Downstream Processing
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Lecture - 25
Precipitation

Today, we are taking up a new topic in the area of downstream, precipitation. Precipitation involves removal of solids from a solution, so you need to achieve certain condition, so that the solids in the solution comes out and as a solid material. Then later on you may purify the solid, you may wash the solid, you may dry the solid, and so on actually. So, precipitation involves removal of the solid, so in many cases the solid make not be 100 percent pure. If you want 100 percent pure solid, then you may resold to something called slightly more complex process called (( )).

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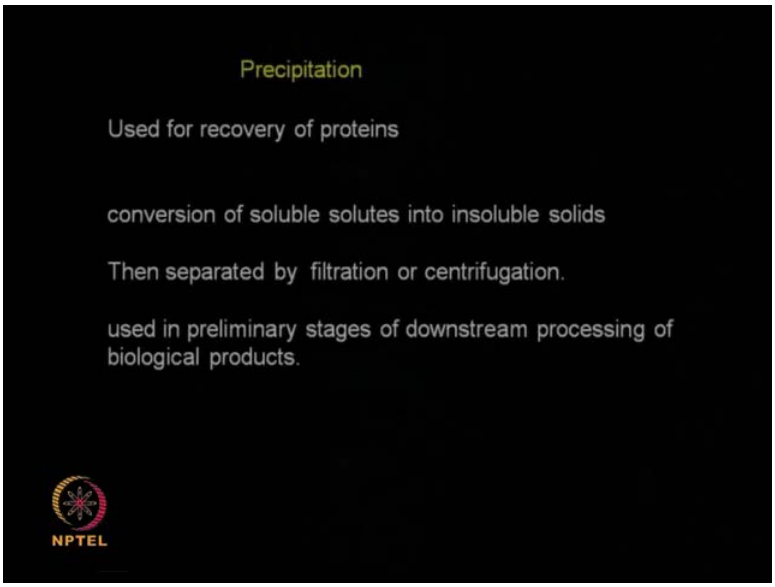

So, in precipitation we can use this particular technique for recovery of proteins. We can use it for even recovery of small molecules and that means metabolize. So, it is conversion of soluble solutes into insoluble solutes. So, we are here in precipitation we are not the both the particle size or the morphology, but whereas crystallization you are most interested in the morphology or the how the crystal does like, the shape of the size of the crystal, the various planes and so on, whereas in precipitation we are more

interested in just removing the solids from the solution, so we convert soluble solutes into insoluble solutes.

So, the solutes are small molecule the solutes should be filth and proteins. Once we do this, you may resold to something called as sedimentation or settling or filtration or centrifugation. So, we have looked at only suppuration process before in early days. So, that is what precipitation does. So, it is used in preliminary or the early stages of downstream processing. As I said we are not bother about the purity of the solid product that is formed, so this process is resorted to in the very early stages. Later on you may do some washing to remove the in impurities or to toxins material or other material that present in the solid.

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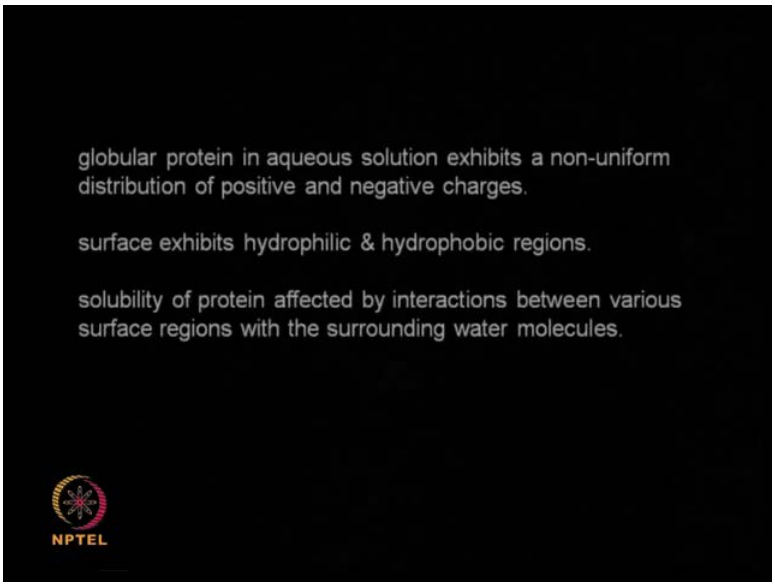

So for example, if we take the globular protein in aqueous solution it is got a not uniform distribution of positive and negative charges. It may have different hydrophilic and hydrophobic regions, because some of the amino acids or hydrophobic some of the amino acids are hydrophilic.

So, the solubility of these protein or affected by interaction between various surfaces regions know you have hydrophobic process portion hydrophilic portion. You have negative charge portion, you have positive charge portion. Then you have the water molecules surrounding it, so ideally you are disturbing these interactions. So, that the

proteins agglomerate and then they come out as a solid form and that is what you are doing in a precipitation, okay?

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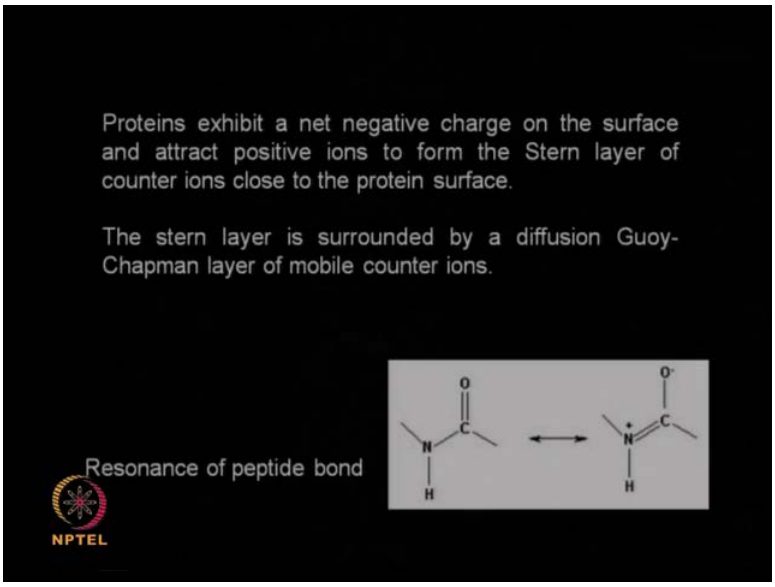

If you look at proteins they exhibit a negative charge on the surface and attract positive ions to forms stern layer of counter ions close to the protein. Why do proteins have charge? If you look at peptide bound you are know what peptide bound and the proteins are made of a load of a tides. So, the nitrogen carbon bound here, it is call the peptide bond and there is a theta bound C double bold O.

So, there is a resonance between this particular configuration and this particular configuration so when this double bond knows down here this nitrogen is going to acquire a positive charge and this oxygen is going to acquire negative charge so this type of resonance is going to give you a negative charge. Of course, it depends on a Ph if too much acidic Ph or too much basic Ph, this particular resonance is going to get disturbed. In the normal conditions, so you are going to have negative charge, so it is going to attract positive ions around it and that is called a stern layer, okay?

Now, on top of the stern layer there is counter ions, because we are having a positive ions, then you may have negative ions around that and that is called Guoy Chapman layer of mobile, counter ions. Those counter ions are mobile, because they are much for away from the protein, so you have the protein and then you have a stern layer and then

you have the Chapman layer, Guoy Chapman layer which are counter ions to the stern layer.

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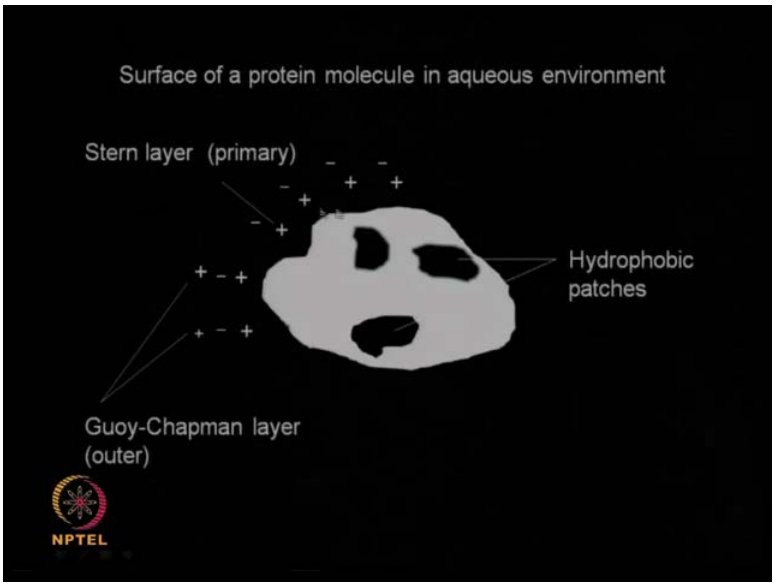

We can show it picture like this in a philic at a protein in an aqueous environment there are hydrophobic patches. Then you have O minuses indivarious places because of does O minuses you are going to have may be yester layer, a primary layer. Then on top of that you are going to have the counter ions much the Guoy Chapman layer much, the out layer.

