Downstream Processing
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Lecture - 39
Absorption, Electrophoresis / SDS PAGE

We have been talking about absorption in the previous class, and we shall continue the same topic. Absorption happens when a gas or a vapor gets captured by a solvent or liquid. Absorption is generally used in the exit of bio reactor or fermenter, because a fermenter may be having gases like carbon monoxide or hydrocarbon vapors, methane H 2 S, and so on.

Gases coming out and fermenter and you do not like to went it out directly in to the atmosphere. You would like absorb them before venting it out, because in some cases it may be very economical to recover some gases or in some cases, because of the local pollution board norms you want to like to keep venting out such gases. So, you would like to use some solvent or liquid which will capture those, and this is called absorption. So, the difference between absorption and adsorption is that this particular is a bulk phenomenon.

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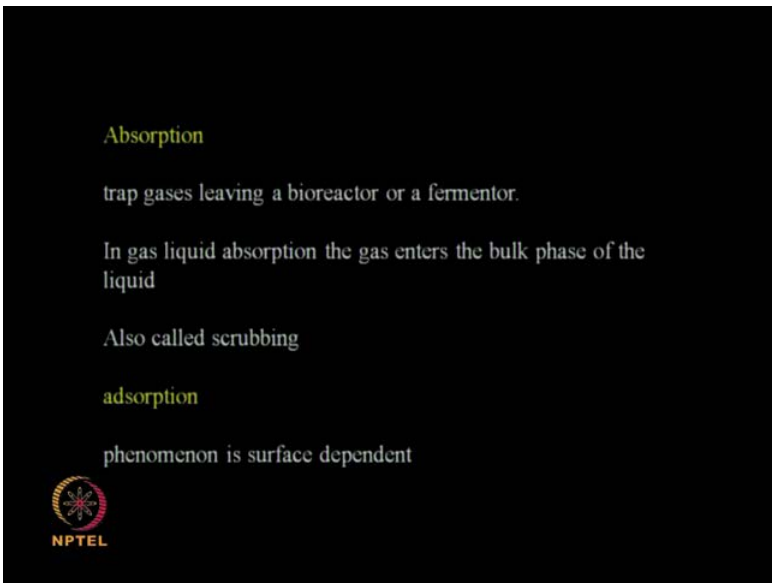

So, we have talking about absorption, it is good for trapping gases leaving a bioreactor or a fermenter. It is very useful in a gas liquid system, so the gas inters the bulk phase of the liquid, it is also call scrubbing. So, what is the different between absorption, that is ab verses adsorption ad is a adsorption is a surface dependent phenomenon.

That means, the gas or the vapor gets adsorbed on the surface of via solid matrix could be a cartelist could be a inert material, so like alumina or activated carbons, zeolites, silica. I am so many different solid material, so that is called adsorption. Whereas, in absorption like carbon dioxide getting adsorbed by sodium hydroxide or mono ethanolamine, florin gets absorbed by may be some bleaching liquid and so on actually, that is called absorption.

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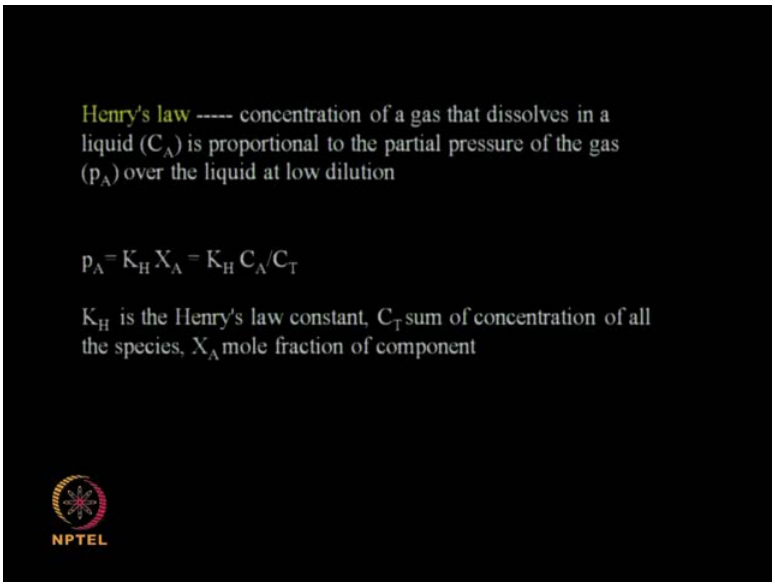

So, it is a very, very important law which relates the concentration of a particular gas dissolved in a liquid to the partial pressure of that particular gas in the gushiest. So, it is relates this equilibrium concentration that is call the Henry's law. So, Henry's law states that the partial pressure of the gas about the liquid P A is equal to that is the partial pressure of the gas in the vapor phase above the liquid will be equal to a constant K H.

Then called the Henry's law constant multiplied by X A, X A is the mole fraction of that particular component in the liquid phase. So, X A can be calculated as C A that is concentration in the liquid phase dived by the total concentration, so C A by C T gives you the mole fraction of A in the dissolved state. So, what this particular law states is

that the partial pressure of a particular component in the vapor phase is a equilibrium with the same component in the dissolved state, concentration of the same component in the dissolved state.

So, partial pressure is equal to K H which is the Henry law constant multiplied by X A that is the mole fraction of the same component in the liquid state. So, that X A is given by C A C S, that is the C A is concentration the solute in the dissolved state dived by the C T, C T is the total concentration because may have many solutes plus you will also have solvent in the liquid forms, so C T takes care of all those things.

So, what it means is there is a linear relationship between the partial pressure of the solute in the gracious stage, we always the mole fraction of the same solute in the liquid state, and they are connected by a constant call Henry's law constant. So, the Henry's law constant depends upon the type of gas or vapor it depends upon solvents, so you will have Henry law constant values is available these data are available in any handbook, thermodynamic related handbooks. And this is valid only for dilute systems, if the system is very, very concentrated or then Henry law constant is Henry's law is not valid.

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Henry's Law constant for gases in water

Gas	$H \times 10^{-5}$ atm/mole fraction	
	(20°C)	(30°C)
N_2	80.4	92.4
CO	53.6	62.0
O_2	40.1	47.5
NO	26.4	31.0
CO_2	1.42	1.86
H_2S	0.483	0.609
SO_2	0.014	0.016

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So, the Henry's law constant for gases in a water, for example at two different temperatures is given here. So, you see nitrogen is around 80.4, there is 10 power 5 atmosphere by mole fraction factor coming here. So, carbon monoxide Henry law constant is low oxygen low, you can see carbon dioxide is low, hydrogen sulfate is

extremely low, sulfate oxide is very, very low. So, what it means is lot of sulfate dioxide will be dissolved state rather then being in the vapor state.

And we can see the Henry law constant increases as we increase the temperature, because the solubility decreases as increase in temperature, so Henry law constant increases as increase the temperature.

So, using this Henry law constant we will be able to given P that is the partial pressure of the vapor in the gas, we can calculate how much that mole fraction of the same gas in the liquid state or vice versa. If we know the mole fraction of the particular solute in the liquid phase we can tell what will be the partial pressure in the gracias state with the help of the Henry law constant.

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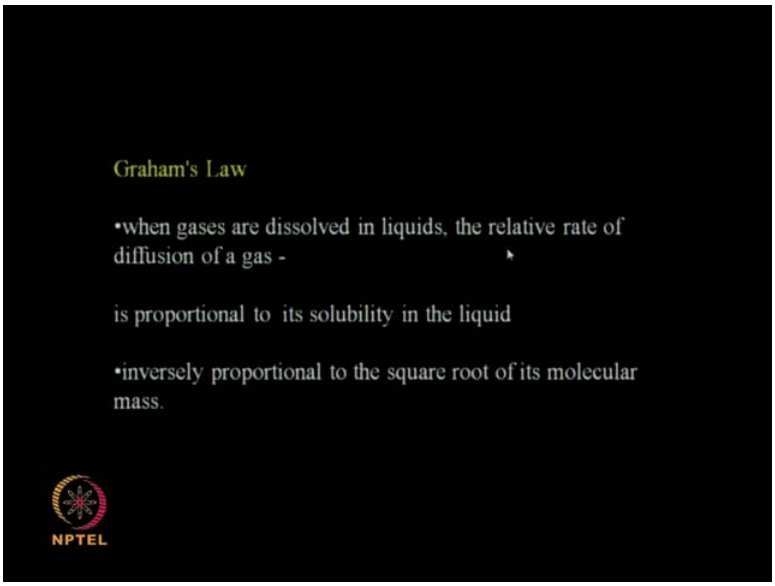

Now, there is another law that is called Graham's law it is use when gases or dissolved in liquids. It tells you the relative rate of diffusion of the gas it tells you relative rate of diffusion of a gas. So, Graham’s law states that the relative rate of diffusion of gas is proportional to, it is stall solubility in the liquid. So, if the solubility is very, very high then the diffusion of the gas into the liquid is also very, very high.

And it is inversely proportional to the squire root of it is molecular mass; that means, the molecular mass is very, very large, then the diffusion is low. When the molecular mass is low, then the diffusion is high, but it is not exactly linear there is a squire root term

comes in there. So, the diffusion relates rate of diffusion of the gas is directly proportional to solubility in the liquid and inversely proportional to the squire root of the molecular mass.

