Analytical Technologies in Biotechnology
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Module - 04
Electrophoresis
Lecture - 02
Horizontal and Vertical Gel Electrophoresis

In the previous lecture, we started a new topic electrophoresis. We discussed about the general principle of electrophoresis. We can little bit revise that. So, when a charged particle moves under the influence of an electric field, the particular phenomenon is called electrophoresis. The charged particle will experience a particular force. It will move as per its charge towards the opposite electrode. So, there is one, which is the force because of the electric field. Another will be the frictional force because of the particle moving with the speed through a media. That particular medium will oppose a frictional force. Together both of these will lead to the resultant electrophoretic mobility.

So, as you have seen the three main factors that affect the electrophoretic run that is the one that is electrophoretic mobility of a particular molecule. Then, the heating problem also has to be solved towards certain extent. Then, third was electro osmotic flow, which might happen due to the support media. As we have seen, the electro osmotic flow, the problem of that has been solved to a great extent in gel electrophoresis.

Now, in this lecture, we will be going to extend of lectures on electrophoresis. We are going to discuss about the gel electrophoresis. Now, what are the main components of gel electrophoresis? Before we go into that, as we go along, we will be going to explain in this lecture that there are two kinds of mainly gel electrophoresis techniques, which are widely used. One is horizontal gel electrophoresis and another is vertical gel electrophoresis. Horizontal gel electrophoresis, the media used is agarose and in vertical gel electrophoresis, the media or support matrix, which is used, is the poly acryl amide. So, let us start with the main components of gel electrophoresis. Now, the main components like, one we are discussing the gels.

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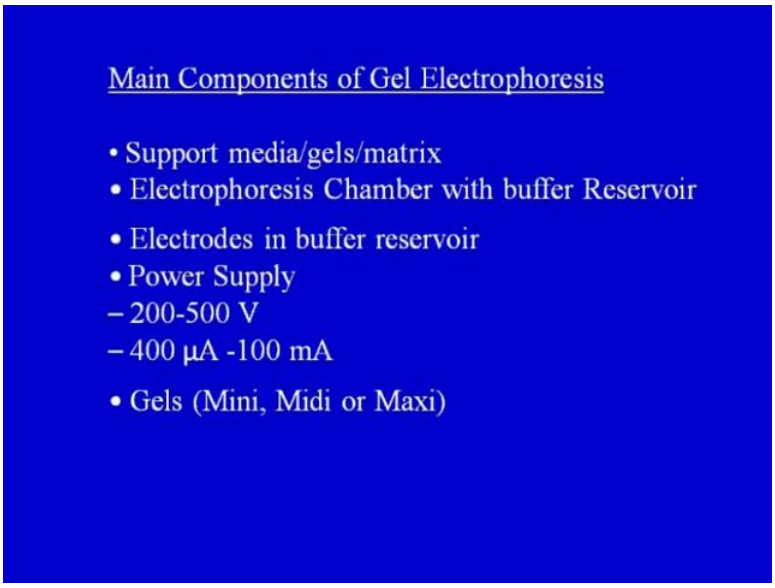

All the support media or we can call it matrix and as I said the most widely used matrix are the agarose and poly acryl amide. Then, with this support material, another thing required is electrophoretic apparatus or chamber with particular with buffer reservoir. So, we will show that in a little while. Then, there are electrodes in the buffer reservoir that is buffer that is positive electrode and negative electrode. Then, it requires a power supply for particular power setting actually; that is can run it on a constant power or can run it on a constant voltage or can run it on even constant current. The power supply could be like it could give a power of 200 to 500 volts or say 400 micro amperes to 100 milli ampere.

So, these are essential components of gel electrophoresis apparatus that is electrophoretic apparatus, power supply and the gels. Now, there could be three kinds of gels like mini gel, midi gel or maxi gel. Mini gels are very small, which could be like say 8 centimeter by 8 centimeter. Larger gels or the maxi gels could be anywhere from 40 to 29 centimeter, so and midi will be between them. So, there could be three kinds and depending on your application, can run either most of the time. Mini gel is the most routinely used. Other gels could also be utilized as per the requirement. So, let us start with support media.

Now, the pioneering work on electrophoresis as we have discussed by Thessaly's and co workers was performed mostly in free solutions, but many of the problems associated

with this approach like the adverse effect of diffusion and convection currents, it could be these things could be minimized by stabilizing the medium. This was achieved by carrying out the electrophoresis on a porous mechanical support, which was then wetted in an electrophoresis buffer and then which in electrophoresis buffer ions and sample ions electrophoresis could occur. So, like this, the problem of diffusion and convection currents could be minimized in a support medium. Therefore, it was needed that from a free solution, one moved to a support media.

Now, earliest support used was like filter paper or cellulose acetate strips were utilized. They were wetted in electrophoresis buffer and they were electrophoresis was run. But, now days, these media are very frequently used. This has been done because of the introduction of and use of gels materials, which led to a rapid improvement in the methods for analyzing macro molecules.

