

**Introduction to Proteomics**  
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**Lecture - 09**  
**One-Dimensional Electrophoresis**

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**Topics to be discussed today:**

- Introduction to electrophoresis
- Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)
- Blue native page (BN-PAGE)

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**Section I**

Introduction to electrophoresis

Let me give you the historical perspective of electrophoresis.

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### Electrophoresis: historical perspective

- Electrophoresis invented by Prof. Arne Wilhelm Kaurin Tiselius in 1930
- He developed moving boundary method to study electrophoresis of proteins
- Rapid developments in electrophoresis and it was widely adopted in 1960s



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This process was invented by Professor Tiselius in 1930. He developed the moving boundary method to study the electrophoresis of proteins. Since then lot of development happened in the field and during the 1950s and 60s this process was highly adopted in different laboratories.

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### Electrophoresis: historical perspective



Dr. Arne Wilhelm Kaurin Tiselius  
Father of electrophoresis  
Nobel Prize (1948)



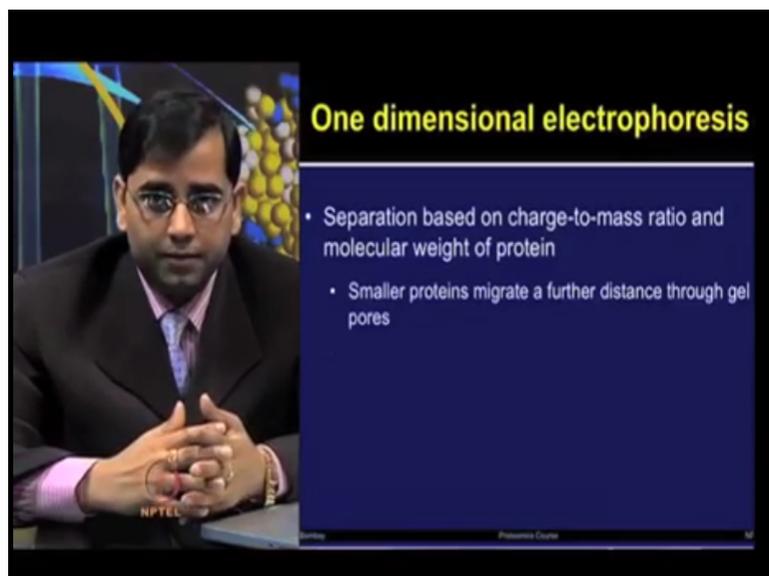
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So here is Dr. Tiselius who is also known as the father of electrophoresis for his great contribution in chemistry. He obtained the Noble Prize in 1948.

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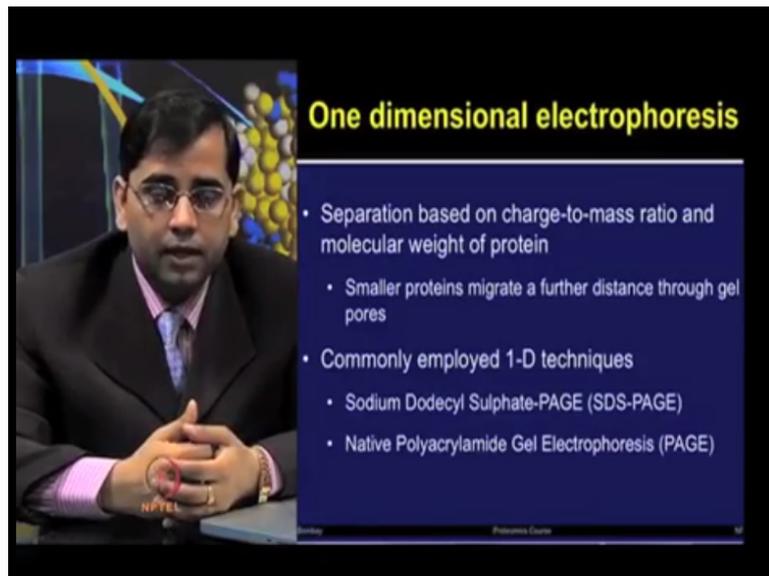
So now will describe one dimensional electrophoresis, some of the concepts involved in that.  
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So the one dimensional electrophoresis, the separation is based on the charge to mass ratio and the molecular weight of the protein. Therefore on a given gel, if you have a smaller the proteins in the electric field, they will move further down and the higher molecular weight proteins they will remain on the top.

So if you apply some standard molecular weight markers which gives you the known molecular weight of the proteins then for a given unknown protein you can determine the molecular weight and it is expected that the higher molecular weight proteins will remain on the top and the smaller molecular weight proteins will reach towards the bottom of the gel.

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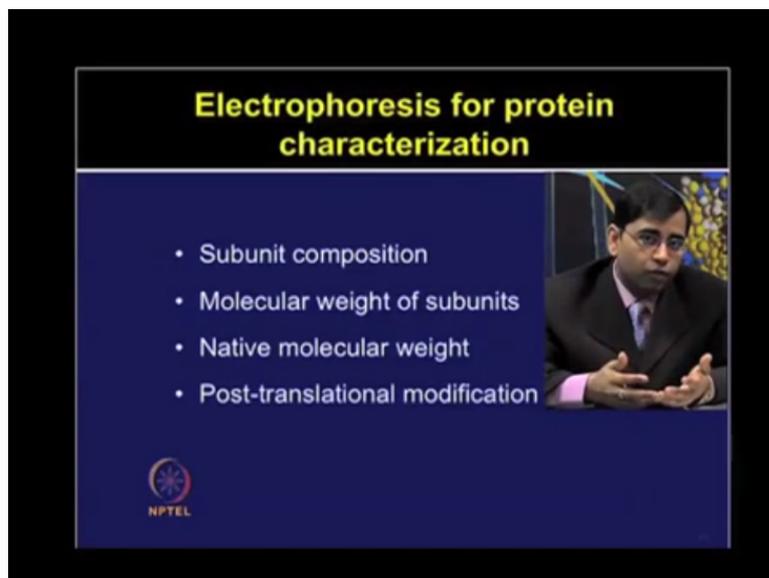


### One dimensional electrophoresis

- Separation based on charge-to-mass ratio and molecular weight of protein
  - Smaller proteins migrate a further distance through gel pores
- Commonly employed 1-D techniques
  - Sodium Dodecyl Sulphate-PAGE (SDS-PAGE)
  - Native Polyacrylamide Gel Electrophoresis (PAGE)

So commonly employed one dimensional techniques include the sodium dodecyl sulfate-PAGE SDS-PAGE or other technique which is used for studying the protein in the native confirmation native form, which is known as native polyacrylamide gel electrophoresis or PAGE.