So, if the protein is like this and there is another protein like that, so they are not going to interact merge if you disturb this layer and you make the proteins agglomerate with each other. Then it may form sufficient large size to molecules, which may then precipitate out of your solution. There are different ways by which you could disturb they these various charge layers sometimes we use Ph, sometimes we use a counter ions, sometimes we use salts and so on. Actually, we will look at each one of them in detail as we go along.

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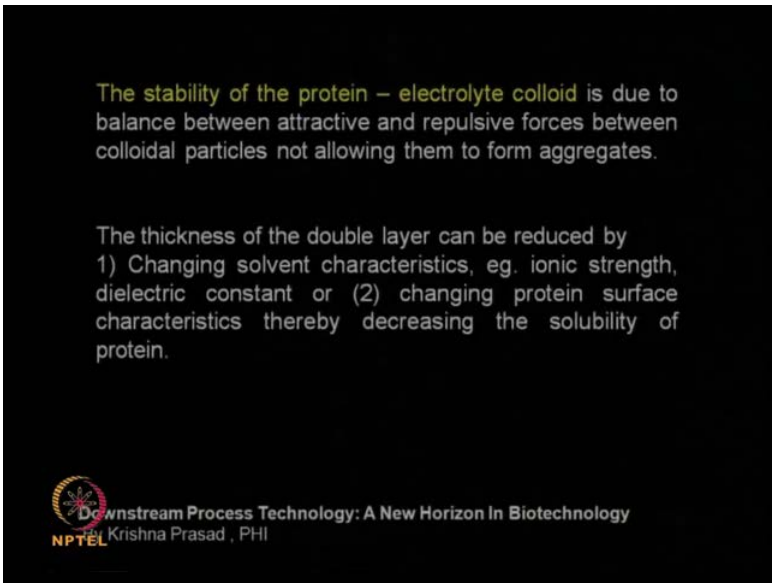

Now, the stability of the protein electrolyte colloid is due to balance between these attractive and repulsive forces between this. So, there is area force which is predating them to aggregate. Now, this thickness of the double layer can be reduced by changing solvent characteristics solvent characteristics could be ionic strength of the solvent the dielectric constant of the solvent or we can change the protein surface characteristics. So, by doing this we are decreasing the solubility of the protein.

So, by doing this we are bringing truth proteins together letting time agglomerate reduce the solubility. Hence, allow the protein to precipitate. If one is interested to know more about this various counter ions. Hence, layers you can look into his particular reference you know downstream process technology, new horizon in biotechnology by Krishna Prasad printer's hall publication.

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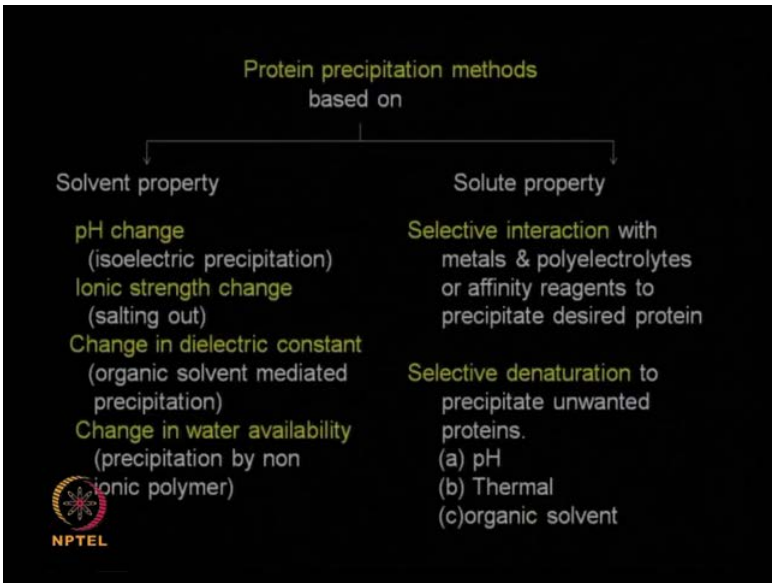

Now, protein precipitation is based on two things one is related to solvent one is related to the solute. Solute means it could be small molecule or it could be protein. So, on now solvent property Ph change, so for example, isoelectric precipitation. What we do? We change the Ph, so that the we reach the isoelectric Ph protein looses is charge, so that could be a precipitation. That is call the isoelectric precipitation. Next one cold be change in ionic strength; that is called in a salting out. What we do? We may add a salt, which is highly soluble and the salt get deserved protein become less soluble it is coming out next called a salting out.

Third approach change in the dielectric constant; that means I can use an organic solvent. I add that organic solvent, so the dielectric constant of the solution changes. So, what happen that protein precipitates out fourth approach change in water availability. I can add a non ionic polymer, so when I add a non ionic polymer into the solution protein precipitate out. So, what we are doing in this side the left hand side, we are changing the properties of the solvent. We are changing Ph, we are changing ion, we are changing dielectric constant, we are changing the availability of the water near the protean.

So, by doing all this we are letting the protein precipitate out. The other side the right hand side, we can change the properties of the protein, so that it precipitate out. Selective interaction, I can add metals all polyelectrolytes or affinity reagents, so that the proteins

get agglomerate and the precipitate out, that is call the selective interaction. Another approach selected de naturalization.

So, I have some unwanted protein. So, when I de nature the unwanted protein it precipitates out. How do I de nature? I can change the Ph when I change the Ph as you all no protein is active only in one selective region of Ph. So, if an, I change the Ph protein make a d nature and then you may allow the protein depreciative or thermal de naturalization.

Proteins are not vary stable at high temperatures, so when I increase the temperature then protein gets d natured and then it may precipitate. I can add organic solvent by adding an organic solvent you are allowing the provident to again get de nature and it get precipitates out.

So, this step of de naturalization it resolved to, if you are not interested in a particular protein. We cannot use this technique if you are interested in that protein, because the protein during the precipitation is completely de nature. So, if you want in active protein, then generally what we do? We use the solvent approach that mean change the property of the solvent.

Now, we can even think about adding need metals or polyelectrolytes or affinity reagents. So, that we can have a selective interaction with the desired protein that we never resolved to this type of de naturalization. That means increasing temperature or changing Ph or adding a toxic organic solvent, which will denature protein so each of the technique. We resold to depending to upon the cost fact depending upon the amount of the protein.

You are interested the stability of the protein, you are interested to remove and what are the other protein present in the broth or other metabolize present in the broth and so on. So, there is nothing like a numerical method, but generally in laboratory conditions, what we do? We use salting out method, actually simple you just add ammonium sulfate and which is highly soluble, so this protein get salting out.

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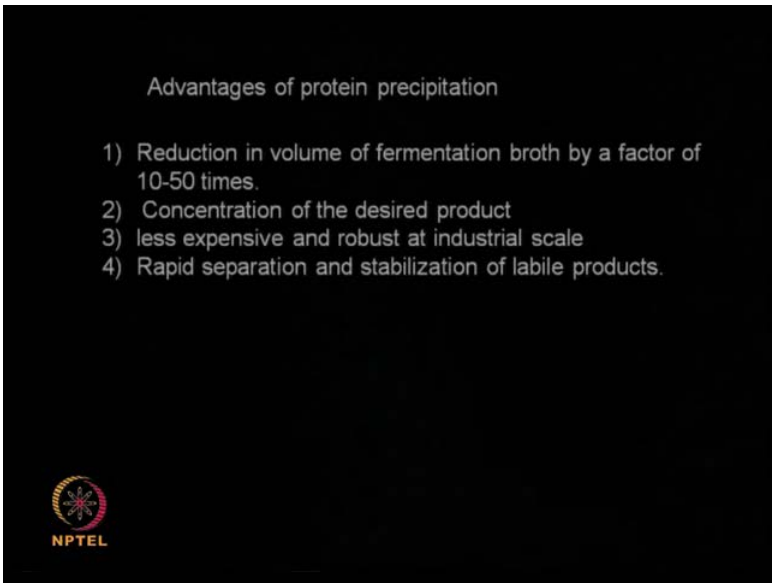

What was the advantages of protein precipitation? By doing this we are able to reduce the volume of broths by a factor of 10 to 50 times actually. So, instead of processing the hole broth by precipitating out. Then removing the supernatant then we can process rest of the impure protein and go about purifying the protein and so on so when we do protein purification we are concentrate in desire product.