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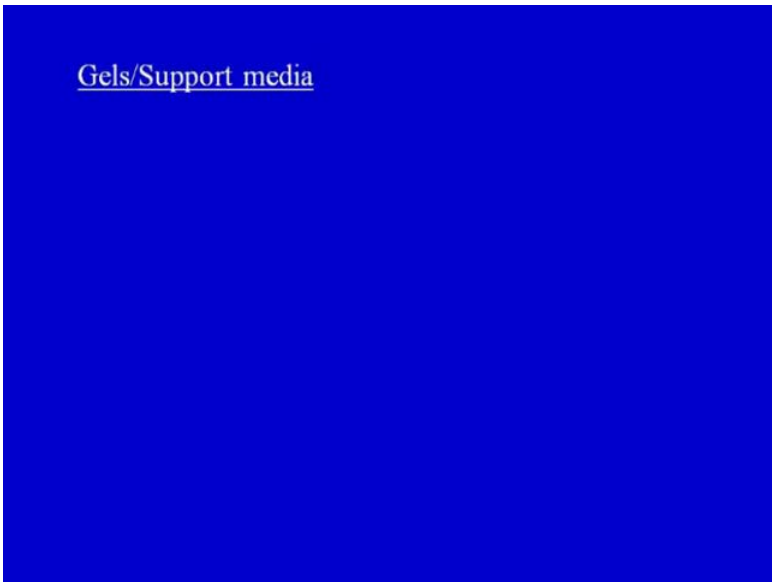

Now, gels are most widely used like I said the rows and acryl amide gels are the most widely used matrices. Now, if you consider the gel structure, so gels structurally are intermediate between solids and liquids. The structure of gels as well as their interaction with macro molecules is not so well understood. This is like mostly electrophoresis which like when molecules run through porous structure where the seizing effect takes place, it is a quite unknown area actually. Now, it is difficult to cast gels of precisely controlled structure because like I said it is a random structure with pores.

Now, two most important and widely used matrices like I was saying are agarose and poly acryl amide. The process of gelation of gels, the individual polymers of both gels combine into fibers and then aggregate into larger bundles joined in random mesh works. So, both gels, they exist as random distribution of solid materials and pores, which we can call open spaces here. They are apportioned in say Poisson distribution of sizes. It is like you have a mean of sizes, but they are not exact equal pores sizes, when you cast gel every time.

So, this has to be understood that there are the kind of mesh work, which combines and aggregate together in a very random fashion and their pore size, you can say the mean of a certain value, but they are not exact pore size. So, travelling of macro molecule through they as also like, you have to, if you want to compare the two gels, then you have to do it through running standards. You might not be able to reproduce the result exactly because every time it will cast a gel, it will be different. So, the idea of gel pores is quite imprecise as I was saying. It refers to the resistance which a gel important to the migration of charges, which varies in shape n size also. You have one is the charge. Another is shape and size will also play a role because of the pore size of the gel.

Now, so gels can be considered as a three dimensional sieve actually. What happens is that the molecules during electrophoresis move through this buffer filled pores of the gel and the ability of macro molecules, say proteins or DNA in particular to migrate through these different size, pores or pore regions will depend on the structure of the molecule. So, if you have a larger structure and particular kind of shape, may be the resistance is more to the movement as compared to the smaller molecule and to the particular shape and the resistance might be less. So, this will certainly affect the migration patterns and the velocity at which these molecules will move. So, gels are of particular you can say, three dimensional sieves, but the movement migration will certainly differ from gel to gel.

Now, there are advantages of gels over free solutions as we have discussed earlier. The porous gels are or they will act as sieves. So, the limit diffusion of the simple molecules like I said electro osmotic flow is minimized or suppressed. Likewise, joule heating is also quite suppressed in gels, but still, the heating does generate and many times cooling operators and cooling systems have to put in to reduce the heat. The separated molecules will segregate into distinct or discrete zones corresponding to their electrophoretic

mobilities when are running them in these gels. At the end of the electrophoresis, when electric field is turned off, individual analytes are constrained and their final position as a distinct band pattern, which can be visualized by say staining or certain other positions.

Now, this one like here, when you have free solutions, certainly diffusion will occur. If you leave it for little longer, then bends might not remain as sharp ends. So, gels have their properties and gels have a particular way to like to separate and analyze the samples and they solve many problems which are faced when you work in free solutions. Now, like we were discussing, there are two widely used gels. One is agarose gel.

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Another is poly acryl amide gels. Now, let us discuss first agarose gels. Now, the pores of the agarose gels are very large or can say larger as compared to poly acryl amide gels. So, agarose gel electrophoresis is the easiest and very common way of separating and analyzing DNA. DNA are large molecular weight to molecules. As far as proteins are concerned, proteins if they very large like say about 500 kb in size or so, then they can be separated in agarose gels; otherwise poly acryl amide gels will be utilized. But, for proteins, agarose gels are also widely used for certain applications like, for example, immuno electrophoresis where they only act as anti convicting support media and where protein movement is not hindered and anti body antigen interactions are seen.