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### Electrophoresis for protein characterization

- Subunit composition
- Molecular weight of subunits
- Native molecular weight
- Post-translational modification

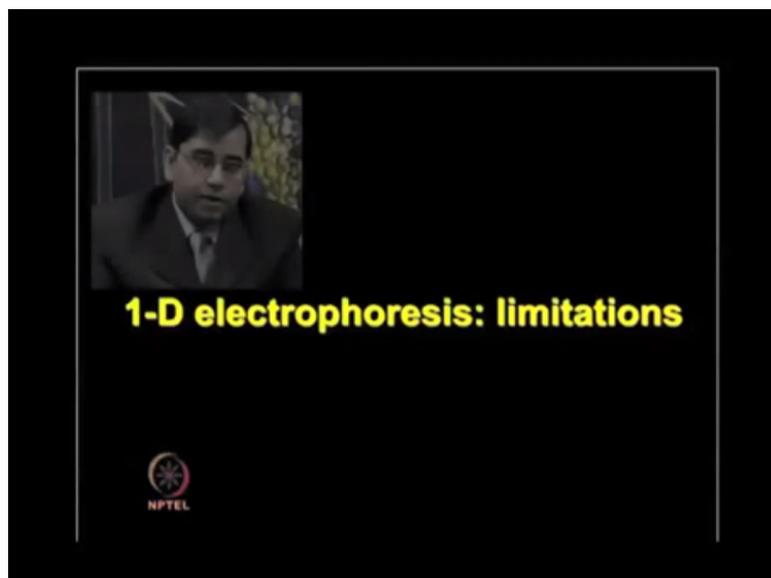


The electrophoretic technique can be used for the protein characterization to study different properties. For example, the subunit composition of the proteins. What is the molecular weight of those subunits? What is the molecular weight of native proteins? Different type of post-translational modification, again only 1 technique may not be able to answer all of this question so often you have to involve more than 1 type of electrophoretic method.

For example, while comparing native and subunit composition forms you need both native electrophoresis and SDS-PAGE. If you want to look for post-translational modification, then SDS-PAGE will be useful. If you want to compare the proteins based on the isoelectric point as well as the separation of isoform or PTM form based on the molecular weight again 2-dimensional electrophoresis will be useful.

So one needs to know the concept and the laboratory way of using these techniques so that by applying a combination of the technique 1 can study different type of properties.

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However, the 1-dimensional electrophoresis has different limitations. You cannot separate your complex proteome the complex protein samples if you have 1000s of protein and you want to study them then 1-dimensional electrophoresis has limitations.

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**1-D electrophoresis: limitations**

- Electrophoresis in a single dimension is useful for separation of few proteins simultaneously
- But large number of proteins can not be separated with good resolution
- Complex mixtures e.g. serum, cell lysate can't be separated

So the 1-dimensional electrophoresis is useful to separate few proteins or a simple protein mixture, but when you are studying the complex mixture then 2-dimensional electrophoresis or DIGE or different type of mass spectrometry based methods will be more useful. So if you have very complex samples like cell lysates or you have serum then separating those and comparing different type of samples on SDS-PAGE may not be a very good way.

In that case, we should apply different methods. So 1-dimensional electrophoresis is very useful and is being used in almost all the laboratories working in the licensed area, but it can give you few questions it can address only those questions, but if you want to get more characterization then you have to apply different techniques.

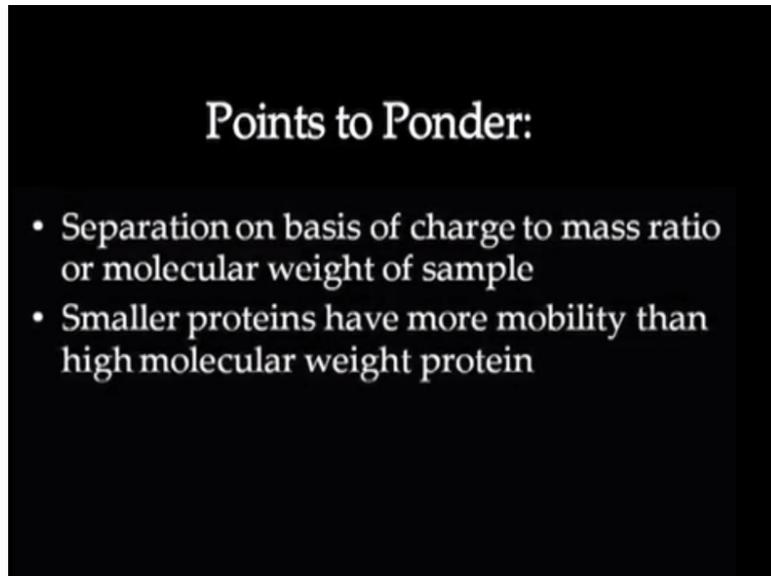
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**1-D electrophoresis: limitations**

- Need technique to provide better resolution at proteome level
  - led to development of two dimensional gel electrophoresis (2DE)

So to overcome these types of limitations and to obtain the better resolution of protein separation, the 2-dimensional electrophoresis was applied.

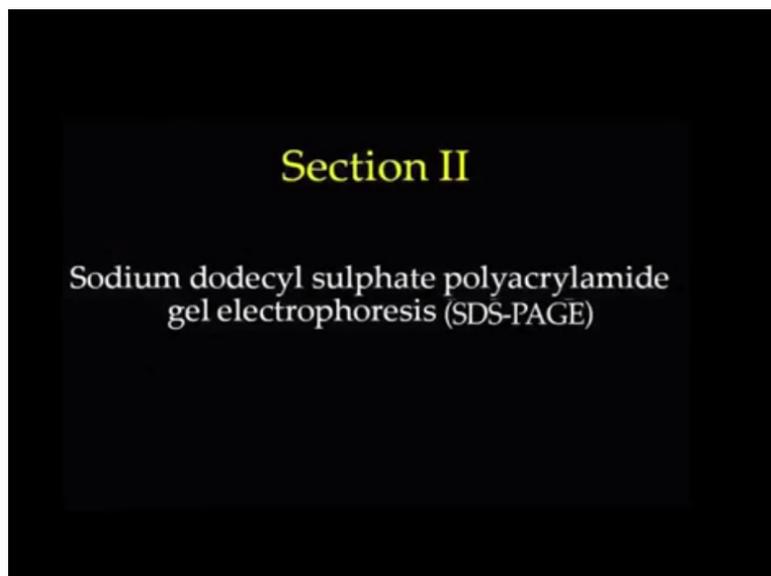
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**Points to Ponder:**

- Separation on basis of charge to mass ratio or molecular weight of sample
- Smaller proteins have more mobility than high molecular weight protein

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**Section II**

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Now we can talk about how to perform sodium dodecyl sulfate polyacrylamide gel electrophoresis or SDS-PAGE.

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## SDS-PAGE

- Widely used electrophoretic technique, which separates protein according to size
- SDS is negatively charged
- Boiling proteins in SDS and thiol agent ( $\beta$ -ME, denatures protein and breaks S-S bond



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So SDS-PAGE is one of the very widely used electrophoretic technique, which separates protein based on their size or the molecular weight. So this molecule SDS or sodium dodecyl sulfate which is an anionic detergent, it provides negative charge. It binds at a ratio of 1.4 gram of SDS per gram of protein to provide almost similar charge to mass ratio. Therefore, your protein separation will be based on the molecular weight in the electric field.