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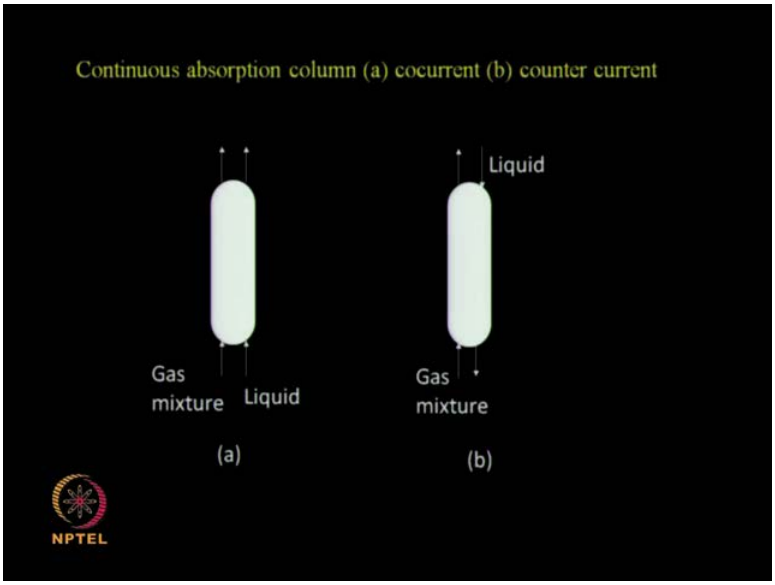

So, absorption is done in many, many different types of system and it is a typical column base system. You have here tall column that is like a desolation column, you have very tall column and the grass mixture may flow concurrent to the liquid that is being used for absorbing or it may be flowing countercurrent.

So, this is the countercurrent system this is a concurrent system. So, the liquids in a concurrent system will be flowing from the top to bottom gas will be rising from the bottom to the top, whereas, in the concurrent system both the gas and the liquid will be travelling of words.

So, generally the countercurrent is preferred as against the concurrent system because of the drying force being much higher in counter current then in the concurrent system. So, these columns are pact I am just like desolation columns, you can have lot of packing you can have plates, you can have c plates, you can have different types of contacting systems.

So, the gas in the liquid coming in good contact because be rate of a absorption is directly proportional to the area interfacial area between the gas and the liquid. So,

higher is the interfacial area between the gas in a liquid, higher will be the rate of transfer of the gas from the gracias phase to the liquid phase. So, you try to create turbulence inside this absorption column, so that the transfer rate is also very, very high.

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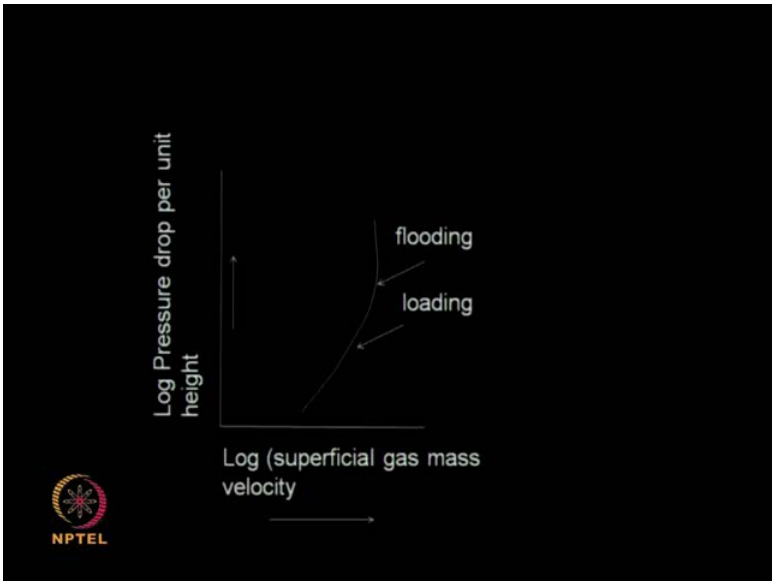

So, these are different approaches by which we try to enhance the rate of absorption, now there is something called the loading and the flooding and we need to understand that. So, if you look at the pressure drop per unit height in a countercurrent system, in a countercurrent system you have the liquid flowing down gas is raising. So, if you look at the pressure drop per unit height as a function of the superficial gas mass velocity, gas is raising.

So, if I keep increasing the gas fluoride that is gas that is riding my pressure drop in the column is going to increase correct that is correct, so but after some time the pressure drop going to increase quit dramatically. And after sometime it just going to be too much because your gas fluoride that is the rising up the column is so much that it will not allow any liquid to come down. So, that particular point is called flooding; that means, no more liquid can travel down because the gas fluoride is extremely high.

So, in a normal situation you have the gas slowly rising, you have the liquid slowly coming down. So, they come in close contact with each other, so there is a absorption technique place, but I keep increasing the gas fluoride that is rising my pressure drop is going to also increase.

That is obvious, but then some particular value the gas fluorite is so much that it is not going to allow any liquid to come down, that is call the flooding. We on flooding you will not have any liquid coming down, so you never operate absorption column, very close to flooding atoll you operate much, much lower may be 30 percent, 40 percent, 50 percent, 60 percent below the flooding velocity.

So, the flooding velocity determines the capacity of the absorption column, so if the flooding velocity is low. That means, I cannot operate my system very high, the flooding velocity is very high, then that mean I can operate the system quit high. So, above the flooding velocity no liquid is going to flow down, so we generally operate much, much below the flooding velocity. Now, there is another point which is call the loading, that is loading is the place where you have optimum amount of liquid flowing down optimum amount of gas raising.

And there is a good contact between the gas and the liquid, that is call the loading here. Below the loading you do not have still optimum amount of gas rising up or liquid coming down, so there is still space for some our liquid to come down. So, the loading is below the flooding and above the loading, you are slowly going to have an increased pressure as you move down the x axis, but beyond that you are going to have just the hole process getting stopped.

So, below this generally it will look like a linear in a log log curve in a log log graph, below the loading we are going to have a linear straight line which connects the gas, superficial gas, mass velocity with the pressure drop in the system. So, you need to understand the flooding velocity for a given system. So, the flooding velocity depend upon the diameter of the column, type of packing, how these things are packed and so on.

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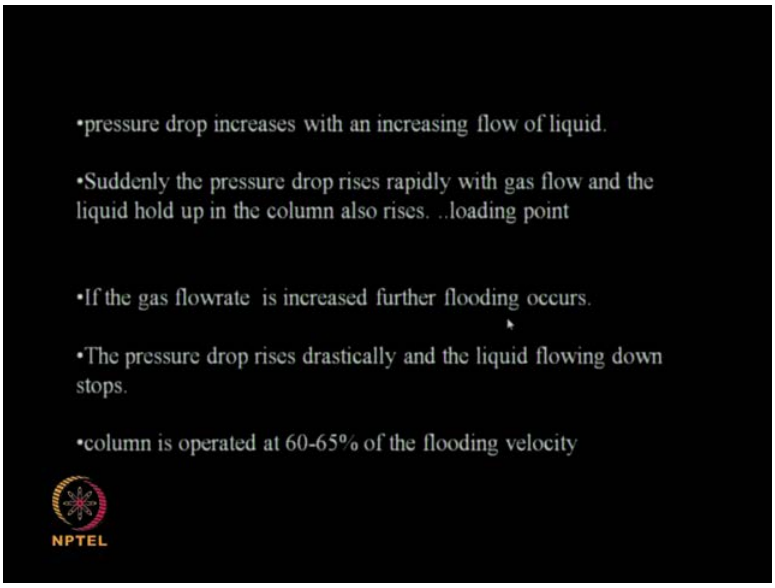

So, pressure drop increase with increasing flow of liquid and suddenly the pressure drop rises rapidly with gas flow, and the liquid hold up in the column also rises, because as we keep increasing the gas flow you are going to have more liquid held a in the column. That means, liquid will coming down slowly, rather than coming down fast, now that is call the loading point.

If the gas fluoride is increased further then you are going to have the flooding point, because at flooding that pressure drop rises, so dramatically, that there is no more liquid coming down at all. So, the column is generally operated around 60 to 65 percent of the flooding velocity, so I tells you the capacity of the entire absorption system.

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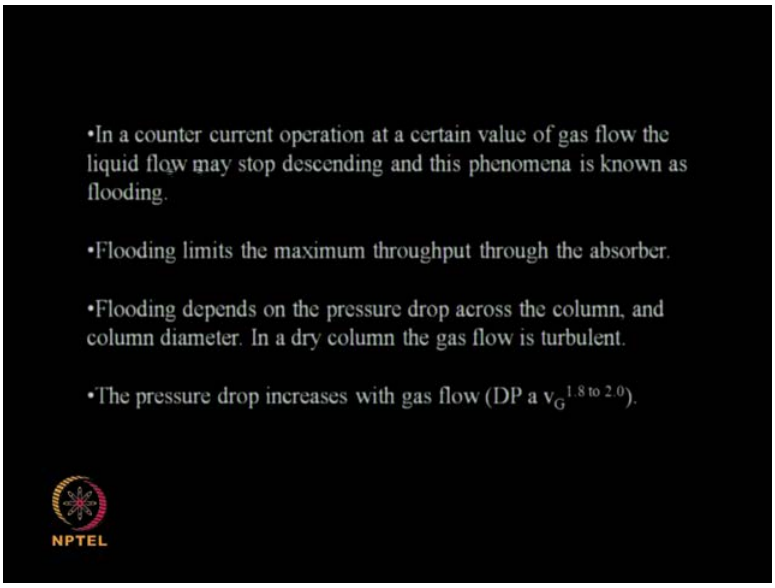

So, these are some fundamentals very basic knowledge about that absorption, in a countercurrent operation at a certain value of gas flow, the liquid flow will stop and that is call the flooding. So, flooding limit is the maximum through put the absorbed; that means, I cannot have more gas rising through the column, so the flooding depends on pressure drop across the column a column diameter and so on. And generally this is an equation for pressure drop, pressure drop is directly proportional to V G raise to the power 1.8 to 2, so with the V G increases pressure drop also increases.