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So, you are not really separating the proteins, but the interactions, you want to see. So, in immune electrophoresis, the agarose gels could be utilized. Poly acryl amide gels are used for the separations of the proteins, which are of the smaller protein molecule as compared to what is used in agarose gels and also very small DNA fragments could be run on poly acryl amide gels like I said the pores on the poly acryl amide gels is smaller.

So, large molecular weight DNA fragments cannot be RUN, but smaller DNA fragments could be seen in like say in DNA sequencing in gels or poly acryl amide gels and where you have small fragments of DNA, where you are trying to sequence. In earlier times, when gels were utilized in the sequencing the DNA, the poly acryl amide gels were utilized. The gels can be used for the visualization. They can be utilized for quantification or even to isolate a particular band of DNA molecules.

Now, most of the time, the gel electrophoresis is in an analytical technique, but it could be made preparative. Small amounts of samples, particularly the nucleic acids could be purified from there and even proteins could be separated or could be extracted from gels for a certain applications like proteins sequencing or in terminal sequencing say an internal sequencing by mass spectrometry. Now, in agarose gels, the DNA could be visualized the gel by the addition of ethidium bromide. So, this binds strongly to DNA by intercalating between the bases. It is florescent meaning that it absorbs invisible UV

light and transmits the energy as visible or a slide. So, when it intercalates electrophoresis and which could be seen and could be visualized here.

So, agarose gels are mostly used with the DNA or nucleic acids. Now, agarose is the linear poly secrete obtained from the red algae or certain weeds. It is made up of basic repeat unit that is agarobiose. Agarobiose comprises of alternating units of gel electrodes and three sets gel electrodes. So, it could be like several algae are the source of this and it could be purified from there, extracted and purified from there. Now, this agarose is supplied as a dry powder.

The agarose variety differs in the physical and chemical properties like gel strength, gel temperature, porosity, electro osmosis, which depends on the purity of the gels. If it contains certain charge on its surface, certainly electro endosmosis problem will occur. Now, most agarose gels are made between say 0.7 percent and 2 percent or it could go up to 3 percent also in certain cases. 0.7 percent gel will show and will be utilized for a good separation of large DNA fragments like say 5 to 10 kb fragments, whereas 2 percent gel will be good for smaller fragments, which are much smaller like 0.2 to 1 kb fragments. So, depending on what kind of fragments you are going to separate, you can you can cast the gel. Mostly 1 percent gel is very common in the agarose gels.

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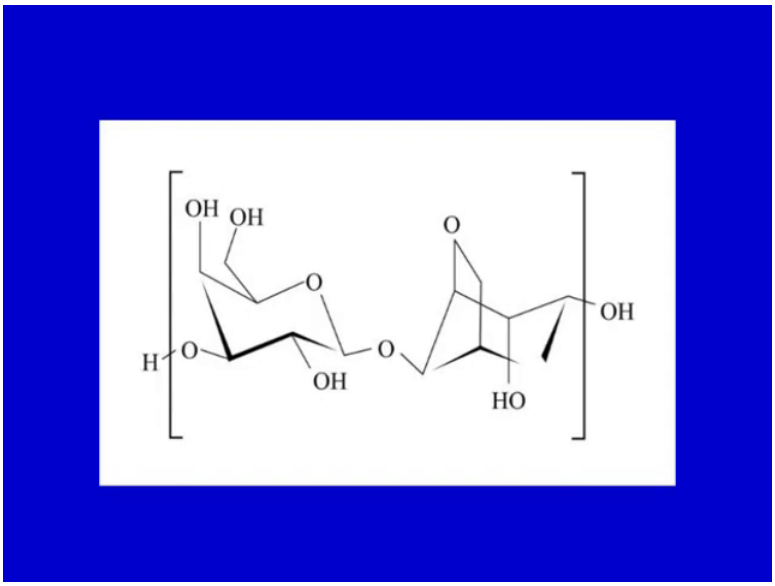

Now, this is a simple schematic of the two units here, which agarose is made of that is the repeating unit agarobiose. Now, the gel properties of the agarose are attributed to

both intra and inter molecular hydrogen bonding within and between the long agarose glean. This is like whole matrix, which is formed is due to the hydrogen bonding pattern actually. So, how this gel is casted actually is shown in a very simple way. How do we make the gel, agarose gel? Now, this is a very simple way.

What is done is you way out required amount of agarose like I said it is supplied as a dry powder. So, if you want to make 1 percent of gel, then you weigh 1 gram of gel for 100 ml. So, you will weigh the required amount of agarose in say conical flask and you will add required amount of buffer which is TBE or TAE buffer. You will mix it well. Now, what is done is you will microwave this, for say one to two minutes and the agarose will be dissolved. It will mix with the buffer. You will then leave it to cool for a very small period of time of few minutes and temperature will be little down like say around 60 degree. So, you can add then ethidium bromide to the gel.