As I mentioned, there is step required to prepare your protein for denaturation so you need to boil your proteins in SDS and beta mercaptoethanol and DTT to denature the protein and to remove or break any disulphide bonds. Now since you cannot do this boiling step in your 2-dimensional electrophoresis where you have already focused the IPG strip in the first dimension.

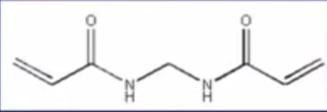
So you need to do the equilibration step. Rest of the steps will be very uniform. So let me describe you the role of the components, which are being used in SDS-PAGE.

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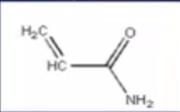
## SDS-PAGE: role of components

- Acrylamide – matrix
- Bis-acrylamide – cross linking agent

N, N'-methylene-bis-acrylamide



Acrylamide



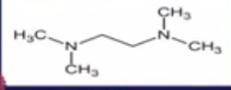
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Acrylamide, it provides the matrix or gelling for making the gels. Now bisacrylamide that is a cross-linking agent, by adding both acrylamide and bisacrylamide in a fixed ratio one can make this gel by adding the gelling agent and the cross-linking agent, but that itself is not sufficient to polymerize the gel. You need to add few more agents, which include APS or ammonium persulfate, which initiates the polymerization process.

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## SDS-PAGE: role of components

- APS – initiator of polymerization
- TEMED – free radical stabilizer, promotes polymerization
- SDS – detergent, makes protein rod shape and negatively charged
- β-ME – breaks disulfide bonds



TEMED

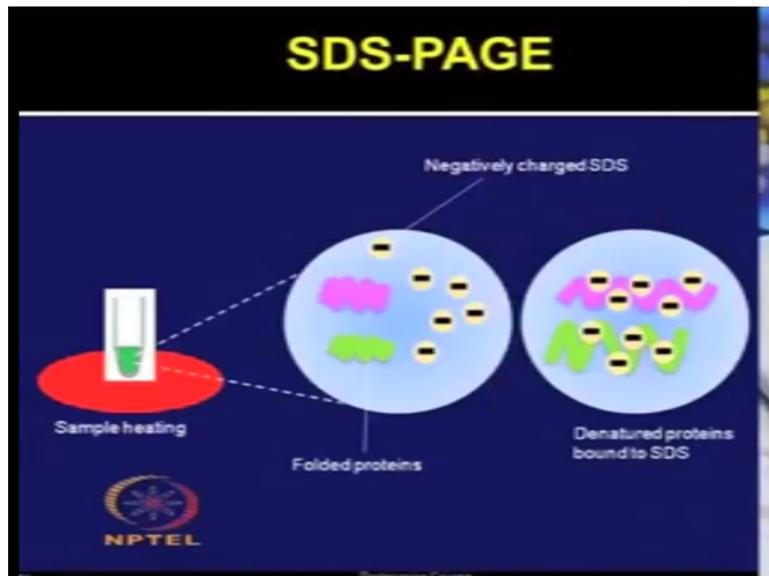


APS

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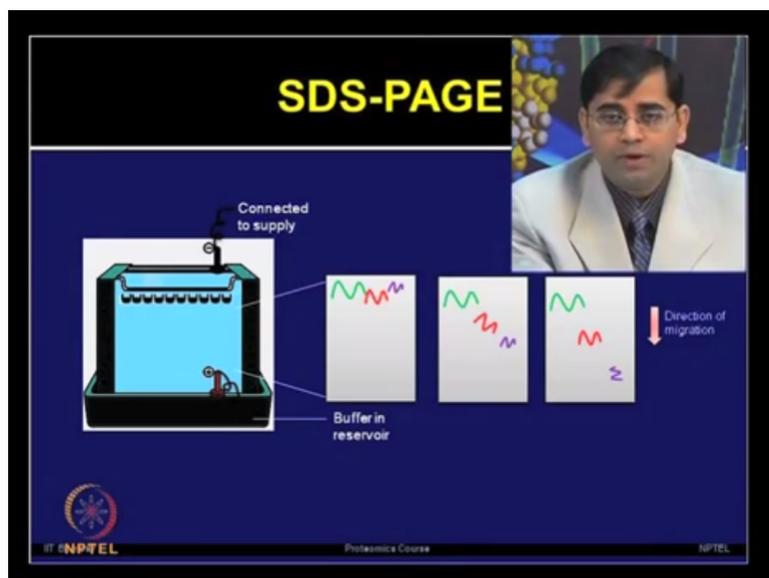
TEMED that is a free radical stabilizer. It also promotes the polymerization of the gel process. So beta mercaptoethanol is not used in making the gel, but for your protein treatment to break the disulphide bonds. So I hope you are adjustable to understand the role of each of these components, which are being used in SDS-PAGE either during the protein preparation or doing the SDS-PAGE or making the gel to separate the protein.

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Now when this step is done you need to boil your protein sample if you are performing a regular SDS-PAGE. The negatively charged SDS molecule will bind to the folded proteins. It will denature to the protein and provide the negative charge. Once this protein preparation is done then you can load this protein on SDS-PAGE gel.

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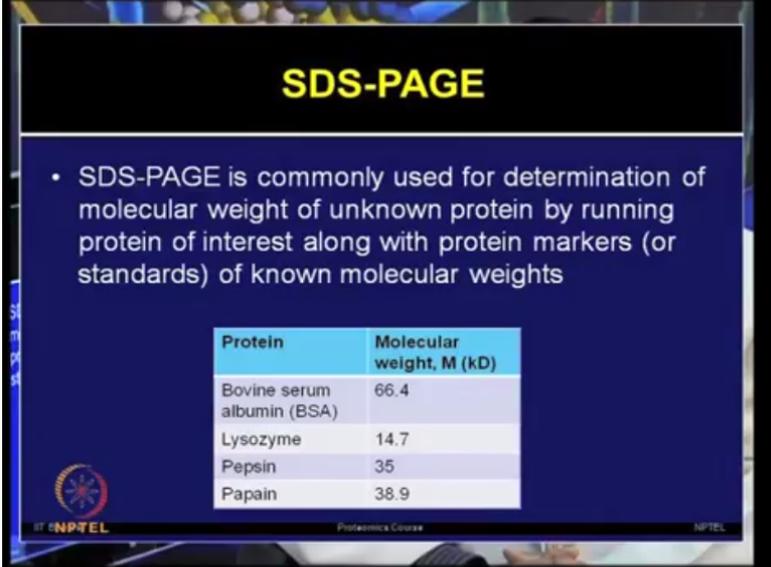


And based on the molecular weight the load size proteins will migrate the furthest and the higher molecular weight protein will remain near the top surface of the gel. So depending upon the protein size you can separate the protein based on the lower and higher molecular weight in this SDS-PAGE gel. You can add the standards or known molecular weight proteins to determine your unknown proteins.