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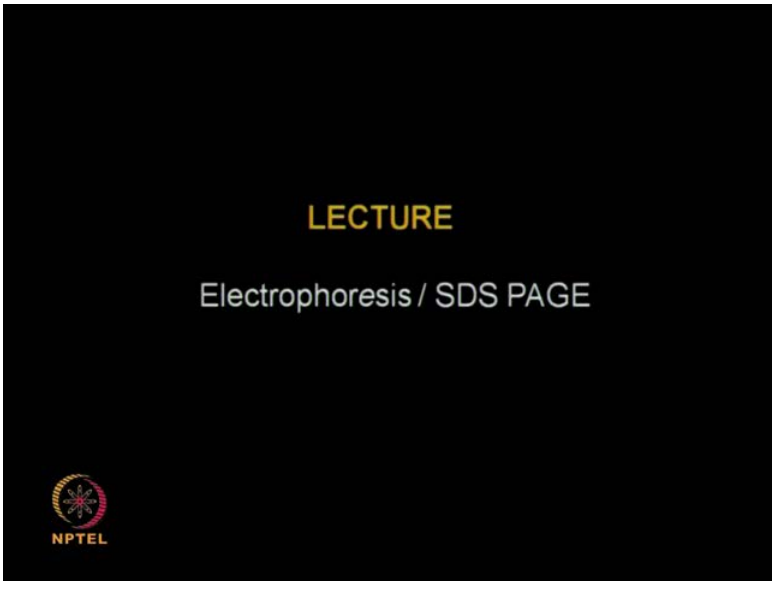

So, for we talked about many downstream including absorption, now there is one very important separation process which is called electrophoreses as well as is S D H PAGE. Although, this is not used for separating protein in large quantity like in normal downstream, this technique is used an analytical tool. To find out what are the various proteins present in a mixture and this technique can be used for separating out proteins based on charge based on molecular weight or size.

So, this is a very, very important analytical tool for separating out or mixture of protein, hence we need to understand the principle behind. It just like H P L C which is chromatographic technique use generally for analytical prepuces not for real large scale purification.

And this is also used as an analytical tool to find out presence of protein, different amounts of proteins in a mixture of proteins. And the principle behind it is based on charge as well as the molecular weight or the size, and generally this is used for polypeptides, this is used for proteins, zensine and so on.

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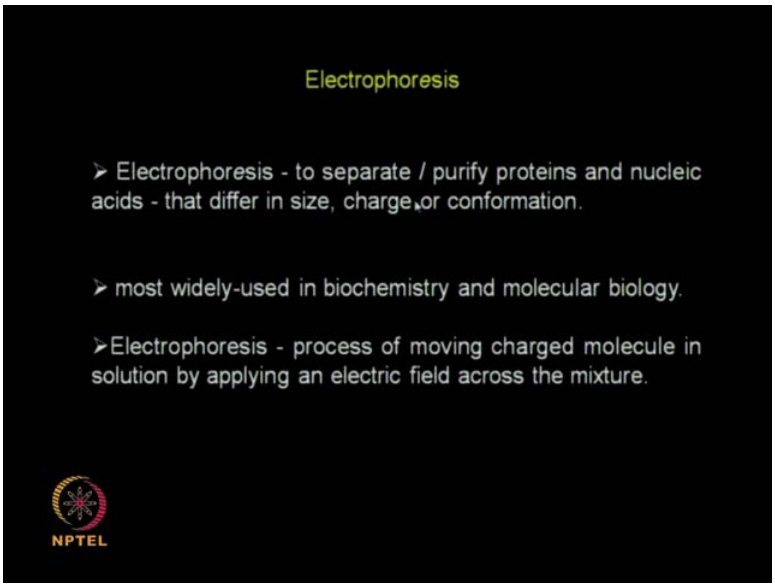

So, electrophoreses it is ment for separating out for purifying proteins and nucleic acids that different size, charge or even confirmation. So, it is very widely used in molecular biology and biochemistry, that is why we need to include it in this particular lecture series as well. Electrophoreses what is does is the charge molecules that is charge

proteins or nucleic acids or peptides start moving, because you are applying a electric field.

So, depending upon the charge on the particular biomolecule, they move faster or slower in the electric field, so they get separated from each other, that is the principles of electrophoreses. So, separation based on their charges and you applying an electric field external electric field.

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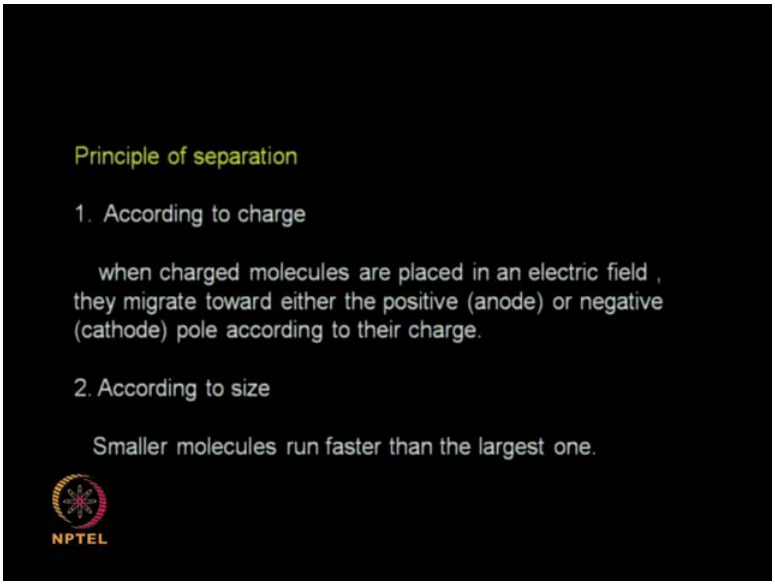

So, it is the principle of separation is according to the charge, so when charged molecules are placed in an electric field, they migrate towards the anode that is the positive side or negative cathode pole according to their charge it is a complementary. Another, approach is according to the size, smaller molecules will defuse faster for a same electric field then the larger one.

So, the smaller molecules would have move further for a given time of applying the electric field and compare to larger molecule. So, larger molecule may not will be much behind whereas, smaller molecules will overtake them and go closer to the electro.

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So, what are the instruments and reagents used you use a buffer, there is power supply because you are all a applying in a electric field. Then there is a supporting media and then we need to detector and quantification also this needed actually, and these are the various parts of a electrophoreses system.

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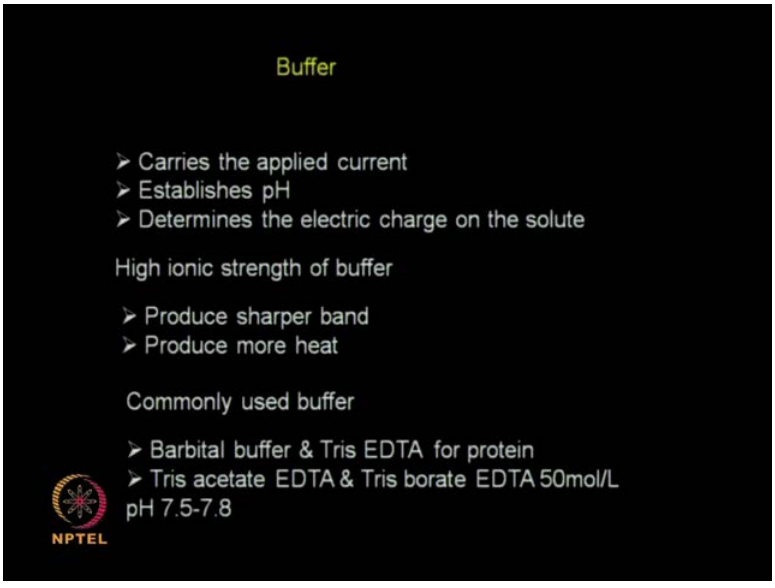

So, what is the buffer? So, the buffer carries the applied current you applying a electric field, so there is a with established P H, and it also determines electric charge depends on the solute. So, if the buffer has high ionic strength then it will produce sharper band, you

will get very, very sharp bands and it will also produce more heat. So, the commonly used buffers are barbital buffer and Tris EDTA, this Tris EDTA is used generally for proteins, and also we use Tris acetate EDTA and Tris borate EDTA around 50 moles per liter and at this p H condition on 7.5 to 7.8.

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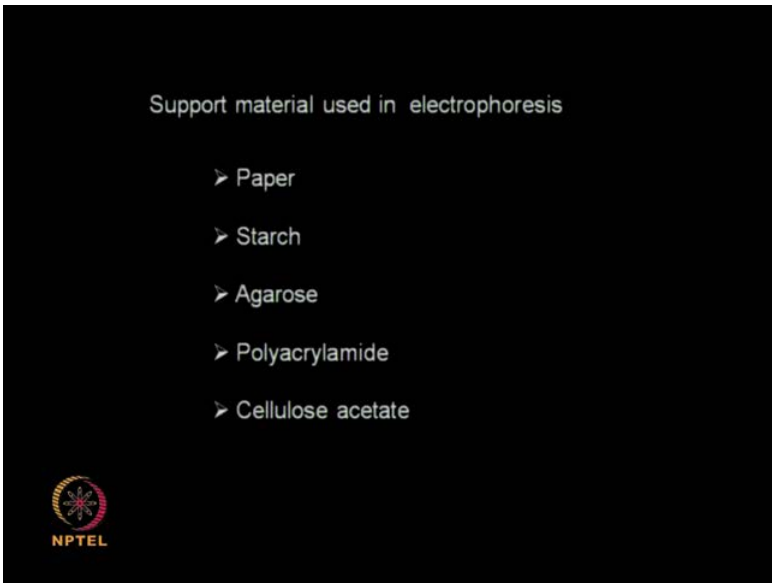

So, what is a support material used, we can used paper we can use starch, we can use agarose, we can use polyacrylamide, we can use cellulose acetate, so large different types of support material or used in the area of electrophoreses.

