hours for getting stained. And after staining it, we have to add the destain to it and after destaining for 2 to 3 hours again, gel looks something like this. As we can see bands are separated on the basis of their mass. That is all for the SDS page. Thank you.

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Hello everyone. Today, we will discuss the overall workflow of 2DE. 2DE, it is very important and powerful gel base proteomic approaches and both of them, they separate proteins on the basis of isoelectric point and molecular weight. So as you all have studied the overall workflow

of 1-dimensional gel electrophoresis, the SDS page method. Dye is just a step ahead of SDS page.

So SDS page separates proteins only on the base of molecular weight. 2D and dyes on the other hand, they separate proteins on the basis of both isoelectric point and molecular weight. So one may wonder what is isoelectric point? So all the proteins, they have a particular pH value at which there net charge is 0. So an isoelectric point of a protein which may vary from 2 to 7, the overall charge on the protein is 0.

So at that point of time, the proteins they have least mobility. So if you have introduced proteins in an electric field, so when they reach up to a pH value which matches with their isoelectric point, their overall charge will be 0 and they will not move any further. They will just stop there. So that is the principle that we exploit in 2D. So as the name suggest, separation happens in 2 dimension.

The first dimension is known as isoelectric focusing and the second dimension is known as SDS page. So first we will see how isoelectric focusing works. So we have commercially available IPG strips. So IPG strips, they are available in various forms. So in this particular case, this IPG strip, this has the pH range of 4 to 7 and it is 13 cm in length. So we have IPG strips which have higher length or they have different pH range like 7 to 10 or 3 to 10, depending on your sample and what you want to see.

So what we will do is, first we will take the IPG strip and we will add a protein solution. So IPG strip, they are commercially available in a plastic, with the plastic film so that this strip is protected. So first what we will do is, this is the tray, we will add a protein sample. On top of that, we will add our IPG strip and the first step rehydration will happen for overnight. So that helps our IPG strip to rehydrate and it will absorb all the protein sample.

So this is our protein sample. It has all the necessary components. A protein is there, urea is there. We have rehydration buffer and we have also added Bromophenol Blue which will just help in the visualization. So I will take the sample. I will add in one of these names. Sample

volume again depends on the overall pH, on the overall IPG strip length. So I will just make sure that there are no bubbles and the sample is spread uniformly.

Now I will take the IPG strip. This is how the IPG strip looks like. You can see there are 2 ends, positive and the negative end. Each IPG strip has a barcode. You can also see there is a pH range mentioned on it, 4 to 7. So what I will do is, I will remove the plastic film, the protective plastic film and I will keep the gel side. So the gel side is being protected by the plastic film and I keep the IPG strip on this tray in a way that the gel is facing the sample.

So I will remove the plastic film. So this is the gel side. So I have kept the gel side on top of the sample. So I will move my IPG strip a little only at the ends I can touch with the forceps because otherwise, the IPG strip, it has pH gradient across. So I do not want to disturb the pH gradient. I will just move it a little so that the sample is spread uniformly. There is no air bubble. So this is first the rehydration step.

Now this will go on for 16 to 18 hours. We will also add mineral oil so that the strip does not dry up. So now we have completed the first step of the 2D procedure which is the rehydration. So this will go on for 16 to 18 hours. So this is the IEF unit in which we will carry out separation in the first dimension. So as you can see we have positive and negative end in this unit. So now we will add a tray.

On this tray, we will add our IPG strip. So now we are placing our IPG strip. Again we have placed a strip in a way that the positive end of the IPG strip is facing towards the positive end of the unit and the negative end towards the negative end of the unit. Now we will start the isoelectric focussing. We have also added paper weights which will soak any salt or all the oils in the sample and help in the proper flow of current.

So now we will also add electrodes at both the ends. So electrodes are same for both the ends and then we will start the isoelectric focussing program which will go on for 15 to 16 hours. So as you can see, these are the various voltage run parameters. Initially, we keep the voltage at 200 volts for 4 hours which will then increase to 500 volts further to 1000 and from 1000, there is a

gradient increase and at 8000 volts, we keep it for 64,000 volt hours.

So this will ensure that a proper IEF run has been achieved and this is an example where there was some problem in the sample and I proper IEF run could not be achieved. You can see this declining red line. It can be because there are any salts or any other components which are interfering. So sample preparation again plays a very crucial role in having a proper separation of proteins in IEF.

As you may have already seen that in 1D SDS page, we add sample buffer which at then breaks all the tertiary interactions within a protein. So in this case also this step is known as equilibration which just prepares a sample for its separation in second dimension. So equilibration solution, they have different components again like urea, they have DTT is there in equilibration solution 1.

So DTT will cleave off all the disulfide bonds. So proteins will have a globular structure. So DTT will ensure that all the different polypeptide chains which are joined together by disulfide bonds, they are broken. And then we will treat our IPG strip with equilibration solution 2 which has all the same components as equilibration solution 1 but the only difference lies is, is that it has IAA, iodoacetamide.

Whereas equilibration solution 1 has DTT. So iodoacetamide will add methyl group and ensure that the disulfide bonds which were broken by DTT, they do not denature back. So we have our strip here as we can see. We will first incubate it with equilibration solution 1. I will take my IPG strip and I will keep it with the equilibration solution 1. And also dap it a little so that whatever oil is there, will go off.

So in the first rehydration step, we had kept the IPG strip in a way that the gel side was facing downwards but from IEF onwards, we will keep it in a way that the gel side is facing upwards because proteins have already separated on the gel strip and we do not want them to get disturbed. So we will make sure that the gel side is always upwards. So we will incubate it for 15 minutes.

Generally, we do it using a shaker so that the solution A is in constant touch with our IPG strip. So once incubation with solution A is over, then we will add solution B. Again we will incubate the strip for 15 minutes under streaking conditions. So this is our cassette for SDS page. We have added plates in this. This you can see is a big plate as compared to the regular size, small size plate which we use for normal SDS page.

We have added our IPG strip here. So you can see on this strip, the proteins have already separated according to their isoelectric point. The strip has different pH ranges and proteins, they have migrated, they have travelled during the IEF run and wherever they found a pH value which matches with their isoelectric point, they stop moving. So there mobility stops. So proteins you will find it all across the strip.

Now the separation is going to happen vertically. Earlier the separation happened horizontally. Now the proteins will move according to their molecular weight. So this is the cassette. We have added our IPG strip. We have also added some Agarose solution to seal it so that the strip does not move here and there and it is static. So we will then just assemble it and then we will keep it in the 2D apparatus.

Now we are keeping our cassette in the electrophoretic unit. Now we will add the SDS buffer and the run will go for around 3 to 4 hours. So once the run is complete, we will again stain our gel the way we do for a normal SDS page gels. We will then destain and then this image is one of the representative images which you will see after you have destained your gel. You can see there are multiple spots on this image and then later you have to annotate these spots using various softwares and identify your protein of interest.

So I hope you had good view of processes involved in performing 2D electrophoresis. You have seen there are multiple steps involved and which makes, you know, the process little tedious but if you have done everything meticulously, then probably you can see some beautiful spots which are separated on the gel.

And those gel images reflects your hard work, your efforts which have been put in to know the protocol, the science, all the steps involved in doing good experiment. And each spot actually reflects what is the isoelectric point, what is the molecular weight of a given protein. So I hope now you have much confident understanding of performing 2D gel based proteomics experiment.

In the continuing lecture, we are going to discuss about some of the pros and cons of using 2D electrophoresis and how it can be made more robust and more qualitative for various other applications. So let us continue the discussion on technologies in the next lecture.