I think it is important to clarify that in 2-DE when you use SDS-PAGE you do not need to make these wells by adding a comb because you already have a flat IPG strip, which has done the first dimensional protein separation. So in this case you need to make a flat comb, you need to make a flat well so that your IPG strip can be placed on top of the SDS-PAGE gel.

Again to remind you here you are only making the resolving gel for better resolution and separation of the protein, stacking part is not done for the 2-D which has already been done during the first process of IEF.

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The slide features a dark blue background with the title "SDS-PAGE" in yellow. Below the title is a white bullet point describing the use of SDS-PAGE for molecular weight determination. A table with a light blue header lists four proteins and their molecular weights in kilodaltons (kD). The NPTEL logo and "Proteomics Course" are visible at the bottom.

- SDS-PAGE is commonly used for determination of molecular weight of unknown protein by running protein of interest along with protein markers (or standards) of known molecular weights

Protein	Molecular weight, M (kD)
Bovine serum albumin (BSA)	66.4
Lysozyme	14.7
Pepsin	35
Papain	38.9

So SDS-PAGE is one of the very commonly used method for determining the molecular weight of a protein of your choice or unknown protein, which you want to know how much molecular weight it is. So if you run the standard protein markers along with your unknown protein you can determine the molecular weight of the unknown proteins.

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I am showing you one SDS-PAGE image in which from the left to the right you can see the first line is standard or known molecular weight. In this well, you can see there are different at least 5 or 6 bands are visible. These are the known molecular weights, sometime starting from 150 kilodalton to 10 kilodalton of molecular weight range. Then we have different wells and in each well unknown protein for which you want to determine the molecular weight can be loaded.

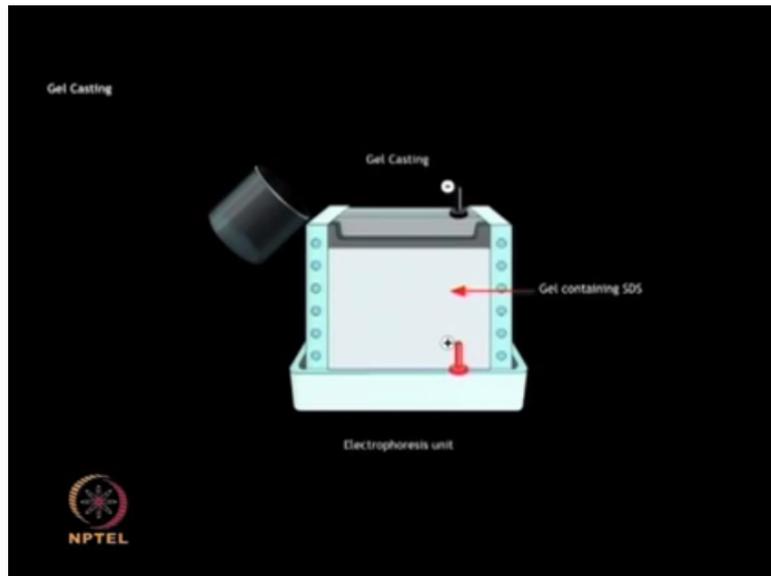
And after staining with Coomassie Blue you can see a pure band here in this case. So one can determine the molecular weight by performing a SDS-PAGE.

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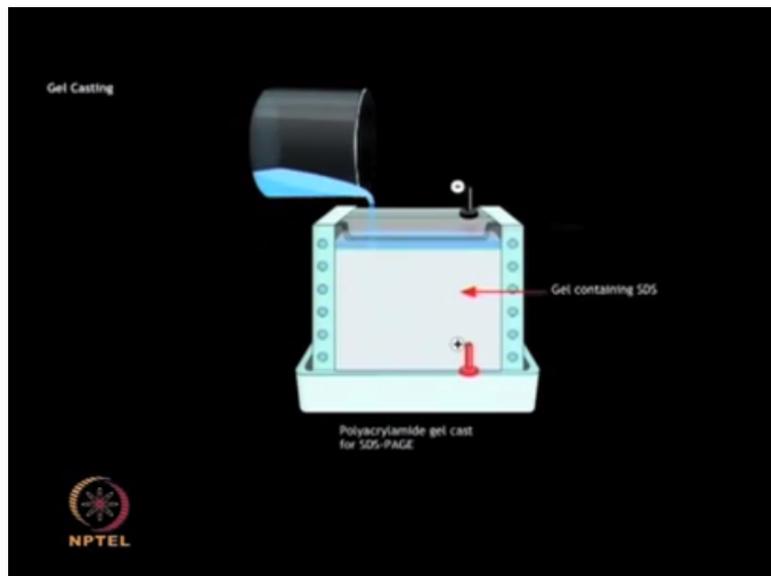
I will now describe you the animation and show you the steps involved in SDS-PAGE. Sodium dodecyl sulfate SDS polyacrylamide gel electrophoresis SDS-PAGE.

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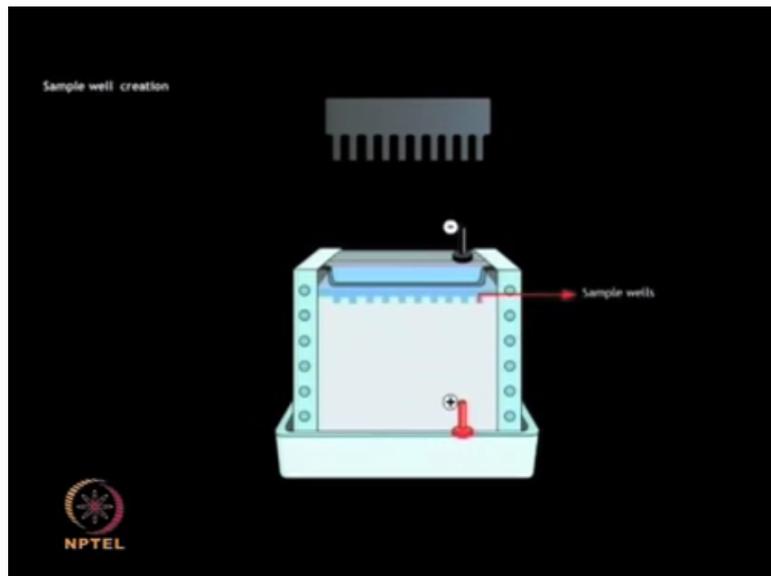
In this animation, you will see various steps involved in separating proteins based on their molecular weight. First of all, the polyacrylamide gel containing SDS is cast between the glass plates as a vertical slab in the same buffer that will use for electrophoresis.

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The molecular dimensions of the pore can be controlled by varying the amount of N-N methylene bisacrylamide with free radical cross-linking being facilitated by ammonium persulfate or APS and TEMED. Next step is to create the wells on these gels.