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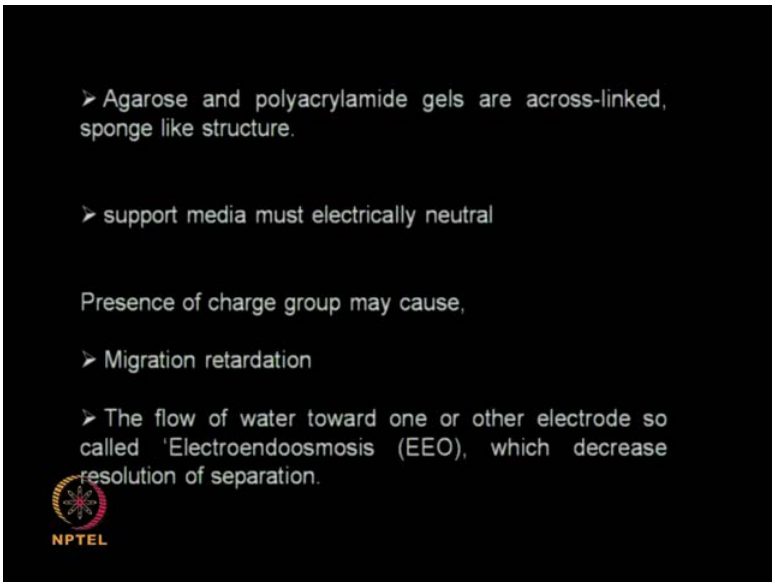

So, agarose and polyacrylamide gels or across linked sponge like structures, so they are cross linked, and another important thing is support media should be electrically neutral, because you are applying a electrical force or potential different. So, the structure polymeric material should not have this own electric field, because if you have a charge group then you may have migration retardation.

It will start retarding the movement of the charged proteins, because they will also have their own chargers. And the water will start flowing towards one or the other electron, and this is called the electroendosmosis EEO which again will decrease their solution of separation. So, when the support material or the base also has it is own charge, then it will allow water start flowing towards one of electrons there by it will decrease resolutions. So, these are the two reasons while the support material should not be having it is own charge.

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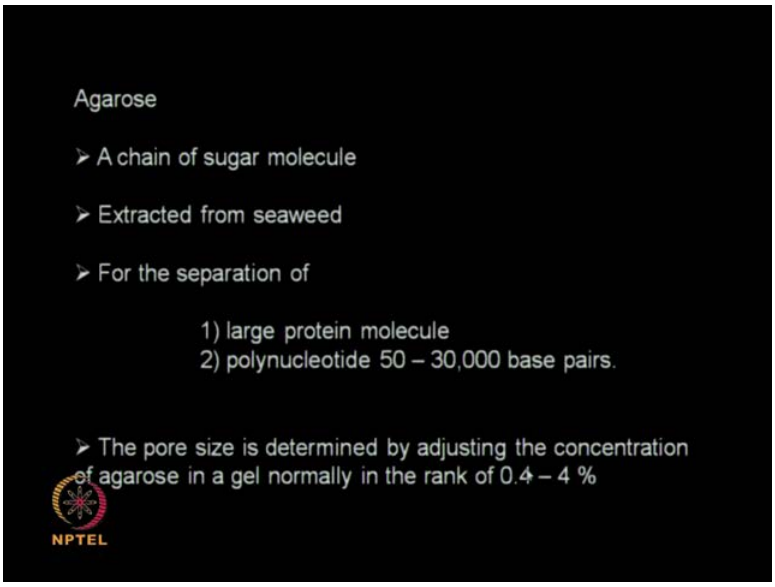

Take in agarose what is an agarose, agarose is a chine of sugar molecule, it is extracted from seaweed. It is very good for separation large protein it is good for polynucleotide like 50 to 30,000 base pair. So, we can adjust the pore size by adjusting the concentration of agarose in a gel. So, generally we can work between 0 to 4 percent thereby we can get wide range of pore size and pore size distribution.

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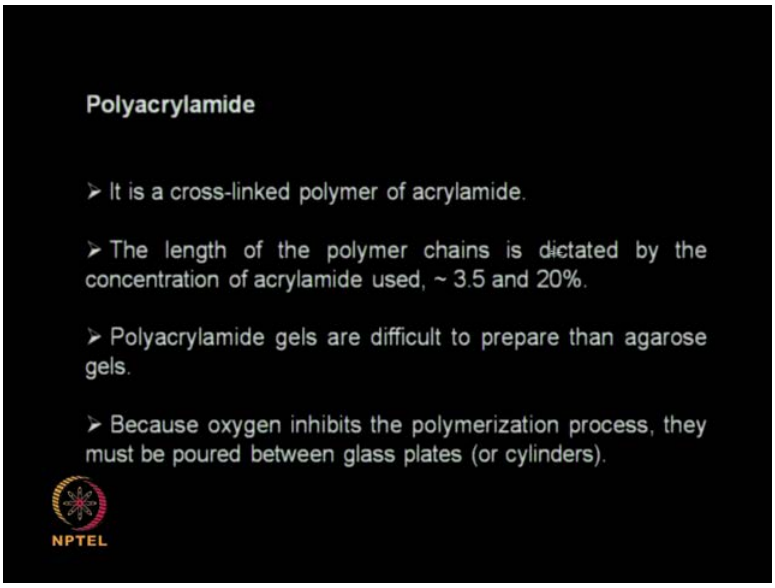

Take polyacrylamide, this is a cross linked polymer of acrylamide, so the length of the polymer change is dictated by the acrylamide that you are using. So, generally we use 3.5 to 20 percent acrylamide, polyacrylamide gels or slightly more difficult then agarose gel, agarose gel much is to your make. Because, oxygen inhibit is the polymerization process, so you need to pore them between glass plates or cylinder, so that they are not in touch with oaring contact with oxygen.

So, agarose gels are much easier then polyacrylamide type of gels, and also polyacrylamide gels have a small range of separation, but it has very good resolving power.

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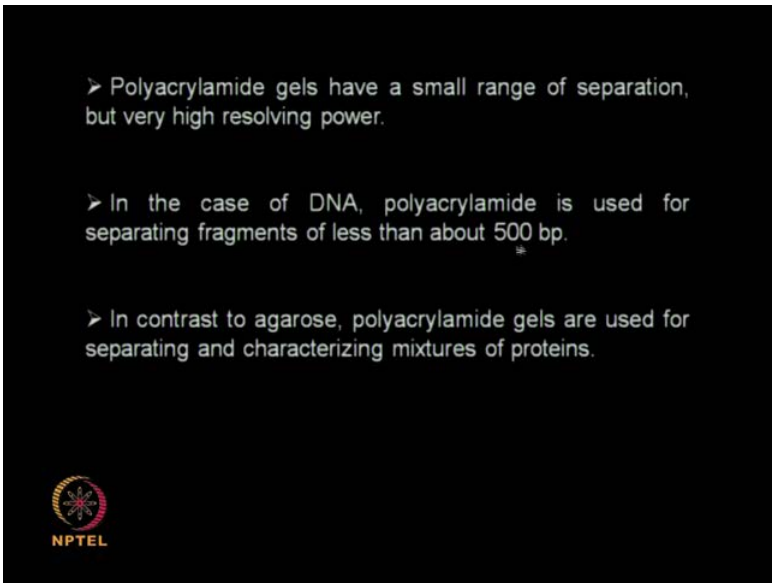

So, if are looking at DNA then polyacrylamide is used for separating fragments of less than 500 base pairs, so there you can to that. Polyacrylamide gels also used for separating in characterizing mixture of protein and contrast agaroes. So, they have got very good resolving power, but the range of separation is very limited.

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There is something called one dimensional electrophoresis, we look at what is one dimensional electrophoreses, in contract to that also have a two dimensional electrophoresis. Just like one dimensional we have two dimensional, so like SDS PAGE

isoelectric focusing, native page these are the three different techniques that are used in one dimensional electrophoresis. That is look at each one slightly in more detail.

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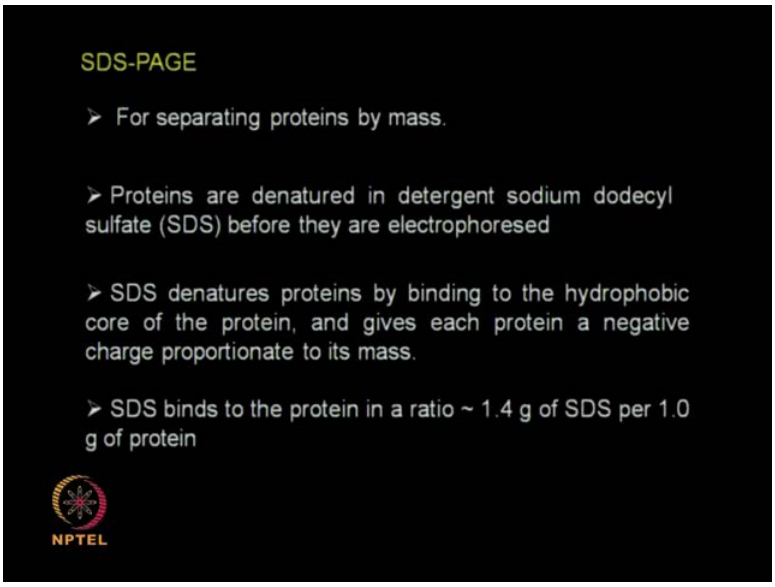

What is SDS PAGE? SDS PAGE is very good for separating proteins based on mass, so what we do proteins are denatured by using a detergent. This detergent called sodium dodecyl sulfate, that is why the SDS consign, sodium dodecyl sulfate. So, when you add to the protein, protein gets denatured and then you send it through a electrophoresis system. So, SDS what does it do?

It denatures protein, because it binds to the hydrophobic pore of the protein and then it gives each protein and negative charge proportional it is mass. So, the protein it is get denature and there is a charge given to the protein based on is mass, and SDS is sodium dodecyl sulfate. Now, SDS binds to the protein in a ration of about 1.4 grams of SDS per 1 gram of protein, so we have more SDS binding to the protein.