Now, ethidium bromide here could either be added to the gel say 1 macro liter of ethidium bromide is 10 milligram per ml and you can stir it or it could be done that can run the gel without ethidium bromide also. You can stir the gel afterwards in a solution containing ethidium bromide. So, both ways, it could be done. So, once you have added ethidium bromide, if you want to then will pour the gel slowly into the tank that is the casting apparatus. I will show you that how it is done. You will push any bubbles or anything and through like you say, you can use tape or certain thing.

Then, you have to insert a comb and the combs are inserted so that they can make slots for sample to be loaded. When gel solidifies, this melted gel when solidifies, then if the comb is there will be slot for well created where you can load your sample and gel, you have to leave it for say thirty minutes or so or say one hour for solidifying.

Then, finally, it is ready to be used. As you will pour, you will put it in the gel running apparatus with electrodes. You will the fill the gel tank with the TAE or TBE buffer, which ever you are tris acetate EDTA or triborate buffer. So, you will tris acetate EDTA or acetate, which ever buffer you are utilizing or submerge the gel in that buffer that is running buffer. Here, it shows very clearly how this is done. So, before this figure, I would like to show you how this whole process has been done here during casting of gel. So, what I was talking about is first thing is take say a conical flask.

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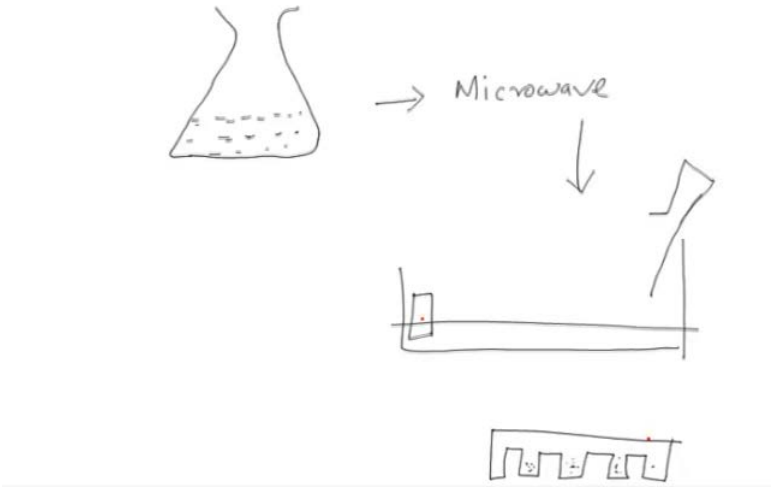

In this conical flask, you will weigh a particular amount of material that is your gel and will fill it with the buffer. Now, this gel is still solid, it will settle down here and will swirl it and you will put it into the microwave. So, that means you have to heat this conical flask, which contains the weighed amount plus buffer, weighed amount of agarose and the appropriate amount of buffer. Once you put it in microwave, the agarose will melt and will mix properly. You will see the melted buffer thick melted solution. Now, after that, you will cool it for a while and then you can add ethidium bromide.

Now, you will add gel apparatus. Now, this gel apparatus will have say, from two sides, it is enclosed, it operates. It is a glass strip with two enclosed sides and other side, you can tape it actually. So, when you put the tape, you can tape this from both sides, then what will happen? It will be enclosed from all sides. Now, this material, you can pour this material in here through, you can put a glass rod or you can, there could be glass; through a glass, you can pour or very carefully sides you can pour this. So, what will happen? This will evenly distribute on to this gelling apparatus.

Now, once gelling apparatus you have put in, if there is bubble, you can see, you can push it or you can remove it through tip or otherwise. Once it is done, there is a comb. What is comb? Comb something looks like this. These are slots here. It looks something like this.

So, these combs like this will not, it will not go into the gel. This area which is like you can say this and these areas will be vacant here or wells will be created. So, this comb can be placed in here in horizontal in one position and this comb here when it is placed in here, then it will create wells. The number of wells will be according to the number of these, how many number and that particular comb and it contains like strips it contains actually. Now, once you have done with this, then your gel is ready after say one hour and it is ready to be used. So, let us go back to our discussion.

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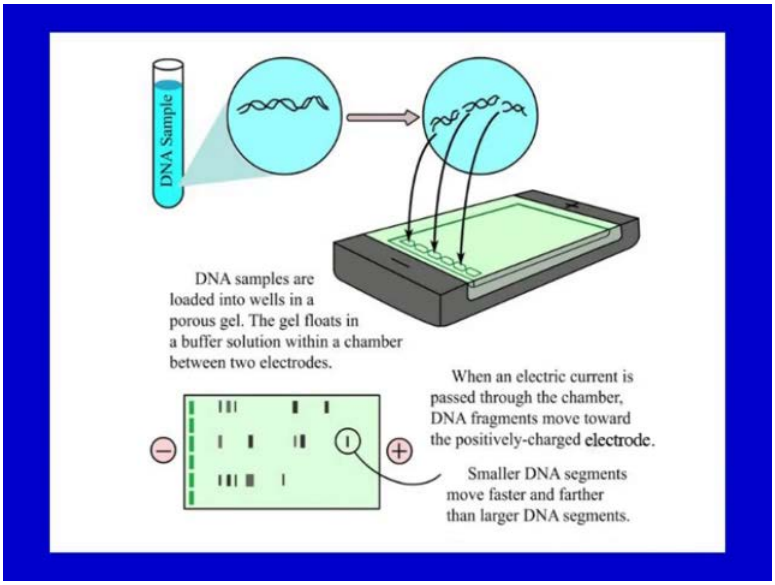