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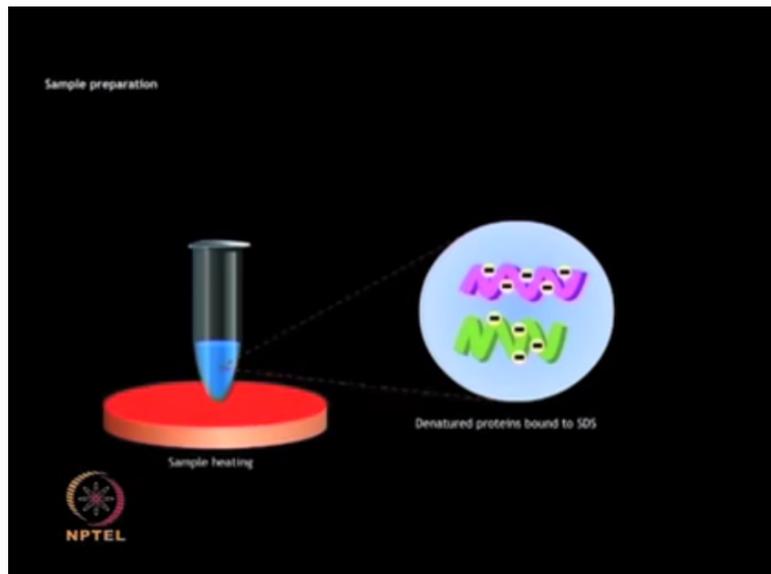
The sample wells of uniform size shape and separation are made using a comb which is placed in the gel as soon as it has been poured.

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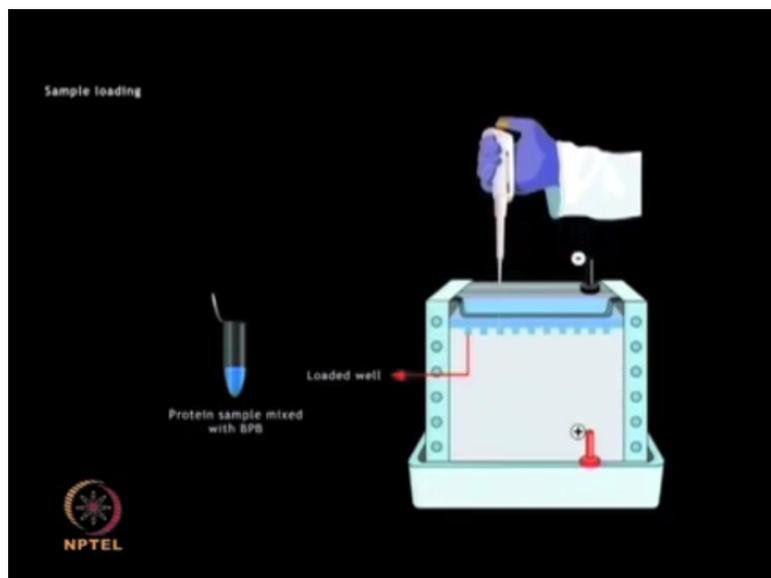
After the gel has polymerized, the comb is removed to provide the gel ready to start the process. Now you can prepare the sample to load on SDS-PAGE gel.

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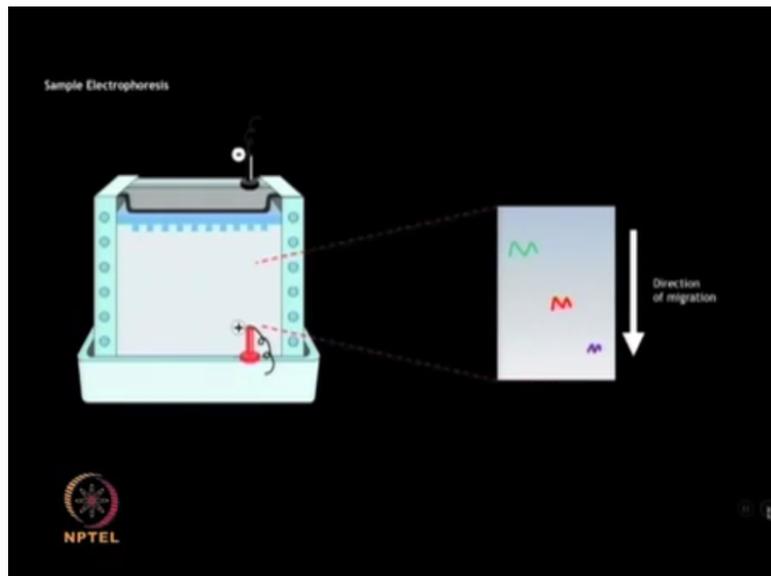
SDS is a negatively charged anionic detergent that binds to protein molecules and causes them to denature. The DTT used to break any disulphide linkages that maybe present during the process you boil or heat your protein samples to denature the protein. Now the negatively charged SDS molecule will bind to the proteins and denature them.

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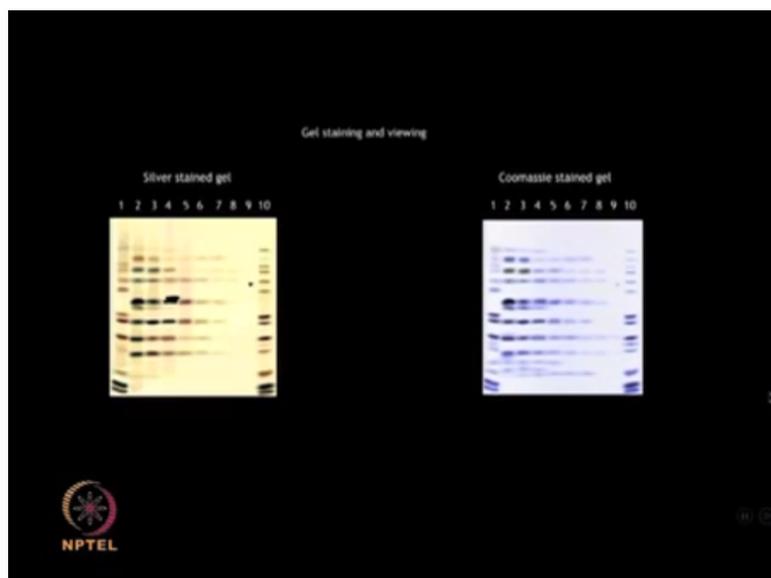
The binding of SDS causes the proteins to have uniform charge to mass ratio thereby allowing the separation purely on the basis of molecular weight. The protein samples can be loaded on the wells by using a micropipette. As you can see in the animation, you can use different wells to load different type of samples. Once all the samples are loaded then you can take this unit and move into an electrophoretic operators.

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It direct current supply between 100 to 350 volts is passed depending upon the size of gel for a time sufficient to separate the protein mixture into discrete bands based on their molecular weight. The progress of electrophoresis can be observed with the help of tracking dye.