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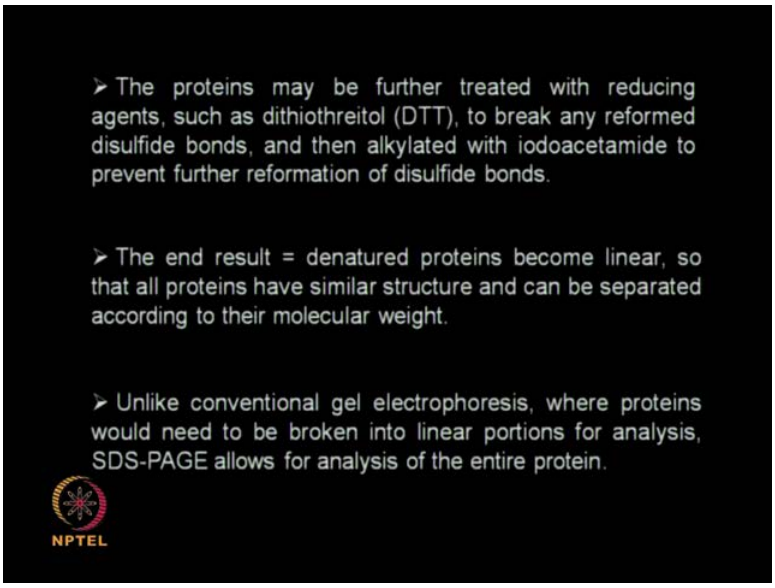

And then after that you can treat the protein with some reducing agents, such as dithiothreitol break any reformed disulfide bonds, and then they are alkylated with iodoacetamide to prevent further reformation of disulfide bonds. So, I am after the SDS treatment we add a DTT, to break any reform disulfide bonds and then we do an alkylation with the iodoacetamide, so that it will prevent the reformation of the disulfide bonds. So, what is the end result we have a denatured protein which is linear.

So, all the proteins will look same they all have linear confirmation and they are all denatured. So, they can end SDS charge to each protein depending upon the mass, so we can separate the proteins based on the molecular weight. So, normal gel electrophoresis proteins we need to be broken into the small, small portions whereas, SDS PAGE we do not have to do that. We can take the whole protein, we do not have to break it into small pieces, that is the advantage with SDS PAGE.

So, what does SDS do? SDS denature the protein and then we do other treatment to break any reformed bonds and prevent any bond reformation. So, all the proteins become linear and proteins get charged based on their molecular weight. So, we can run a electrophoresis and the separation is based on the molecular weight. Whereas, in the conventional gel electrophoresis we have to break the protein into small, small bids linear portion. Here, we do not have to break the protein at all it can be entire protein can be viewed is the SDS PAGE system.

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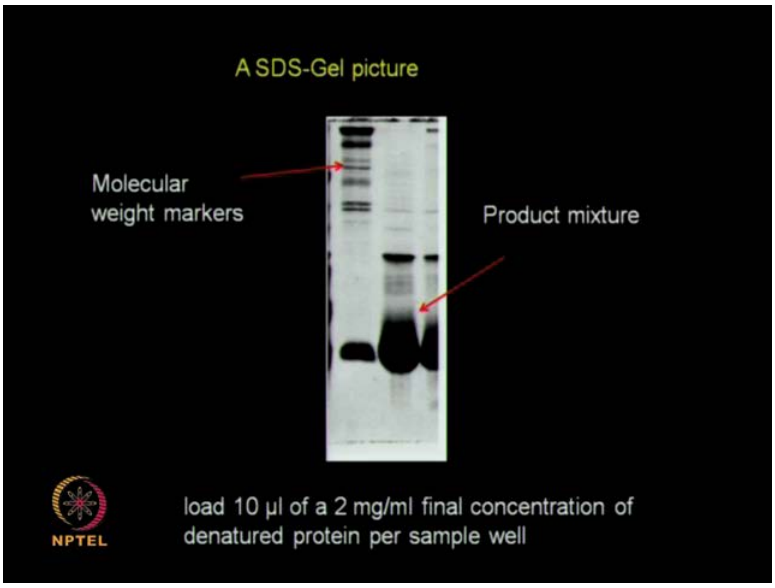

So, this is a typical picture of a SDS gel picture, so this is your product mixture the proteins get separate like band here, you have a molecular weight marker. So, you have a marker which contains of none molecular weight. So, they will form band here and by looking at where, your protein is located is tell the molecular weight of this band or this band or this band and so on. So, generally we are loading about 10 micro liter of a 2 m g per m l, final concentration of the denatured protein per sample value.

So, we can run many gels we will have a molecular weight marker and we can tell whether a protein of particular molecular weight is present in a mixture or it is absent in a mixture, like it is very, very easy to do. And depending upon the band strength, we can tell the concentration as well. So, we can say this particular protein a amount is much higher than this protein and so on. So, that is the advantage of looking at the SDS gel picture, this is a typical gel picture.

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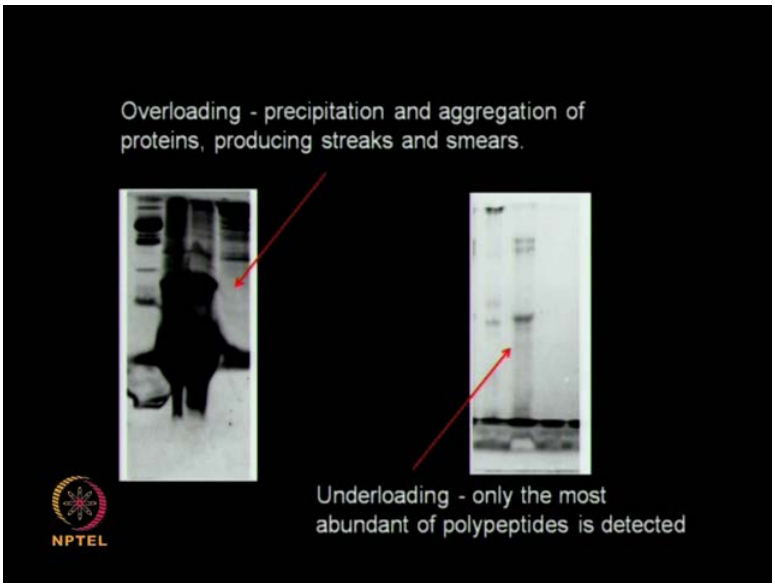

Sometimes you may end up putting too much of this sample, so you will have a very, very thick un separated value and this is like over load this called over loading. So, when you over load what happens the protein mixture gets precipitator or aggregation of the protein takes place. So, you have streaks and smears like this you know this is under loading.

So, only most abundant proteins are seen other proteins are not seen, so you need to strike a balance between over loading and under loading. So, you should be able to all your proteins not a thick smears, but as bans, but you should not miss out the smaller proteins also. So, you need to what do once on whys until you are able to get a good SDS picture p H picture. So, what are the stains used for a detecting protein.

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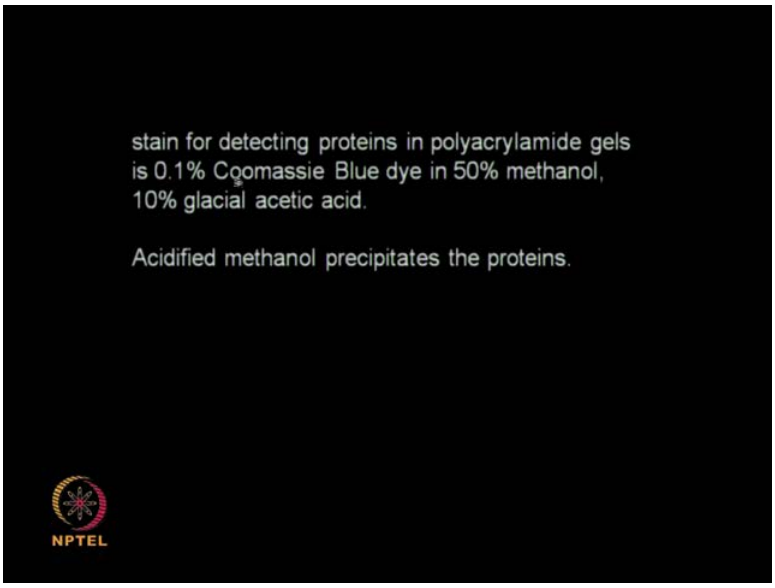

If it is in a polyacrylamide gel you may use 0.1 percent coomassie blue dye in 50 percent methanol 10 percent glacial acetic acid, we acidified methanol precipitate the protein. There are different gels different stains or used, but this is a standard stain which is used for viewing the gel bens.

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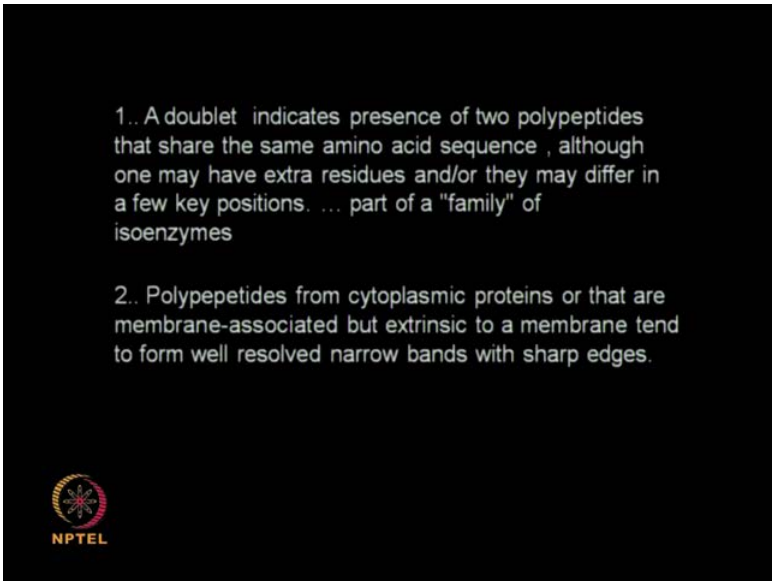

There are depending upon the type of bands that are form, depending upon the quality we can make lot of conclusions, here in the next few slides I am going to talk about some other conclusion, you can arrive at based on looking at the gel picture. So, if you have a

doublet; that means, you have two bands close to each other we can say the presence of two polypeptide that share the same amino acid sequence. So, they may be sharing the same amino acid depends, although one may have extra residues under they may differ in a few key positions.

So, few residues may be different or some different are there key position, so they could be a family of isoenzymes. So, if you see doublet then you can tell actually, polypeptide from the cytoplasmic proteins or those which are member an associated, but extrinsic membrane tend to form well resolved narrow band with sharp edges. So, if you have proteins in the cytoplasmic or they are extrinsic membrane, they will form very good narrow bands and sharp edges.