As I originally said the protein after precipitation is not going to be 100 percent pure you may have to go further into purifications stages to really get it ready pure, but you are able to get concentrated product. Now, we can do different downstream approaches to purify further (( )) it is a less expensive technique very very expensive, very very less expensive and it is very robust in industrial scale, because ultimately we need to considers scaling up. We want to do it in 100 liter 1000 liter and so on.

So, when we do that, sought of large scale operations precipitation is a robust technique, which can be scaled up to any level. We can achieved rapid separase separation quickly precipitation happens and then you got a filtration. Then you can stabilize the labile product later, I told you. So, these are the advantages of provident precipitation, okay?

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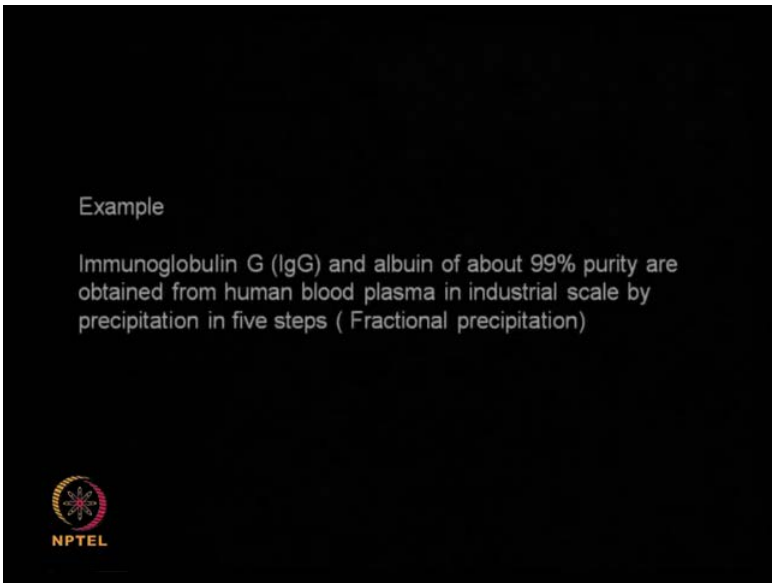

What example if you take the immunoglobulin G lgG and album of about 99 percent purity are obtained from human blood plasma in industrial scale by precipitation. It in five steps that mean we do on precipitation. Then again you precipitate like that you do it five times and then you are able to get almost 99 percent pure proteins from your human blood plasma in this. This is done in very large scale industrial scale without any problem. If you want to do any other separation means it may be very, very expensive.

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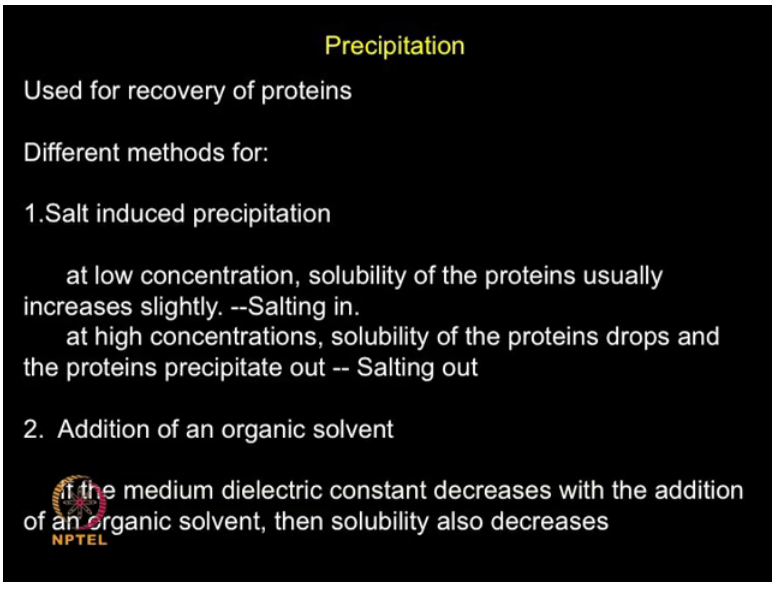

So, precipitation it is used for recovery of protein the methods, which we can reserved to for example, salt induce precipitation or addition of organic solvent. In salt induce precipitation, one interesting thing is this like salting or also you can have salting in at very low concentration of the salt solubility of the protein. Usually increases slightly no many add salt, because of the changes in the ionic strength sometimes the protein may solubelize more, but that is only initial stage.

So, you could have a salting in but at high concentration solubility of the protein drops dramatically and that protein precipitate out that is called a salting out. So, you can have a low concentration of the salt little bit of salting in and that high concentration is a salt you may have salting out addition of a organic solvent.

So, the medium dielectric decreases with the addition of organic solvent that means when I add a solvent and the dielectric constraint of the medium decreases. Then the solubility of the protein in that solvent also will decrease, so the protein will precipitate out. So, you need to select solvents, which will decrease the dielectric constant of the medium. So, we need to have some idea about a dielectric constant various solvents that can be added.

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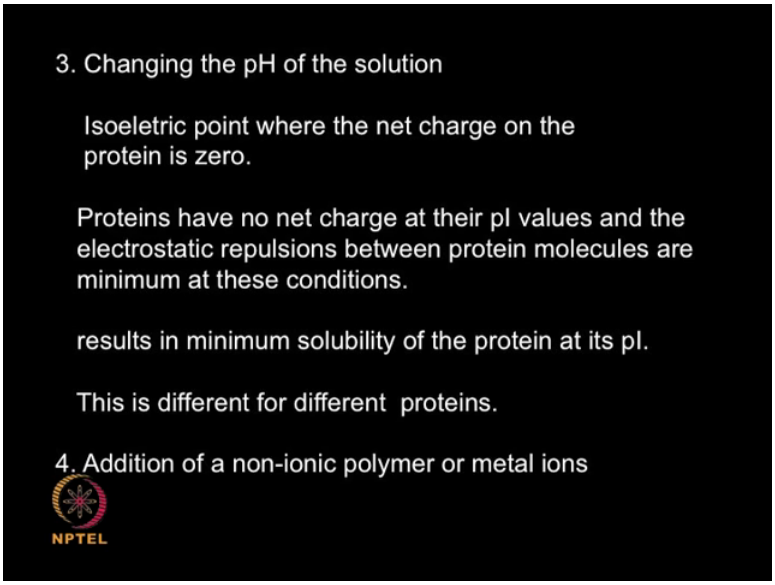

Of course most important point you do not want solvent which you are adding. So, de nature the protein, so you need to keep that point also it mind. Third approach changing the Ph we all know what is the isoelectric point that is the Ph at least recharge on the

protein is 0. That means protein will have no net charge at that p l values and electrostatic repulsions between the protein are minimum at these conditions, okay?

So, the solubility of the protein also is minimum at its p i and this p i values differ with different proteins for different types proteins. We will have different types p l values the next approach. We can do addition of a non ionic polymer or metal ions, we will talk about this also in detail. So, we can add a polymer which is not ionic or we can add a metal ion which will also change the properties of the protein and will allow the protein to precipitate out.

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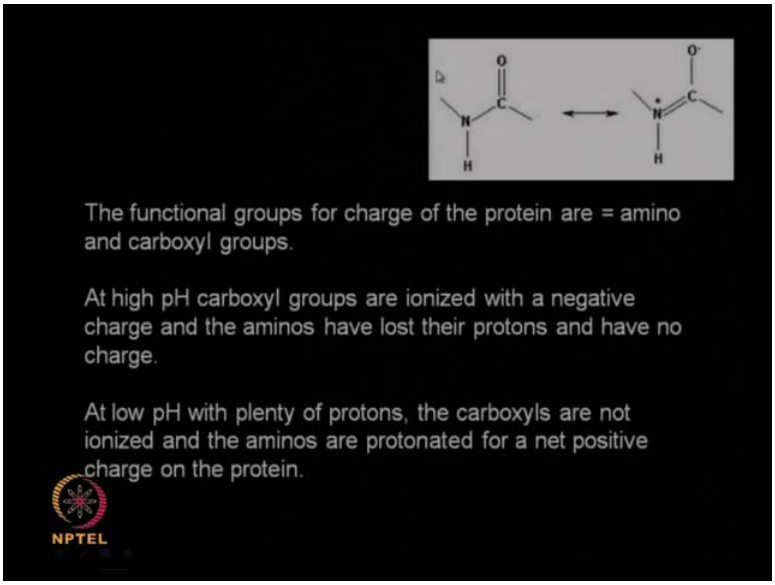

We look at the peptide bond once again we N C double bond o and because of the resonance we are going to have a N plus and a O minus, so this is in a resonansce stage. So, the nitrogen that is the amino side and the carboxyl pure they are going to give the charge. At high pH that means in alkaline conditions the carboxyl groups are ionized, you have a negative charge at the high pH, alkaline condition and ionized will have loss there protons there will not any charge.