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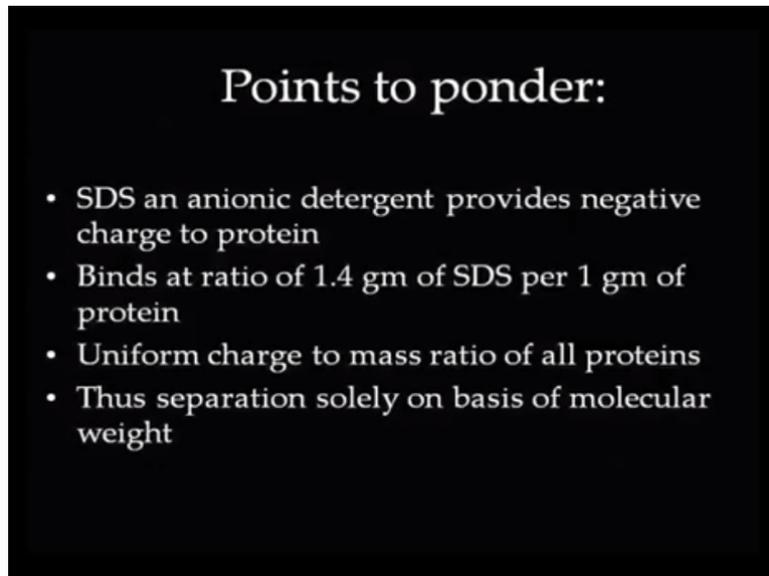


The larger proteins are retarded in the gel and remain close to the point of application while the smaller proteins migrate further along the gel. The gel can be then stained with either Coomassie or silver stain and viewed to observe the various discrete protein bands present in each of the sample. For example, the left image here is silver stained gel and the right image is of Coomassie stained gel.

You can load the molecular weight marker or the standells as shown in the first and the last and the 10th well and then unknown protein sample for which you want to determine the

molecular weight can be separated in different wells. So I hope after looking at this animation now you are clear how to make a gel to separate the proteins based on their molecular weight and I will show you later on 1 laboratory demonstration how to use SDS-PAGE gel for separating proteins in 2-dimensional electrophoresis.

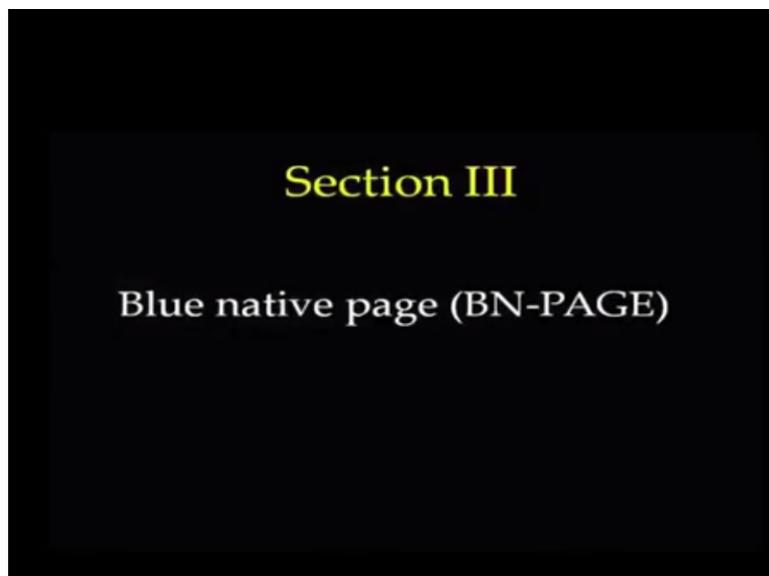
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**Points to ponder:**

- SDS an anionic detergent provides negative charge to protein
- Binds at ratio of 1.4 gm of SDS per 1 gm of protein
- Uniform charge to mass ratio of all proteins
- Thus separation solely on basis of molecular weight

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**Section III**

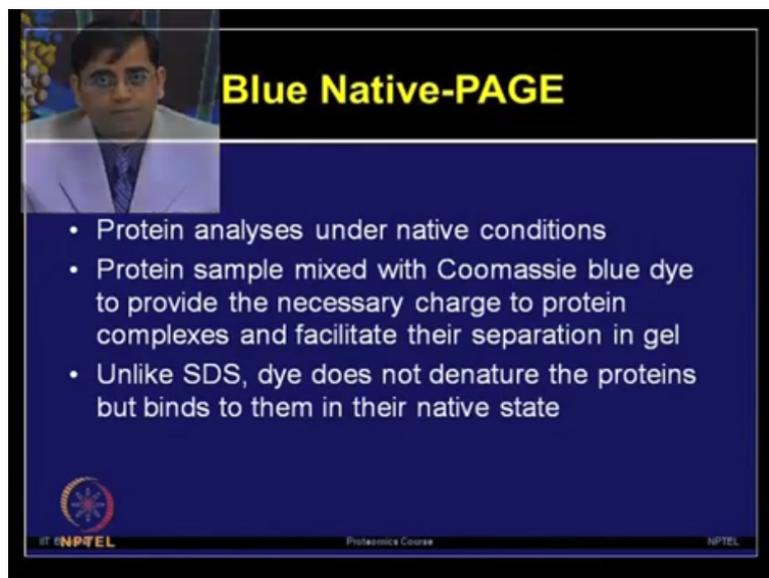
**Blue native page (BN-PAGE)**

So let us now move to a variation of SDS-PAGE known as blue native PAGE. Blue native polyacrylamide gel electrophoresis. Now in SDS-PAGE we are providing a denaturing environment. We want to denature the protein whereas in the blue native PAGE we want to separate the proteins in the native condition.

And by applying these 2 techniques together by using SDS-PAGE and blue native PAGE one can obtain very unique and different information often related to the isoforms, subunit composition, molecular weight and different type of post-translational modification. This information can be obtained either alone from SDS-PAGE or from blue native PAGE or by combining these 2 together to get the integrated information.

So I will briefly describe you here the blue native PAGE. Please keep in mind this is not going to be used for 2-D, but often people also use the native form for the 2-D separation.

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**Blue Native-PAGE**

- Protein analyses under native conditions
- Protein sample mixed with Coomassie blue dye to provide the necessary charge to protein complexes and facilitate their separation in gel
- Unlike SDS, dye does not denature the proteins but binds to them in their native state

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So in the blue native PAGE, the protein analysis is performed under the native condition. Here you are not going to boil your protein sample with SDS and providing the different type of denaturing condition. So you need to add a sample with Coomassie dye, which provides the necessary charge required for protein complexes to separate in the gel, but as I mentioned unlike SDS this dye will not denature the proteins, but it will bind in their native state itself.

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## 2D Blue Native-PAGE applications

- Multi-protein complexes (MPCs) identification & analysis requires separation in native conditions
- In BN-PAGE electrophoretic mobility of MPCs determined by negative charge of bound Coomassie dye, and size & shape of complex
- Provides integrative view of protein interaction networks, protein function & cell behavior



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People apply the 2-D blue native PAGE for various applications such as studying about the multi-protein complexes or MPCs. So identification of multiple protein complexes is not possible by using the SDS-PAGE or denaturing environment. So MPCs can be identified by using the blue native PAGE.