So, here if you can see in this figure like I was showing, gel is casted here. You can see here, these are the wells here which are of a few numbers here. Now, you have DNA sample and there can be more than one DNA sample or there could be like different samples here or one sample loaded into each well. Now, this sample could be treated and you can get like say this has been, this gel sample has been restriction digested. What is created here is that from one DNA sample, you have got many DNA samples and DNA fragments. Now, these DNA fragments need to be separated here. So, what will happen? You will with a pie pit, you will load this into here. Now, when we are loading this, the loading is done.

The sample is prepared in a certain high density solution like sugar sucrose solution or glycerol or something else could be utilized so that the sample settles down and does not flow diffuse in the buffer. It settles down at the bottom of the well. Then, there are two

electrodes here, negative and positive. It is run in the gel apparatus and what you get is a banding pattern and this bending pattern, where, what will happen? Your DNA fragments since they are negatively charged, they will move towards the positively charged electrodes. Smaller DNA fragments will move faster as compared to the larger DNA fragments. On that basis, the separation will take place. This you can analyze in UV laminator and or you can even cut the gel and you can get the DNA extracted.

So, agarose gels are used for electrophoresis of mostly nucleic acids, but you can also use them for proteins also. Like I said for proteins, the pore size of 1 percent agarose gels are larger relative to the sizes of proteins and agarose gels are therefore used in techniques like immune electrophoresis or say iso electric focusing. Likewise, they could be used were proteins are moving unhindered. There are other aspects of like when you are trying to purify or extract the DNA material from the gel, agarose gel, and then low melting temperature agarose gels are available, which is like the melted say to 60 to 65 degree Celsius.

They could be very useful to extract the separated DNA samples and you can recover and purify those in purified form very easily like without you have to, in other gels, you might have to melt them at higher temperature and recovery is low. But, in here, the recovery is very high. Now, because of poor elasticity of agarose gels, mostly the electrophoresis is done in horizontal gels. So, because of this problem in agarose gels, it is horizontal gels and not vertical gels. This was about the agarose gels.

Now, the second most widely used gels and particularly for proteins gels are the poly acryl amide gels. They are routinely used in many teaching labs and research labs. So, the electrophoresis performed in acryl amide gels is frequently referred to as poly acryl amide gel electrophoresis of page, page. Now, here like if you could recall the agarose polymerization like melt and then you solidify and through hydrogen bonding, the whole structure or like matrix structure is formed here co polymerization of acryl amide and n n prime methylene bis acryl amide performed.

So, bis acryl amide is formed into two acryl amide molecules by methylene group and they are used as a cross linking agent. So, what is done is the acryl amide monomer is polymerized and they polymerized in a head to tail fashion. So, the long chains are formed. So, the long chains are formed and in these long chains, insertion of bis acryl

amide occasionally into the growing chain results in two cross linked matrix of fairly a defined structure. So, the polymerization of the acryl amide is an example of free radical catalysis.

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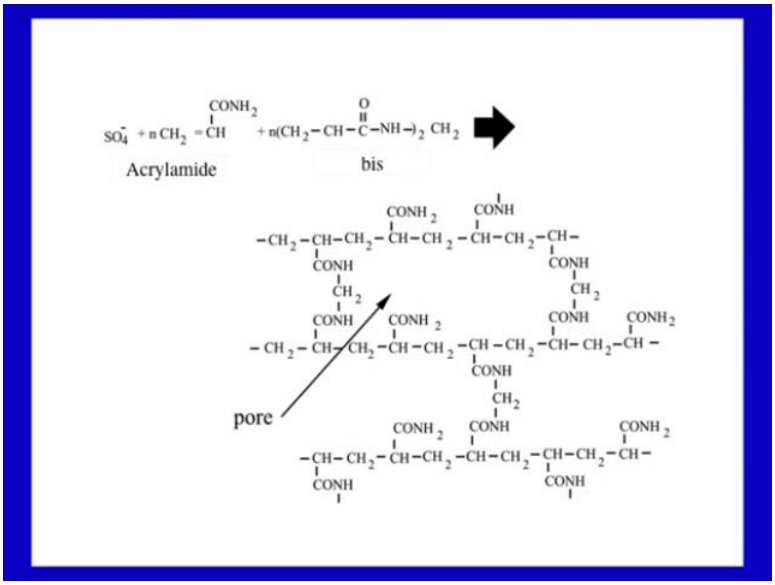

This figure here shows you, this is acryl amide molecule. What happens is that this is bis acryl amide and long chains of acryl amide are formed and like these two chains and in between, bis acryl amide gets inserted. As the cross linking occurs, depending on the concentrations, the pores will be created. So, polymerization of acryl amide is an example of free radical catalysis.