So, at high pH generally will find O minus at low pH with plenty of protons low pH is accessory condition. So, you are going to have plenty of protons so what will happen? O minus will disappear, the carboxyl will not get ionized, but the aminos are not protonated so will have n plus, so there net positive charge on the protein. So, an acidic condition you will have net positive charge, because the O minus would have become neutral and

only the nitrogen will have positive charge. At high pH in the alkaline condition, what will happen? The positive charge on the nitrogen would have disappeared, because we are in alkaline condition and the O minus will remain. So, you will have a net negative charge, so pH will alter the charge on the surface of the protein.

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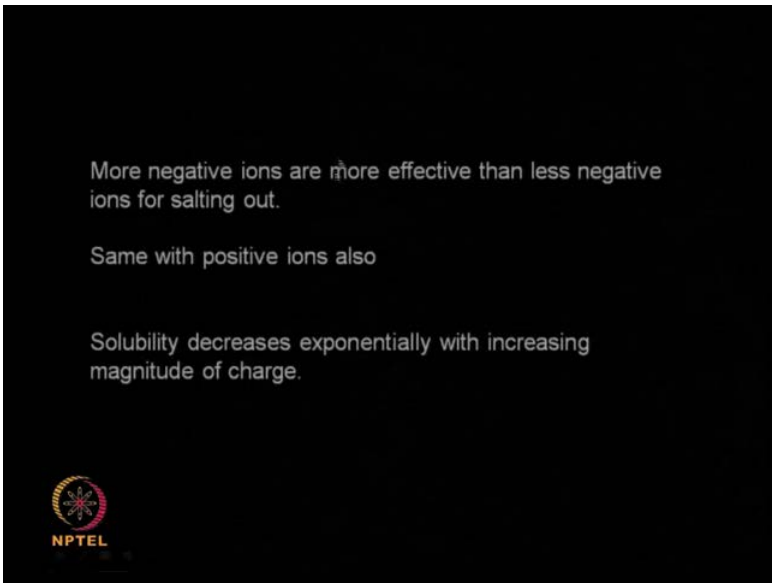

So, more negative ions are more effective than less negative ions for salting out. So, it is better to have more negative ions, then less negative ions for salting. Similarly, it is better to have more positive ions then less positive ions for salting out, okay? We look at a problem on that, so it is better to have more negative ion and less negative ion better to have more positive ion. Then less positive ion for salting out now solubility decreases exponentially decreasing magnitude of charge. So, if the charge more and more than the solubility will decrease in exponential fashion.

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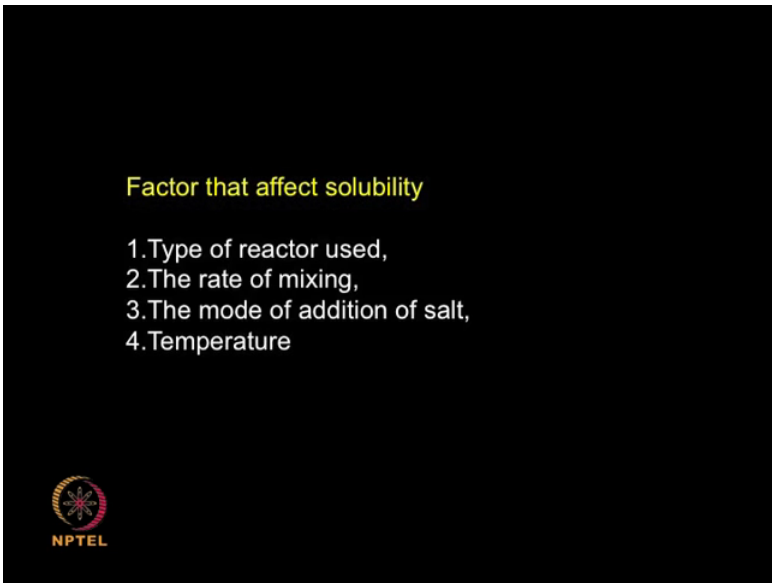

What are the other factors, which affects the solubility type of reactors using I am, using use centrically reactor or I am using tubal reactor and which more continuous removed rate of mixing? How fast hesitation is or how slow is hesitation? What is the length to the diameter to the reactor and so on? Mode of addition of salt, how I may adding? I may I adding a different lots or I may adding in one lot, I may injecting in different place in temperature as you know solubility is a function of temperature.

So, what temperature what I may operating I may operating at low temperature or I may operating at higher temperature. So, all these factor affecters solubility that means, I am designing a crystallizer. I need to consider all the aspects as well, so I am designing a solid liquid suppurating precipitation, I need to consider all these factors.

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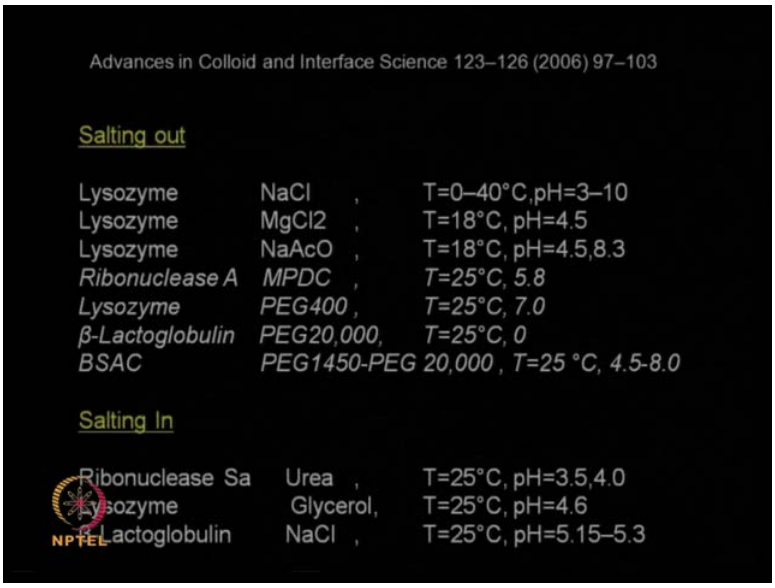

This is data taken from paper call advantages on colloid and interface signs two 2006 look at this the various salt one can think about for salting out proteins and various salts one can think about for salting in. You are also given the temperature and pH conditions for example, lysozyme. I can sodium chloride zero to forty degrees pH of 3 to 10 or I can use magnesium chloride or I can use sodium acetate.

Similarly, if I am interested salting out ribonoclease a, I can use some other salt. Here you see, I can use polyethylene glycol various of molecular weight PEG 400, PEG 20,000 and so on. For precipitating out or salting out lysozyme, bicto lactoglobulin so on.

Actually, so that temperature conditions also slightly varying pH conditions slightly varying. If I am interesting in salting in; that means allowing more protein to soluble eyes that also can be achieved. I can use glycerol and I can salt in lysozyme here and again sodium chloride salt in beta lactoglobulin. These are the conditions 20 25 degrees 4.6 and so on. So, and this particular table tell you what are the various salts that can be used for salting out or salting in, okay?

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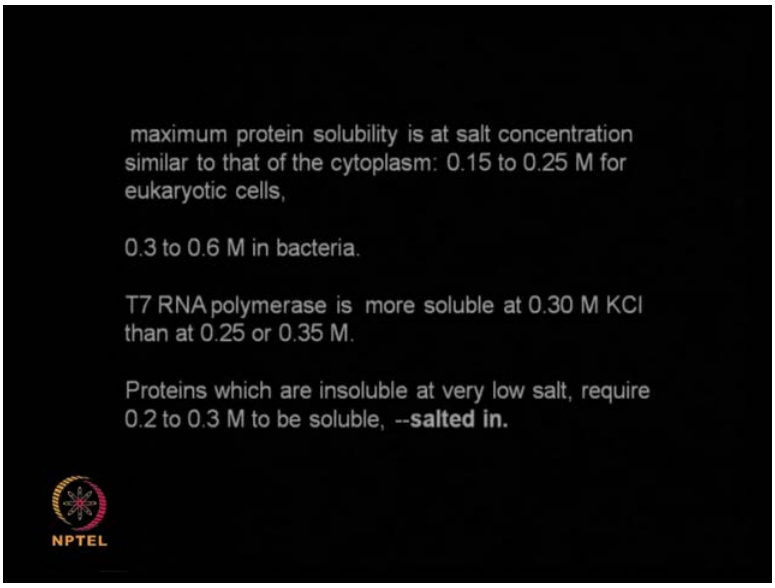

For example, if you look at various proteins maximum proteins solubility's at a salt concentration similar to that of the cytoplasm; that is 0.15 to 0.5 molar for eukaryotic cells and 0.3 to 0.6 molar for bacteria. RNA polymerase is more soluble at 0.3 molar k c l than at 0.25 or 0.35. So, we need a ready optimum concentration of KCL to the solubility of this T 7 RNA polymer.