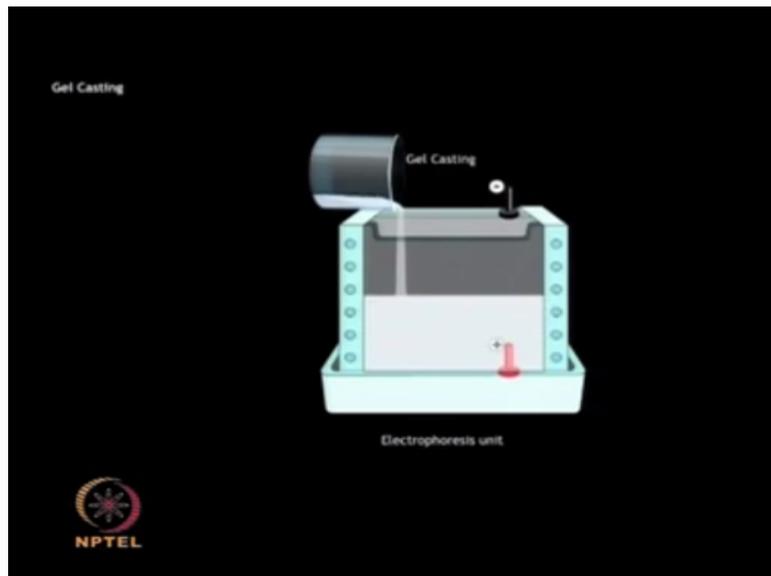
In blue native PAGE, the electrophoretic mobility of MPCs or multi-protein complexes determined by the negative charge of bound Coomassie dye and size and the shape of complexes. So this technique can provide the integrated view of protein function.

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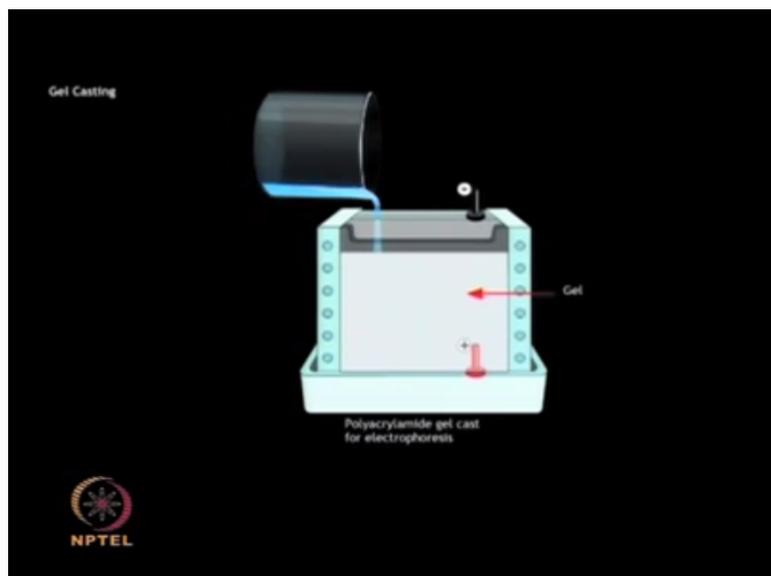
I will now show you an animation how to perform the native polyacrylamide gel electrophoresis, blue native page or BN-PAGE.

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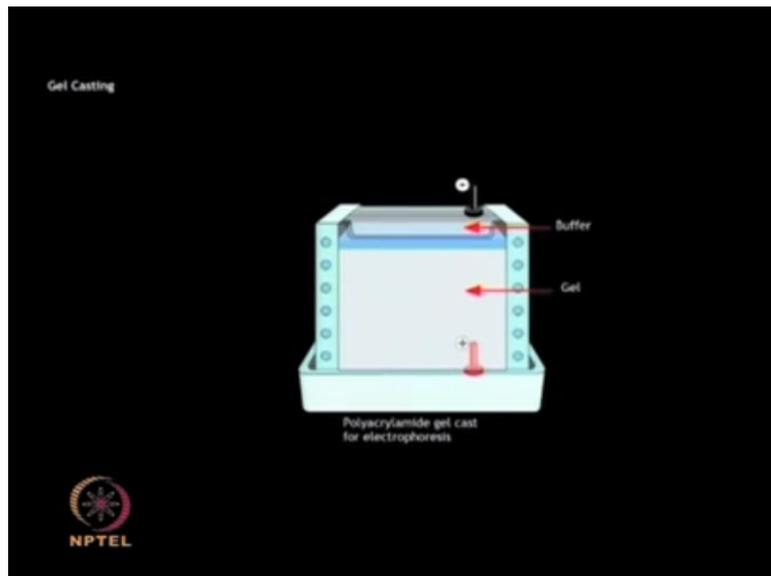
The polyacrylamide gel is cast between the glass plates as a vertical slab in the same buffer that is used for electrophoresis. The gel is prepared by free radical induced polymerization of acrylamide and bisacrylamide in a suitable buffer.

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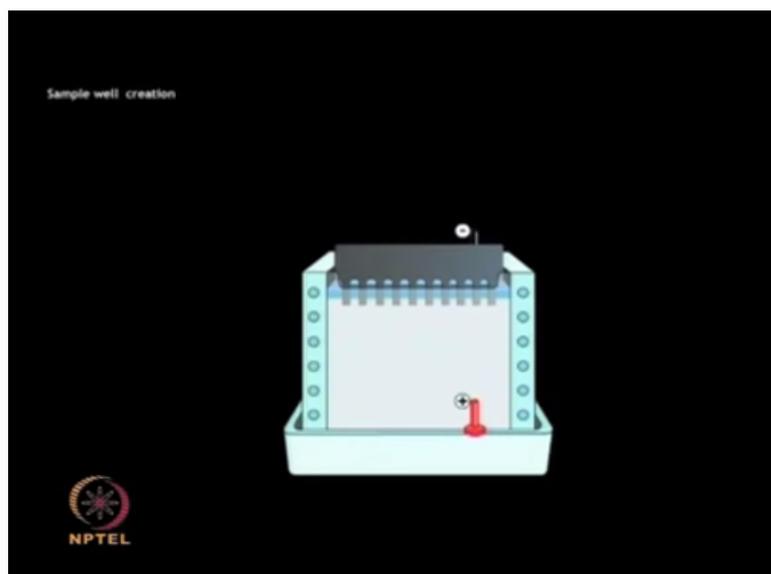
Ammonium persulfate and TEMED are added to facilitate the generation of free radicals and cross-linking.