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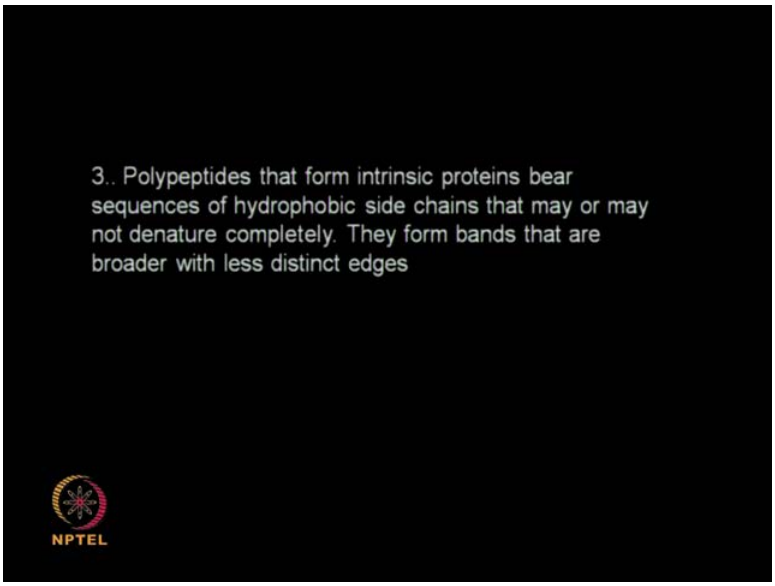

And if we have polypeptide that form intrinsic proteins or bear sequences of hydrophobic side chains that may not or may denature completely will form broader bands, and the band edges will not be very dusting. So, if the proteins are intrinsic or if there have too much of hydrophobic side chains and they are getting denature, then you will get a thicker band and edges are not very short. And bands the consistently show up following electro pores of a sample, we can say it presence, representative the polypeptide the characterize the sample. So, it keeps on coming consistency, so then you are sample is characterized by the particular polypeptide

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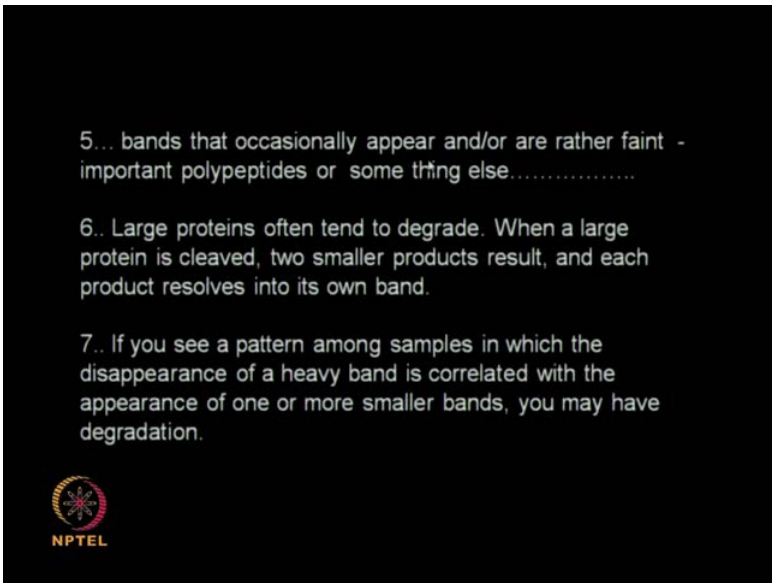

Bands that occasionally appear or they are very, very faint, they are important polypeptide or they could be when spurious, so we do not know. Now, large proteins some can get degraded, so when a large protein get degraded, you may get two small product and each product may form a band in your SDS PAGE picture. So, if you see sometimes a large band, heavy band disappearing and then formation one or more smaller bands, then you can be sure that the large protein is getting degraded.

So, you ran a gel you see a thick band and after sometime you do not you see a thin band and formation of yours bands, when you can say that particular protein has degrade to form the smaller degraded product.

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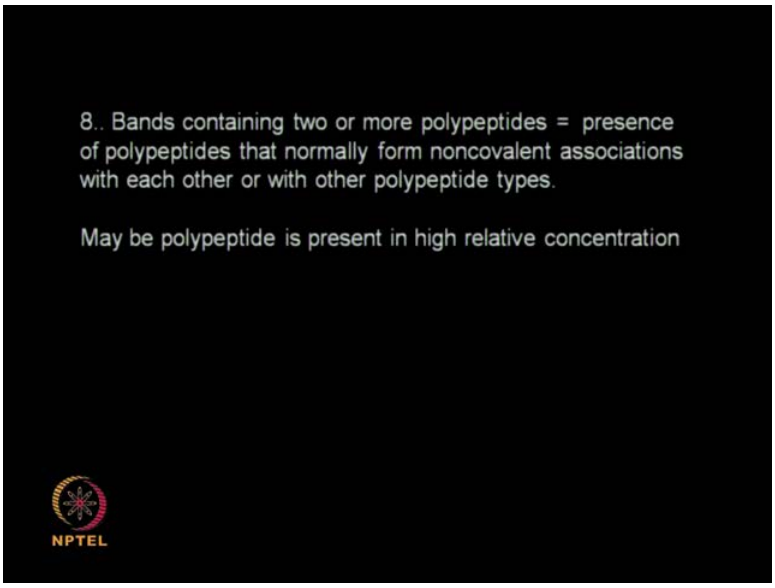

So, bands containing two or more polypeptide, so we have presence of polypeptides that normally non covalent association with each other or with other polypeptide. So, with they are not forming a covalent bond, so you will defined band containing two or more polypeptides or the polypeptide is present in high relative concentration.

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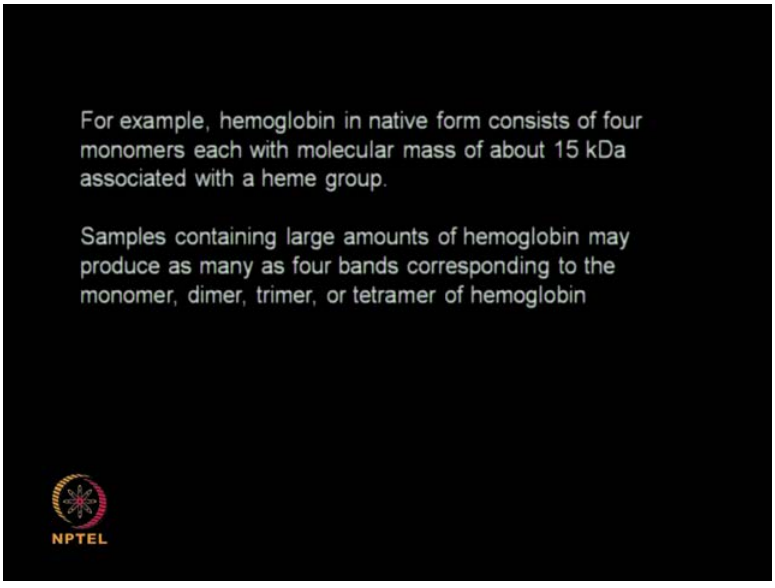

Like, hemoglobin in native form consists of 4 monomers, each with 15 kilo dalton associated with the heme group. So, sample containing large amounts of hemoglobin may produce 4 bands corresponding to the monomer, dimer, trimer or tetramer of

hemoglobin. So, because it spidrominent and it is got 4 monomers forming a single system, which are connect by heme groups. You may have 4 band with respect to monomer, drimer, trimer or tetramer of hemoglobin. So, you need to look at in your bands in your SDS PAGE and try to understand or make sense out of the composition of the mixture.

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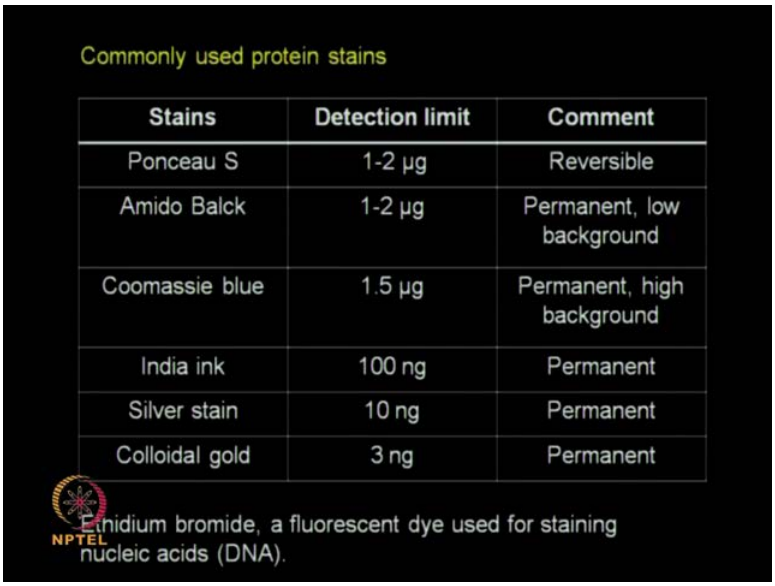

So, commonly use the protein stains, we have Ponceau, Amido black, coomassie blue, India ink, silver stain, colloidal gold and so on. You also use Ethidium bromide it is a fluorescent dye for shining nucleic acid as well. So, these stains some of them reversible and most of them are permanent; that means, they are not reversible that all it forms a permanent stain on your SDS PAGE.