Protein which are insoluble at very low salt concentration may require 0.2 to 0.3 molar to be soluble, this is more like a salting in type of approach. So, you see this particular slide gives you bulk park figure of the concentration of a the salt require to achieve other salting out or salting in, okay?

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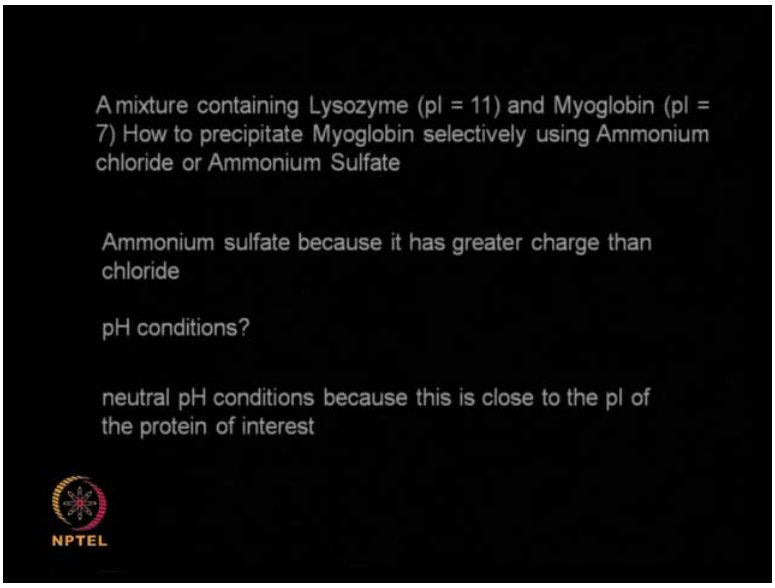

Let us look at a very simple problem. So, I have two proteins lysozyme, myglobin; both have different isoelectric pH. You all know what is isoelectric pH? That is the pH at which charge the charge is 0, so lysozyme de isoelectric pH is 11. Myoglobin isoelectric pH is 7, how to precipitate myoglobin selectively using ammonium chloride or ammonium sulfate? So, which one is better, ammonium chloride or ammonium sulfate? Ammonium sulfate is better, because it has got greater charge. Then chloride we just saw couple slides back is better to have higher negative charge.

Then lower negative charge it better to have higher positive charge. Then lower positive charge, so ammonium sulfate is better than ammonium chloride, okay? What is the pH condition which is which pH do you like to operate at? Neutral pH because it is closer to the p i form myoglobin. This is a protein of our interest and not this because the problem says how to precipitate myoglobin selectively, okay?

So, if I have mixture of lysozyme and myoglobin, I would use ammonium sulfate and I will work around pH of 7, understand? So, we need to look at the charge on the salt and select, we need to look at the p l value for the protein and select the operating pH. Of course, in some times the m resolved cost factors may come in to picture, but that is later, but if you just consider only the physical principles then this how you approach this problem.

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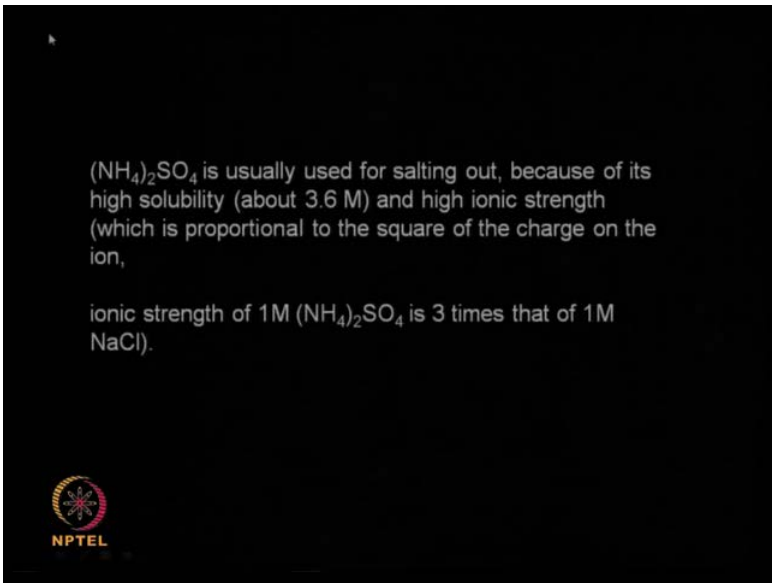

Why do you generally use ammonium sulfate? In our labs we told take ammonium sulfate, because of it high solubility. It is highly soluble in water about 3.6 molar and high ionic strength which is proportional to the square of the charge on the ion, right? So, ion is strength of 1 molar ammonium sulfate is 3 times that of 1 molar of sodium chloride.

So, it is always better to use ammonium sulfate than sodium chloride this not two times, but it is 3 times, because we, I also talk to that it is not directly propositional to the charge. But it will be much more exponential so in most of the cases that is why it reserved to ammonium sulfate as the regent for salting out proteins.

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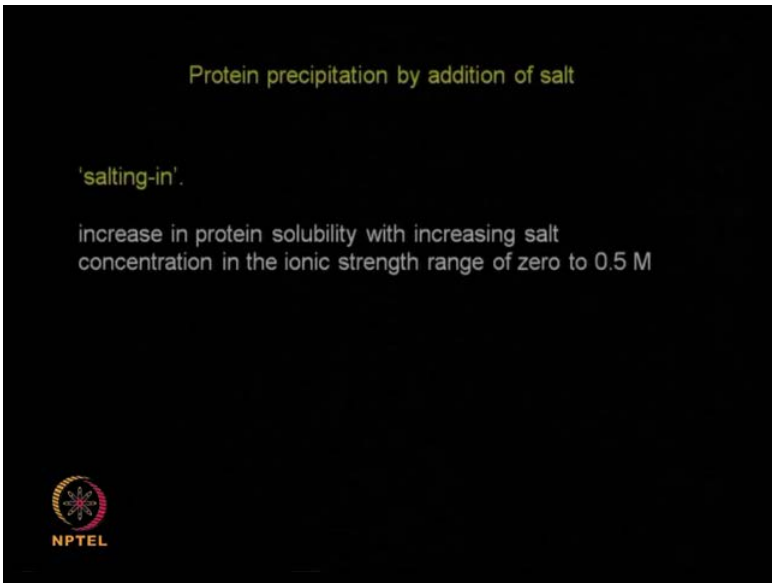

So, if I want increase protein solubility with increasing salt concentration. Generally it happen that very low salt concentration zero to point five molar ready lower concentration.

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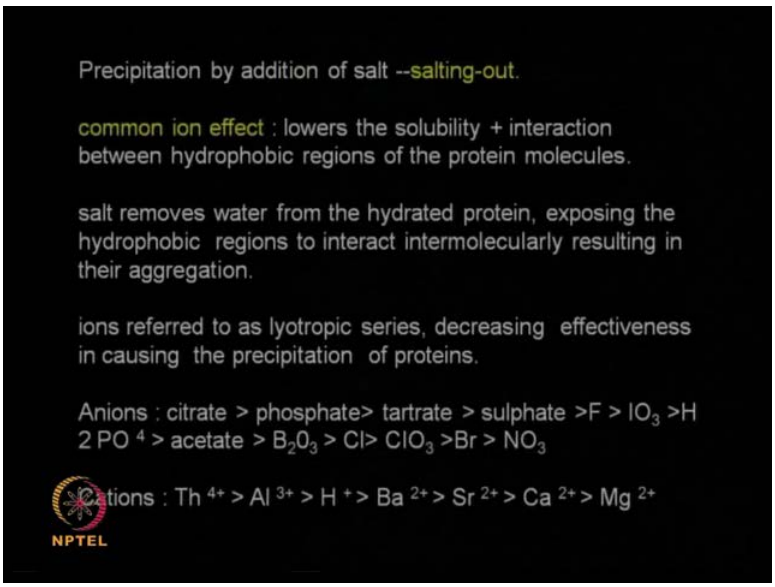

So, for salting out so what is the principle there is something called common ion effect must have studied that longed in back? So, in common ion effects we have lower in the solubility interaction between hydrophobic regions of the protein salt removes water from that hydrated protein. So, it is exposing the hydrophobic regions so the hydrophobic

region interact. Then the aggregate hence the solubility goes down in hence they come out as solubility.