In here, the polymerization is initiated by the addition of ammonium persulfate, which acts as an initiator and TEMED as an accelerator TEMED is tetramethylethylenediamine. Now, TEMED catalyzes the decomposition of the persulfate ion to gives a free radical, a molecule with unpaired electron. Now, this free radical is a highly reactive species, they will catalyze the cross linking in head to tail manner.

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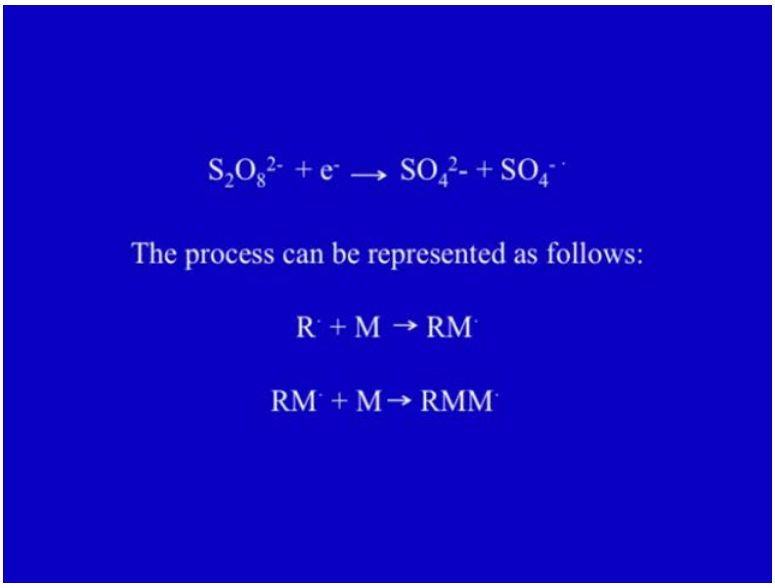

So, what happens is if we can see here on the screen, so you have a persulfate ion and what is decomposed into here there is a single unpaired electron. So, free radical is generated. Now, if M is the acryl amide, it will present the macro molecule and R is the free radica that the generation of, it is a kind of chain reaction, which leads to the formation in cross linking in chain manner. So, it goes on actually and in between, this acryl amide is introduced. Now, here one has to remember that like it is a free radical reaction, gel solution will be degassed for proper polymerization like oxygen will remove free radicals and it might hamper with the polymerization.

Apart from this persulfate and TEMED polymerization, there could be photo polymerization could also be utilized. It can be an alternative method that can be used for polymerization of acryl amide gels. So, ammonium persulfate and TEMED are replaced by riboflavin. Now, when gel is poured, it is placed in front of bright light for say two to three hours and photo de composition of riboflavin generates a free radical that initiates polymerization. So, both ways you can do a polymerization of acryl amide gels. Now, poly acryl amide gels are corrected in terms of percent t, which we weight percentage of total monomer including cross linker.

So, here acryl amide monomer is t and cross linker is c. So, percent c is the proportion of the cross linkers has the percentage of total monomer. So, the percent t means, it carries both monomer as well as the cross linker, percent c is the proportion of the cross linker

in the total monomer. So, the pore size in the gel can vary by the changing the concentration of both acryl amide and bis acryl amide. So, what happens is that when percent t increases at a fixed low concentration of percent c cross linker, then the number of chains increases and pore size decreases. Now, when percent c is varied at constant percent t, pore size decreases to minimum at about 5 percent c, but it then increases with further increase in percent c.

What happens is that it can be due to the formation of shorter and thicker of the linear chains of polymer. So, you can increase the cross linker extent only. Now, monomer solutions are made as concentrated stock solutions. From stock solutions, you will make various percentages of acryl amide gels like for protein separation, 30 percent t and 2.7 percent c stock is preferred; for nucleic acids, 30 percent t, 3.3 percent c cross linker are used.

The final concentrations of say 0.05 percent for ammonium persulfate and TEMED are used for resolving gel. We will be discussing about resolving gel. For stacking gel, 0.05 percent APS and O.1 percent TEMED can be used. So, these are the different varies concentrations. Every lab standardizes through protocols the running conditions for electrophoresis gel.

Now, electro acryl amide gels will be prepared between 3 to 30 percent acryl amide. The low percentage gels are used for free migration of proteins like for example, simple IEF or stacking gel in STS stage. Both techniques we are going to discuss in later lectures or to separate DNA molecules. Gels between 7.5 or so and up to 20 percent used for separation of the proteins, whereas smaller pore size which introduce a seizing effect and proteins are separated on the basis of charge. So, most of the acryl amide gels are vertical gels and vertical slab gels are very common where many samples can be rubbed in identical conditions.

They are the routinely used apparatus for the analysis of proteins as well as for separation of smaller DNA fragments, say in DNA sequencing gels. Now, there are tube gels also which are used quite a bit, but the only problem is in tube gels one is very sample can be operated at the time. These particular gels are used for as a first time in machine for two d gels. So, that could also be used. We will be discussing two d gels

also in a while. So, this was little bit about that what are the different aspects of poly acryl amide gels.