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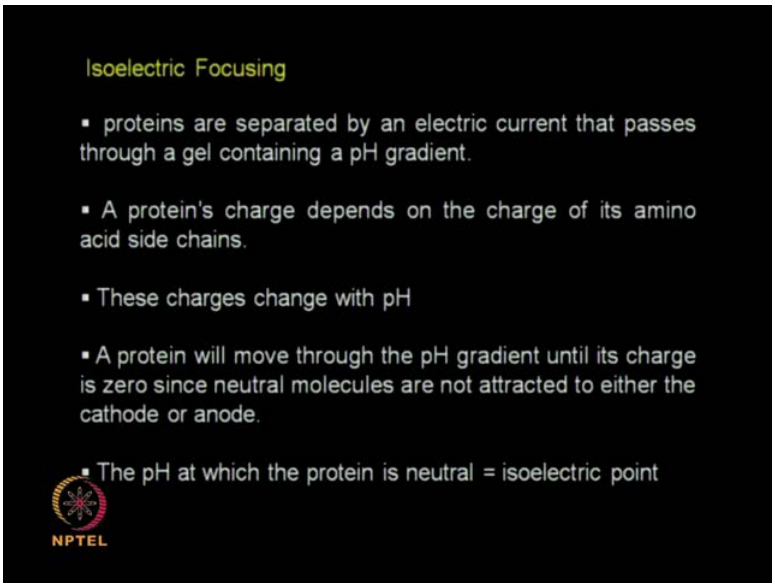

Next one dimensional electrophoresis call the isoelectric pore focusing. So, here proteins are separated by an electric current, that passes through a gel containing a p H gradient. The protein charge depends on the charge of it is amino acid side chains, and these charges change with p H. So, when you apply a electric current in a p H gradient the protein will move through the p H gradient until it is charge is 0, once the charge become 0 the protein will not move, because it will not get attracted either by the anode or the cathode.

And this is how it get separated? So, the p H at which the protein is neutral is called the isoelectric p H or isoelectric point, we talked about isoelectric p H long time back. And protein when it has a charge and when you are apply a electric current will move and when it reaches is isoelectric p H that the charge is 0. So, it will not move towards either the anode or the cathode. So, you separate the proteins mixture based on the individual isoelectric p H, so if a protein has a p H of isoelectric p H as 6.5, another one having isoelectric p H is 7.

Then the one protein will move until it reaches the p H value at 6.5 other one will move until it reaches the p H value of 7. Because, the charge of the protein at p H 6.5, for the first protein will be 0 and for the second protein the charge will become 0, when it reaches the p H at 7. So, the isoelectric focusing is base on separation based on it isoelectric p H values. So, here you have the gel containing a p H gradient and you are

applying an electric current. So, you have immobilized p H gradients or you like IPGs, they are called immobilized p H gradients or used for IEF.

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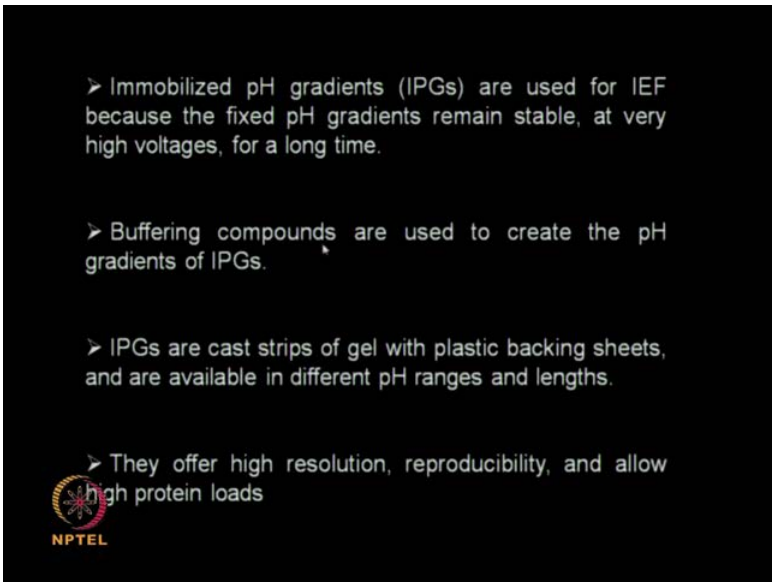

Because, the fixed p H gradients remains stable, even at a very high voltages and for a very, very long time, you are also use you can use buffering compounds to create the p H gradients of immobilized p H gradients. So, IPGs are nothing but cast strips of gel with plastic backing sheets and you they are available in different p H ranges and lengths. So, I can create a matrix with different p H values by just buying the IPGs with different p H ranges. They offer very high resolution, the reproducible, and you can use a very high protein loading in this particular isoelectric focus technique.

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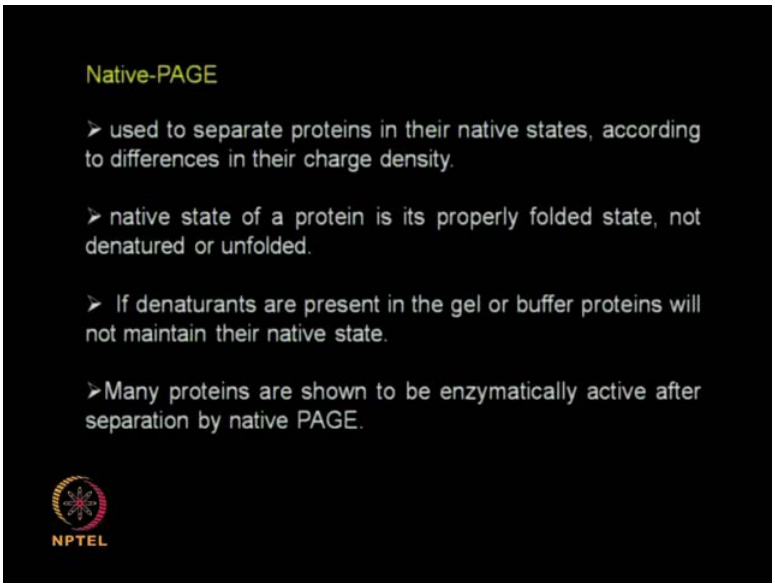

The third one dimensional technique is called the Native PAGE. So, this is used to separate proteins in their native states, according to the differences in there charge density. Native state is the active; that means, protein is properly folded it is not denatured like SDS PAGE system or it is not a unfolded.

So, if denaturant or present like a surfactant or a you neither acidic or charged medium the protein loses it is activity, so they get denatured. Even, after separating the various proteins using a Native PAGE system, we can take out those proteins or enzymes and use it for further studies, because they still retain their activity. So, that is the advantage of Native PAGE because the various components still retain their three dimensional structure and hence their activity.

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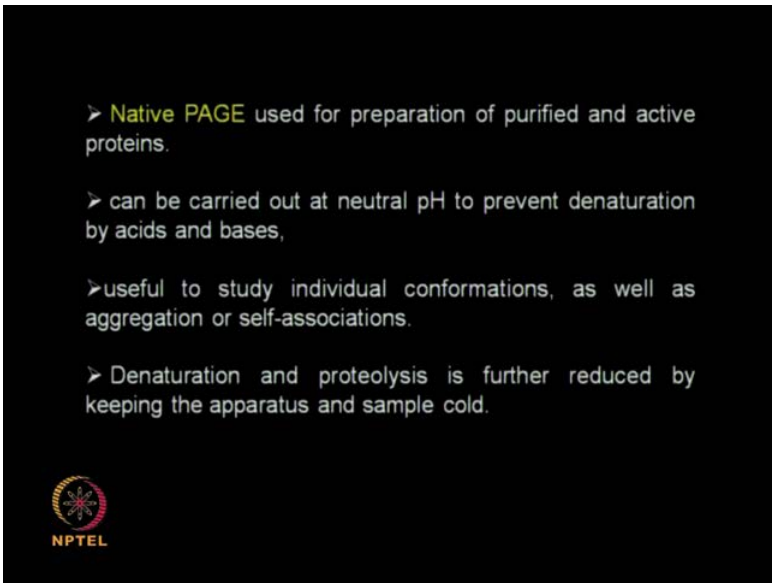

So, we can use Native PAGE for preparation of purified and active proteins, and generally it is done at neutral p H, so that there is no denaturation by acids and bases. It is useful to study individual conformations as well as aggregation or self associations, and we maintain the activity or we prevent the denaturation or proteolysis by carrying out the whole Native PAGE separation process under very cold condition; that means, under very cold condition. So, that the protein does not get denatured or deactivated.

So, these are the 3 different one dimensional technique which we talked about, one is called the SDS PAGE, the second iis called isoelectric focusing, third one is called Native PAGE. So, the SDS PAGE is based on the size of the molecule, in SDS PAGE we are completely denaturing the protein, so you are studying the denatured protein. In isoelectric focusing the separation is based on the isoelectric p H, that is the p H at which the charge on the protein is 0.

Why do proteins get charge because the amino acids in the protein are charged. And the 3rd approach is Native PAGE where, the protein activity or the structure three dimensional structure is maintained, here you are not adding any denaturing agents. So, that if you want to study the activity of the separated protein, then this is the best technique. Now, having talked about one dimensional it is go to two dimensional electro psoriasis this called 2D PAGE. So, here you are separating based on two different

principles, and they are triangles to each other in the first dimension. You put in isoelectric focusing, so the proteins are separated based on their charge.

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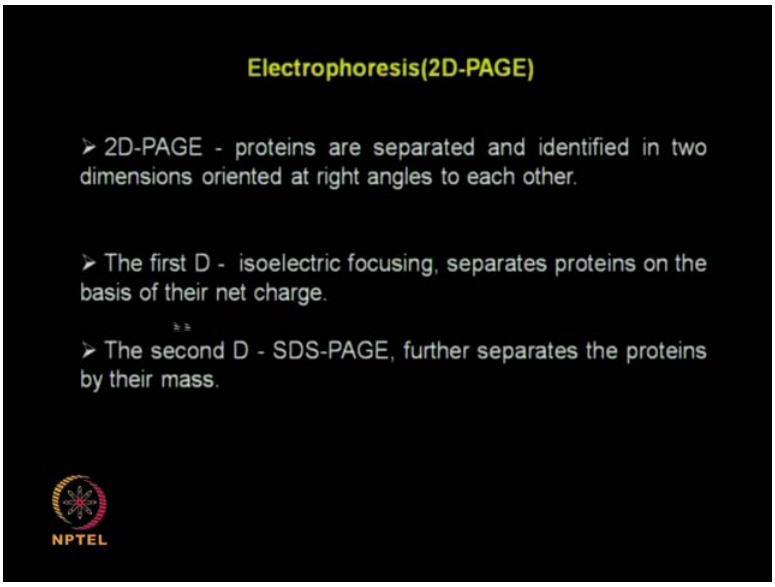

The second dimension the proteins are separated based on their mass, so first you separate them based on the charge, then you separate them based on the mass. So, instead of getting a band which is in one dimension you are going to get spots in a two dimensional paper. That is what is the principle of a 2D electrophoresis for a 2D PAGE. So, a typical 2D PAGE picture may look like this, one is the separation like a p H this side and this could be a molecular weight.