So, ions refer to as lyotropic series it is decreasing effectiveness in causing the precipitation of proteins. Suppose I take anions citrate greater that phosphate greater than tartrate, greater than sulphate, greater than fluoride and so on. You take the cations, then thorium, aluminum h plus, barium 2 plus write down to calcium to magnesium. So, they come mater the level tropic serious. It is decreasing effectiveness in causing the precipitation of the…

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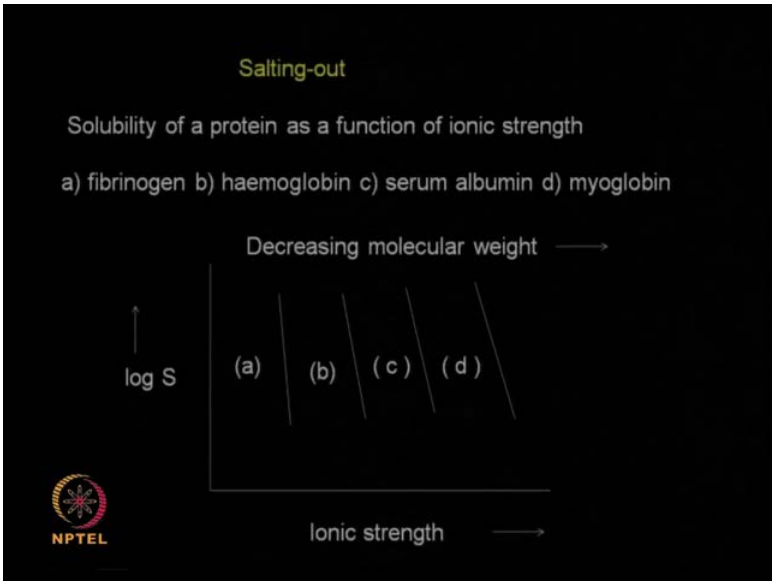

So, if you look at solubility of a protein as a function of ionic strength, so you may have graph like this; that is solubility with increasing an a strength. This could be for fibrinogen this may be for hemoglobin. This could be for serum albumin, this could be for myoglobin. So, decreasing molecular weight, so the molecular weight is very, very large. Then it is match is precipitate out where compare to very, very small molecular weight. So, molecular weight also takes place in the precipitation process.

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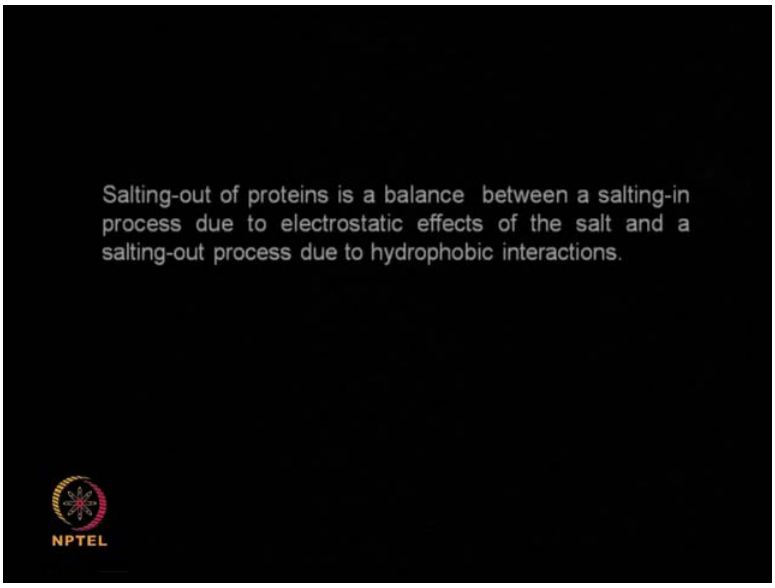

So, basically salting out of protein is balance between salting out process due to electrostatic effects and salting out process, due to hydrophobic interaction. So, both player very important role electrostatic verses hydrophobic interactions and at very high concentration of the salt protein just precipitate out.

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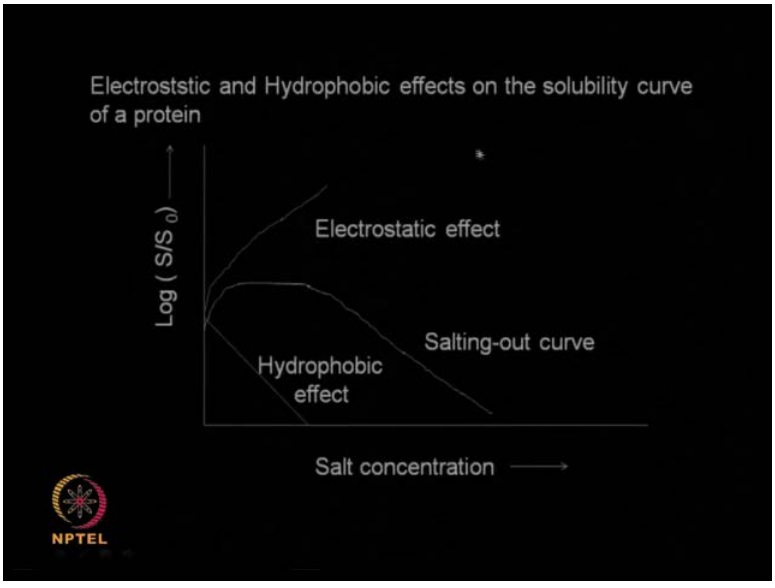

So, you may have a salt concentration is increased electrostatic force is increasing as salt concentration. You may have hydrophobic effect so both may be combining together to give you m salting out. So, initially solubility will increase and then solubility will

decrease that is what very, very low concentration 0.3 molar or around that any region the addition of salt will allow the protein to solubilize higher. Now, that is why you will generally have a graph like this it may be very, very close to you to the left hand side. But still, you will have little bit of salting in taking place depending upon the type of salt which we are using.

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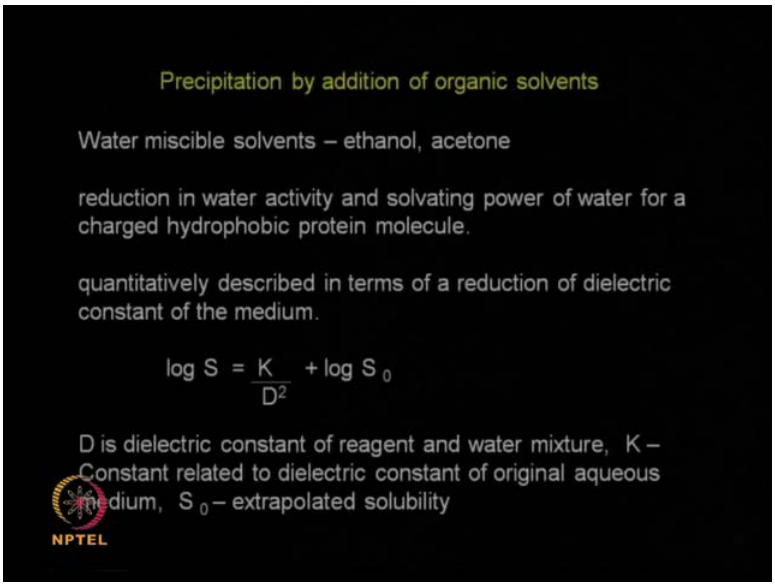

So, if you take another method that is precipitation by the addition of organic solvents. So, we can consider organic solvent like ethanol acetone and so on. So, by adding this solvents, we are changing the dielectric constant by changing the dielectric constant the protein solubility goes down. I mention very early, lower the dielectric constant lower will be the solubility, hence the protein will precipitate out.

So, what I am doing? When you are adding this type of organic solvents reduction in water activity and solvating power of water for charged hydrophobic protein molecule. So, it is described this way log S solubility equal to K by D squire plus log S naught D is the dielectric constant of regent and water K is some constant related to dielectric constant of original aqueous S naught is the extrapolated solubility. So, if I keep increasing D, I will be decreasing the solubility.