Now, how do proteins will migrate through the gel and how this acryl amide gels are run actually? So, when you have casted the acryl amide gels, let me show you here on your screen how the whole thing works here. So, we have seen for agarose gel, the acryl amide gels little bit require more processing here.

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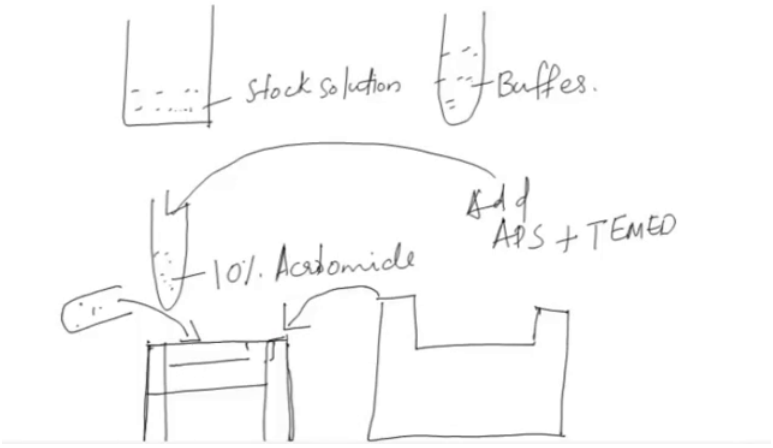

Like I said, the first thing is that you have a stock solution actually. So, you have a particular stock solution. Now, this is the stock solution like I said, it is 30 percent acryl amide with say 3 percent or so bis acryl amide. What we have done is you have made this stock solution. You can store it and you have to make different percentages of gels as per requirement like for example, if you want to make 10 percent gel, so what you will do is and there is stock solution is of acryl amide.

Then, there will be buffers. We have to take these buffers, which are like buffers which could be running buffer like electrode buffer and could be gel buffers where you have to make the gel. These buffers we will be discussing later on. There could be like a condition like a stacking gel and resolving gel or it will be a continuous system where a single gel is utilized. So, what you will do as per your calculations, you will mix this say you are making 10 percent gel, 10 percent acryl amide gel, then the final concentration

like, so what you will do is you will take the buffer, you will take the certain amount from stock solution and acryl amide solution.

You will be mixing here so as to that final concentration is 10 percent. So, once this 10 percent you will add here, the two things, one is APS and TEMED. Like I said, TEMED is the accelerator, so as TEMED is added, this whole process starts very fast and very small amounts are added. Now, once you have added these things to the 10 percent gel, which make all already added buffer all, then very quickly, you have to pour this into the casting apparatus. These casting apparatus if I say this is like these are two, if slab gel electrophoresis, then there will be two plates. One will be something like this and another will look something like this. So, this plate, this plate will be put on top this plate with spacers here.

Spacers are very thin like strips, which are rubber or other. These spacers which are only a few like say 0.5 mm or 1 mm, they will be placed here and this plate is placed on top of this. So, what you will find that the plate is put on top and they are being separated by the spaces. So, there is a gap between the two plates. This has to be understood that the two plates are put on each other; they have the gap because of the spacer. Now, what will have happen? This material has to be poured in here. So, you will pour your material here and you have to be very careful while pouring. If this is a single gel like only resolving gel, it will be put at the top. If it is a stacking gel and resolving gel, resolving gel will be put first, say up to this height and then stacking gel will be put in here.

So, this will be discussing later on. Once you have done this, then remove here any bubble put say layer of organic solvents are on top of it just to make a inform shape here, rather you know disturb bad about bad. Then, finally, it will solidify very fast in say, fifteen twenty or thirty minutes. Once it is casted, then it will be put into the running apparatus, which contains electrodes placed for the electrode buffers. The other thing is that now here before as usual, you have poured this, you have to put combs.

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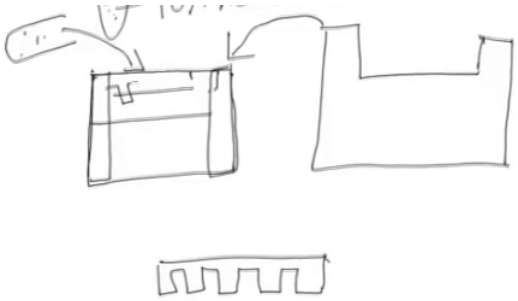

Again the combs as I have shown you in agarose gels, this comb will be put in here and these combs will create this in the gel so that you can load your samples. Let us go back to our discussion. So, how do you the proteins on this and how do they migrate?

So, what is done is say a viscous protein solution is layered on top of the gel in a small well, as I told you how well is created, which are molded into the gel during the polymerization process. Then, bottom and top parts of the gel are inserted into reservoirs containing the buffer solution and the appropriate electrode. The electric field is applied and the proteins migrate to the hydrated gel. The components of the buffer gel are combined to the protein for good run. The ph of the medium must be such that the proteins have the charge, like so you want transfer them from negative electrode to positive electrode, so they have to have a particular ph, particular charge. That will be done as per the ph of that particular buffer.