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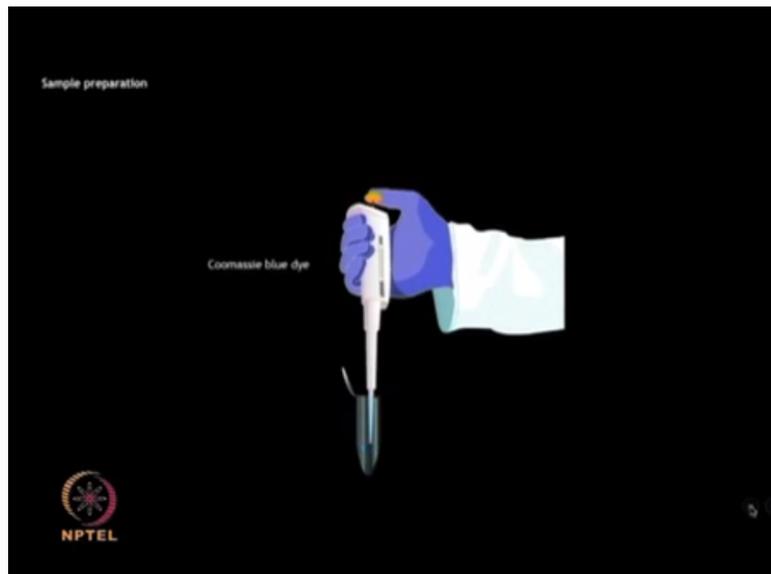
The molecular dimensions from the pores can be controlled by varying the amount of N,N-methylenebisacrylamide. Once the solution is poured then the sample wells can be created.

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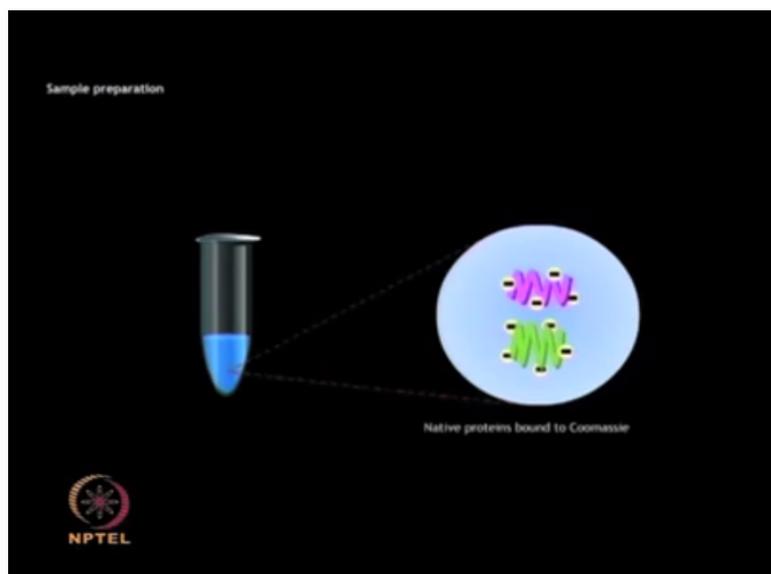
The sample wells of uniform size, shape and separation are made using a comb which is placed in the gel as soon as it has been poured. After the gel has polymerized, the comb can be removed to provide a gel ready for the process sample preparation.

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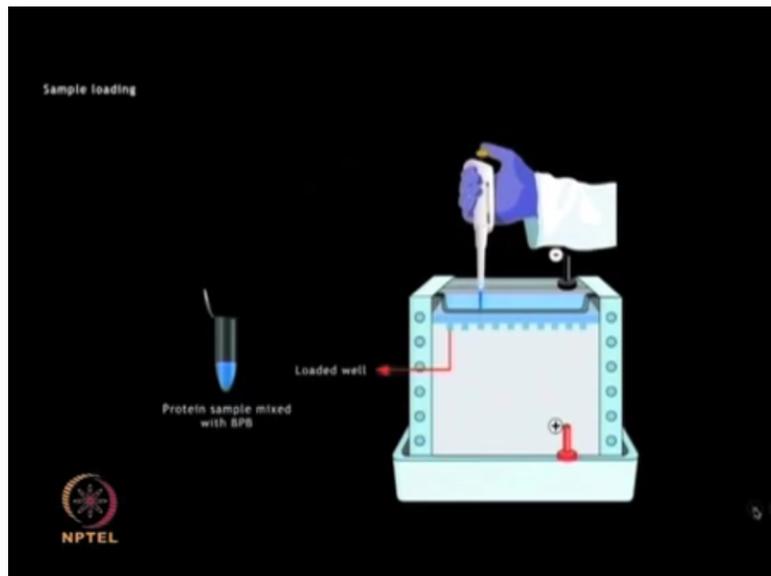
The protein sample present in a suitable buffer is mixed with Coomassie blue dye, which provides the necessary charge to the protein complexes thereby facilitating their separation in the gel.

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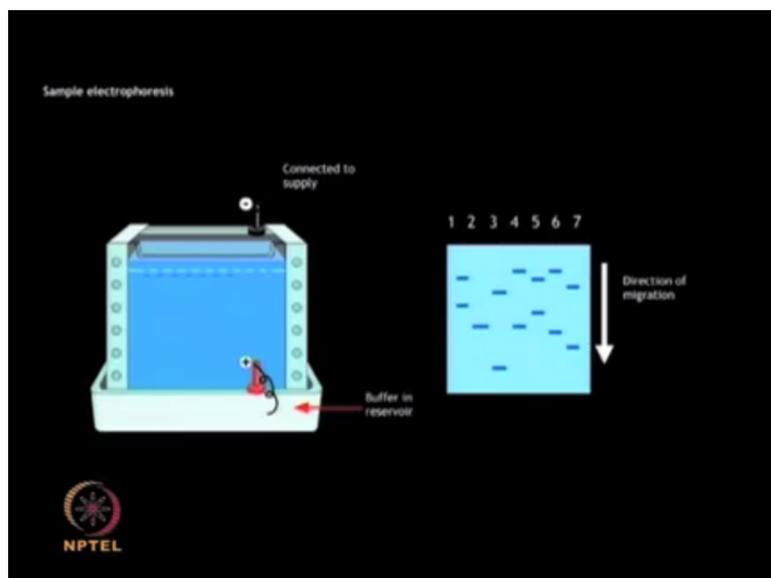
Unlike SDS, the dye does not denature the protein, but binds to them in the native state.

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The protein samples are then loaded into the wells with the help of a micropipette as it is shown in the animation you have different wells to load different protein samples. You can use one of the wells to add known or extended markers and other wells can be used to separate the unknown proteins.

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Once all the samples are loaded then this unit can be placed into electrophoretic unit and a direct supply of around 100-350 volt can be passed depending upon the size of the gel for a time sufficient to separate the protein mixture into discrete band based on their mass charge to mass ratio.

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The progress of electrophoresis can be observed with the help of tracking dye. The larger proteins are retarded in the gel and remain close to the point of application while the smaller proteins migrate further along the gel. The gel can then be stained with Coomassie brilliant blue and viewed to observe various discrete protein bands.

After watching this animation, I hope you are able to understand how to perform native polyacrylamide gel electrophoresis and these concepts can be applied for using the 2-DE as well.

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**Points to ponder:**

- Coomassie dye added to samples to give necessary charge
- Separation on basis of charge and molecular weight

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## Summary:

- Electrophoresis is employed in protein annotation for example:
  - Subunit composition
  - Molecular weight of subunits
  - Native molecular weight
  - Post-translational modification

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