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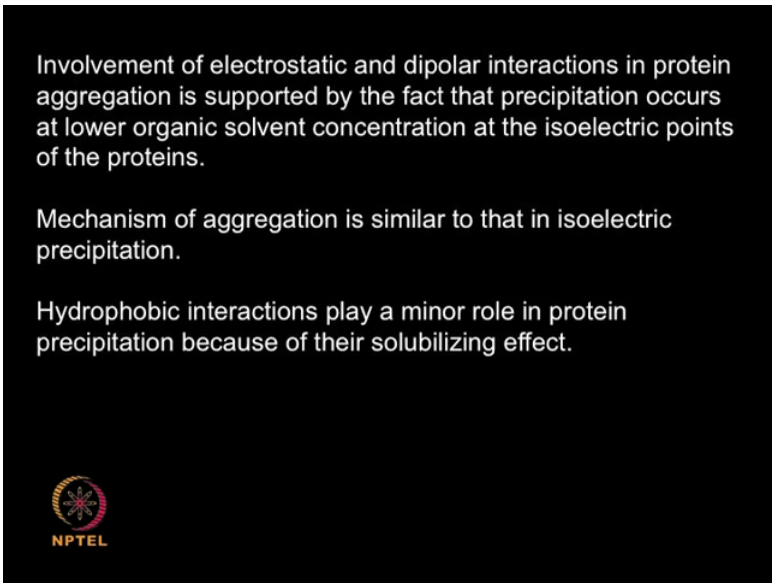

So, involvement of electrostatic forces dipolar interaction protein aggregation is supported by the fact that precipitation occurs at lower organic solvent concentration at said the isoelectric points of the protein. So, the mechanism of the aggregation is similar to that in isoelectric precipitation. So, hydrophobic interactions play a minor role in protein precipitation because of their solubilizing effect here.

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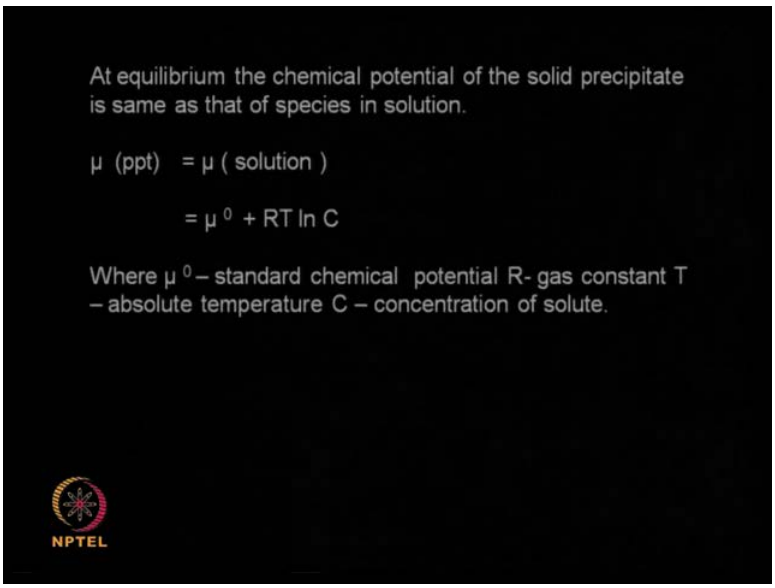

Now, at equilibrium the chemical potential of the solid precipitate and is solute which in the solution have to the equal rate mu precipitate equal to mu in solution. Now, mu in

solution can be given not like that mu not plus R T lon C R is your gas constant T is your temperature mu naught is the standard chemical potential and C is your concentration at the concentration is very, very low. We can call this equation is the RTC if you remember rant of equation. So, the chemical potential of the solute in the solid phase is equal to the chemical potential of the solute in the dissolved phase, so this is at equilibrium.

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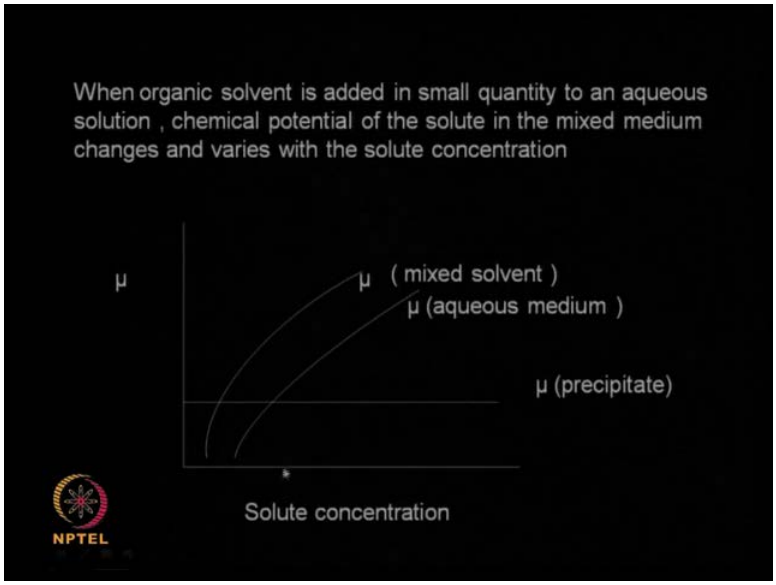

So, if you have solute concentration and mu that is the chemical potential and organic solvent is added in small quantity to an aqueous solution chemical potential of the solute in the mixed medium changes and varies with the solute concentration. So, you have the aqueous medium and then you have the mixed solvent as the solute concentration increases. But the chemical potential of the precipitate is always constant, because it is a solid material actually. So, if you look at the chemical potential diagram, this is how if you look like.

So, for the aqueous medium this is the chemical potential graph as a function of solute concentration, when you are adding a solvent the chemical potential in the mixed medium of the solute in the mixed medium in the changes. That also will have this type of this shape, whereas for the solid precipitate chemical potential will remain constant. That is why it is parallel to the x axes.

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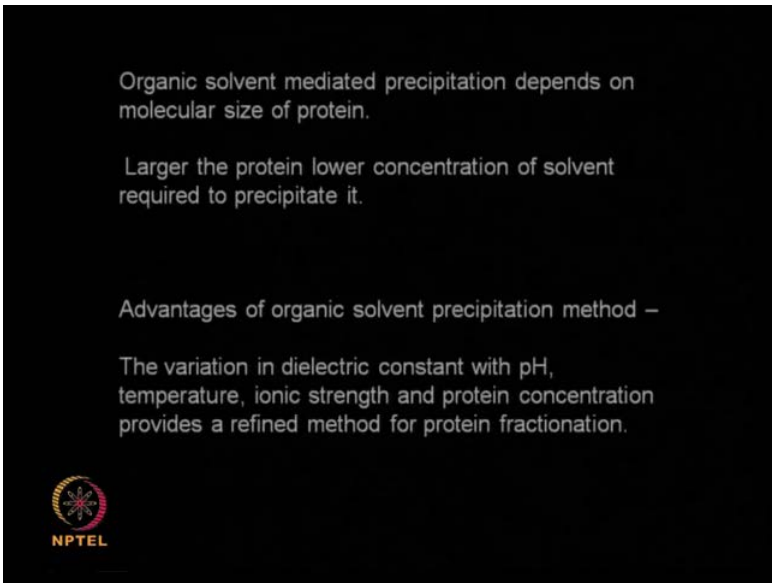

So, when you are using organic solvent to precipitate precipitation depends on the molecular size of the protein. So, if the protein is larger, then I will required lower concentration solvent to precipitate out. So, if the precipitate if the protein is smaller, then I will required more quantity of the solvent. So, it is very ideal for very large proteins know you just have to add little solvent and the protein precipitate out. So, what are the advantages quit a lot the variation in dielectric constant with pH temperature ionic strength. Protein concentration provides a refined method for protein fractionation, so that is main advantages of this type of technique.

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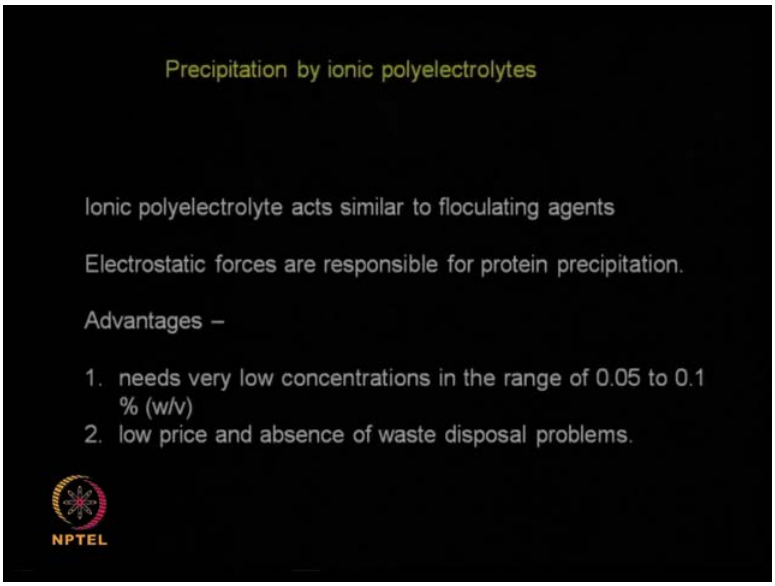

Another method by which by can achieve protein precipitation is adding ionic polyelectrolyte. So, it is almost like flocculating agent what does the flocculating agent do. Suppose, you have suspended solids they are not settling down because they are very lied.