The equipment for like this I was showing you to conduct page is very simple. It consists of a mold put on the gels that is the casting one, then an apparatus to hold the gel and buffer and power supply as we were talking about capable of delivering the required voltage current.

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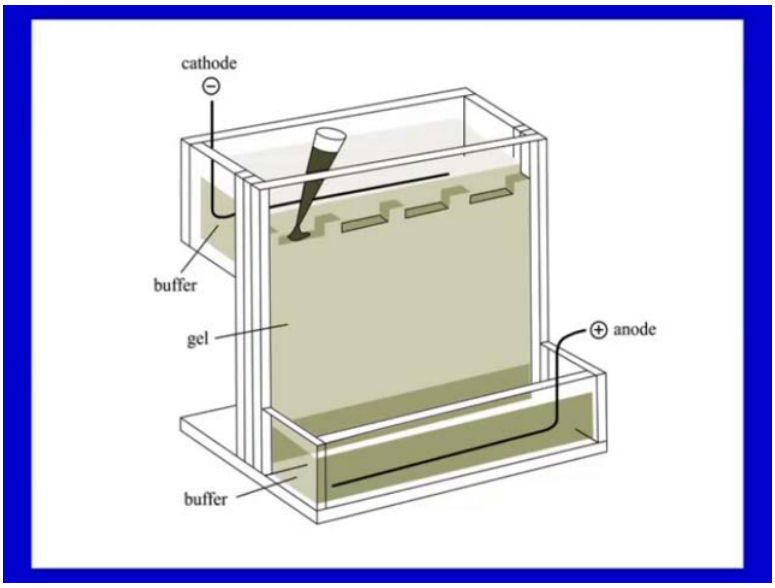

So, typically this is how it looks likes actually. If you see here, this particular one on the screen, this is tank buffer that is the top tank buffer and bottom tank buffer. So, this contains, this here contains the electrode buffer or you can call it running buffer. As this tip is showing here through pipette, you can load these samples in this wells very smoothly so that it diffuses out and settles down to the well.

This is your gel which has been casted well settled created by your comb. As you switch on the electric current, they will start. So, if it is a negative electrode, this is positive, all negatively charged species or proteins samples will move towards the positively charged electrode. According to their size and the charge, they will separate out.

So, remember smaller protein molecule will run faster and the larger protein molecule runs slow because of the resistance of the gel depending on the pore size here. So, that is how the whole separation will occur. We are going to discuss this quite a lot as we go, as we discuss STS page and other techniques in here. So, this is very simple electrophoresis or vertical gel electrophoresis apparatus. Likewise, tube gels could be run in here, rather than the slab gel or this plate gel in over tube and only a single sample could be run. The whole operators will look something like this.

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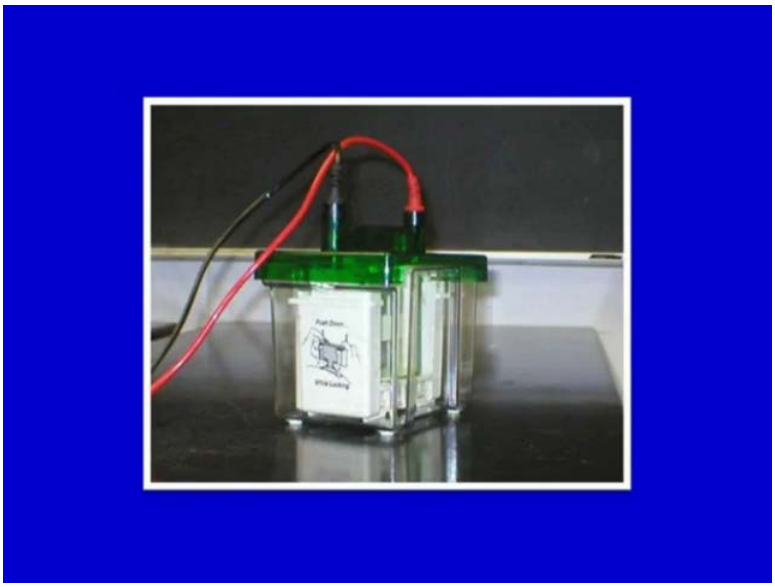

You have enclosed buffer chamber and these are the electrodes, two electrodes here. The gel apparatus is kept on here. As you switch on the electric current, this whole pattern will; the protein migration will take place and separation will take place. After the protein has been run, you can remove the gel plates from here. Gel plates could be separated and the gel could be put in the staining solution like say solution containing the Coomassie or any other dye and that could be visualized.

So, today now we have discussed about vertical gels and horizontal gels. The horizontal gels are agarose gels and vertical gels are poly acryl amide gels. We have learnt today about how they are casted and how they are polymerized and how they are run, what different kinds of samples are being run on this gels and details about that. In the next lecture, we are going to start with say STS page technique and also since modern STS page is a discontinuous system, we will little bit deal about continuous and discontinuous buffer systems. So, we will end this lecture here.

Thank you.