So, you will not get discrete bands, but you will get resolved spots this is via 2D system works, typically this is way it looks like. So, one dimension may be because of the charge other dimension is because of the mass. So, each spot may hopefully represent one single protein, but if the spots are very big, you can be sure that there are many protein which are unresolved.

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So, probably these are resolved proteins, we can take it out and then look at the characteristics by looking at the molecular weight using a mass spectrometer.

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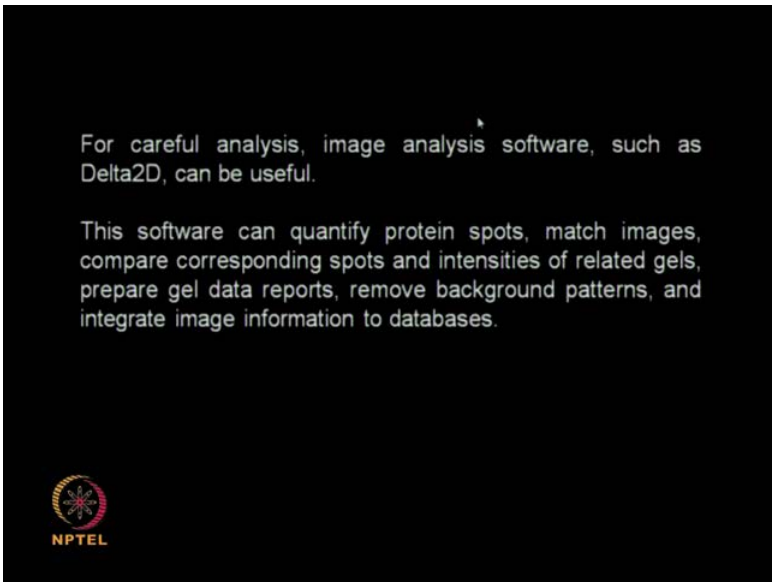

May be can also use image analysis software, so if you are comparing 2D pictures from different parts of the human system, we can use image analysis software and see what are the differences in the image type. And there are many softwares, but one of them could be software could be called delta 2D, this can quantify protein spots it can match images, it can compare corresponding spots, and intensities of related gels. So, if you

have two gels, it can compare the pictures of the two gels, and say what is the difference, we can prepare gel data reports, it can remove background patterns integrate images and so on.

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So, there is lot of image analysis being had needs to be done, if you are looking at a pictures like this or you can even take out one single scratch, one of these spots collect it. And we can run (( )) or we can run s LCMS to see the molecular weight and of this particular spot. So, that is the advantage of a two dimensional electrophoresis.

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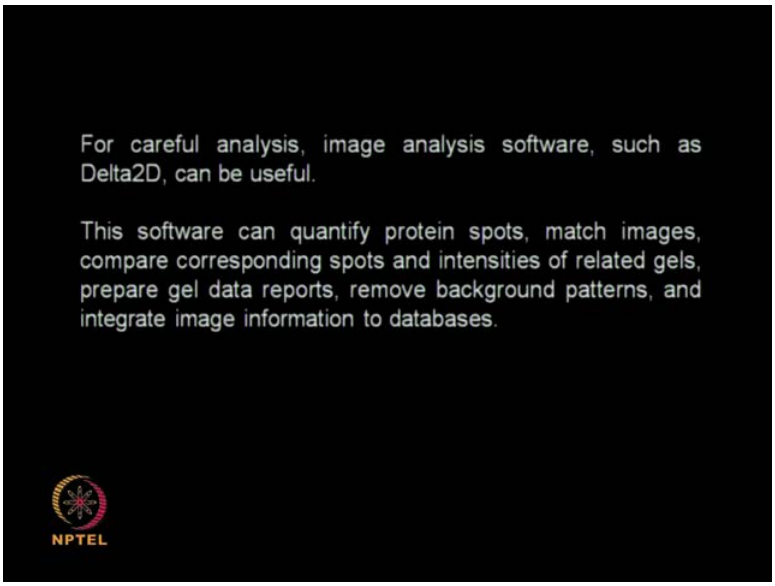

Now, they both the one dimensional and two dimensional electrophoresis is meant as an analytical tool for separating out proteins or nucleic acid or biomolecules. That are present in a mixture and the separation is based on charges and the molecular weight. So, in the first dimension separation is done through the charge and the second dimension is because of the molecular weight.

So, this is typical analytical tool in extremely useful especially in the area of proteomics, especially in area of molecular biology, drug design and so on. So, that is why we are spending some time to understand what it means and so on actually.

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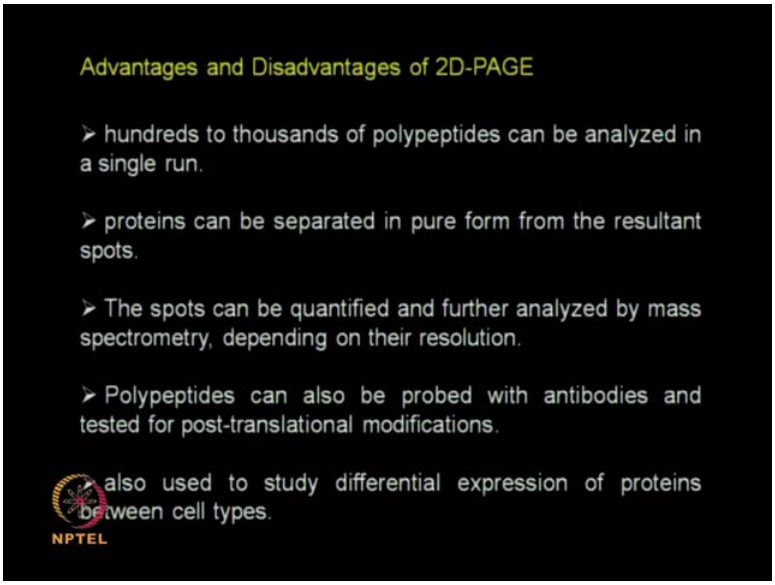

So, what are the advantages and disadvantages of this 2D PAGE. So, we can look at hundreds and thousands of polypeptides because if you look at here, a sample of from a tissue sample from a human or animal. We are talking in terms thousands of polypeptides, so we can separate them using a 2D PAGE in one run.

And then we can separate out the proteins in pure form, as I said we can scratch one of those spots and then we can do further analysis. We can use it for looking at the amino acid sequence, we can look at molecular weight running a LCMS or maldi tof and so on.

And then we can quantify the spots; that means, we can tell what is the concentration of that particular spots? Then we can look at the polypeptides using antibodies and we can look at the post translation modifications, so that is another thing. So, then we can also

study differential expressions of protein between cell types, so if you have two different cell types you run at 2D PAGE of both the cells. And then we can compare and you see what is the difference between these two cell types by looking at those these spots and spot distribution.

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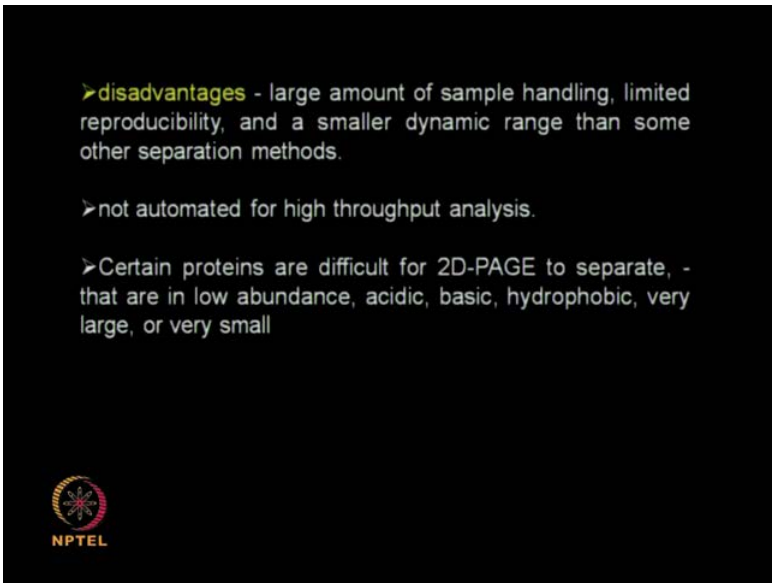

Of course, there are many disadvantages also there is large amount of sample handling reproducibility is a problem, I run two times this 2D PAGE, you may get entirely different setup patterns. So, that is because some proteins may get expressed more, some proteins may get under expressed. So, the problem of if you get a very dark spot is it the concentration of that particular protein is more or is it the expression of the protein is more.

So, that particular doubt remains always in a 2D page, so you need to analyze large samples or spots, limited reproducibility and very small dynamic range then most of the separation methods. And it is not automated like a high through put screaming, so that is another problem. Where if the protein is of low abundance or if it is acidic or basic or hydrophobic or very large or very small, then you will not be able to separate them, and also view proteins in a 2D PAGE system.

So, these are some of the advantages of the 2D two dimensional electrophoresis, but still it is extremely useful technique which is become very ubiquitous and in the area of proteomics as well as in molecular biology both the PAGE system. That is why we are

trying to spend some time on understanding briefly although not very much in detail, but briefly the usefulness, the silent features of this particular analytical technique. So, we have covered quite a lot of ground in the past several classes and we will continue further in the next class on the area of downstream processing.