They are not agglomerating because there net charge, which are repelling each other so we add something called polyelectrolyte, which will disturb the charges or neutralize the charges. So, what will happen those flouting flatulence flocculating. They will comes together and they forms flogs and they form flogs weight will be higher than, they means settle down that is the process called flocculation.

So same principle resolved to in this particular approach where you are adding ionic polyelectrolytes. So, electrostatic forces are responsible for protein precipitation, so when you add ionic polyelectrolyte, you are disturbing this un allowing the proteins to come to each other. Then settle down, so we will require very lower concentration of polyelectrolyte very small 0.05 to 0.1 percent, low price sometimes.

We can use very cheap polyelectrolytes to achieve this type of suppuration. There are no waste disposal problem also know, because you add the polyelectrolyte protein precipitates. Then we do not need to add some other some other chemical, because the quality you are adding very less.

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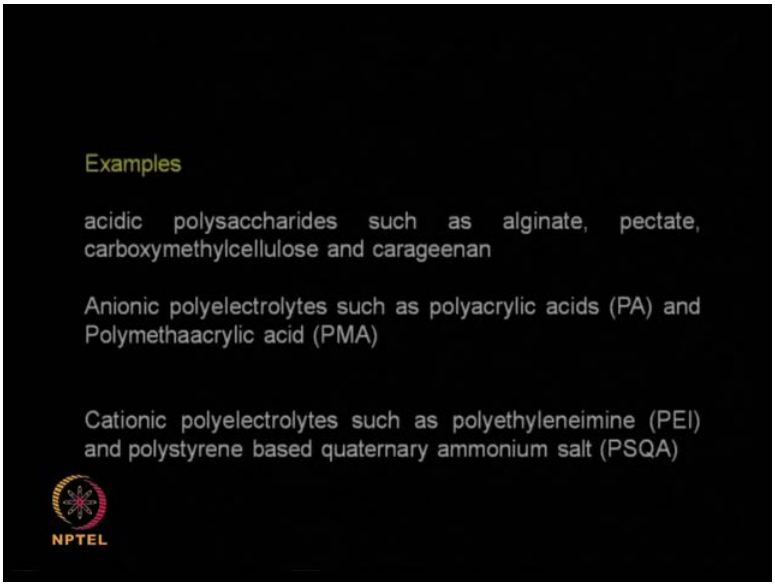

For example, we can add alginate pectate, carboxymethylcellulose, carageenan all these are polyelectrolytes. They are called acidic polysaccharides, they can help in the precipitates. We can also add ionic polyelectrolytes, such as polyacrylic acid or polymethaacrylic acid. We can even add cationic polyelectrolytes, such as polyethyleneimine bolystyrene based quaternary ammonium salt.

So, all these are cationic material and polyacrylic acid or pollymethaacrylic acid are ionic material or we can add acidic neutral material like alginate or carboxyl methyl cellulose and so on. All these are electrolytes, which will help in the flocculation of the protein and allow it to settle down. The quantity required here may be very, very less and compared to salting in where you may have to add large amount of ammonium sulfate.

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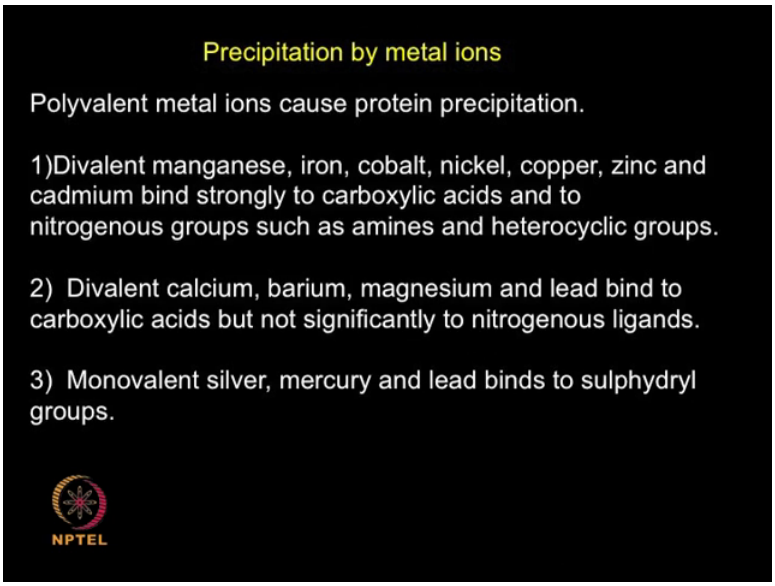

You can also do this precipitation by using metal ions. For example, you can use polyvalent metal ions which will help in the aggregation and settling down of the protein molecules. For example, if I use divalent manganese iron cobalt nickel copper zinc cadmium, there will bind strongly to the carboxylic acids you have a O minus right. Sometimes they will also add bind to the nitrogen groups in the amine and heterocyclic groups divalent calcium barium magnesium and lead bind to carboxylic acid.

But not significantly to the nitrogenous ligands monovalent silver mercury and lead binds to sulfhydryl compound or groups. So, we can consider radius polyvalent that

means divalent or monovalent mettles, which will selectively go and bind to the O minus or the N plus regents. Then it will resort to the precipitation of the protein.

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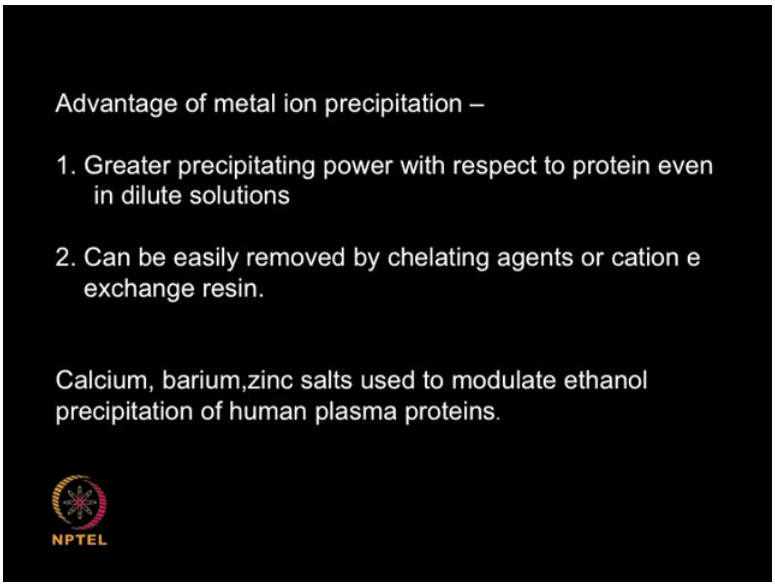

What are the advantages? They have greater power precipitating even dilute protein solutions. I am can be process using this metal ion precipitation, so can be easily removed by chelating agents or cation exchange resin. So, once the metals go and bind to this places when I can use ion exchange column and the protein. It is removed calcium barium zinc salts used to modulate ethanol precipitation of human plasma proteins. These are good examples of where metals are used moving a human plasma protein.

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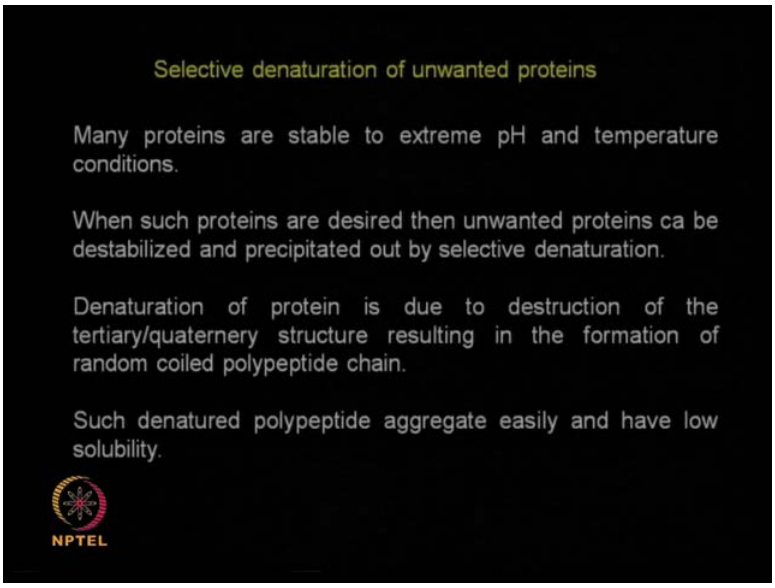

Selective denaturation of unwanted protein, so if you are not interested in certain protein you want it to be removed it is not getting removed, because of the ionic charges and electrostatic charges. What you do you denature the protein? So, we can, it is de natured it will dust precipitate out.

So, this is good if you are not interested in the product where as whatever I talk before, if you are interested in the particular protein. So, for example, I have some proteins, which are stable that extreme pH and temperature there are impurity proteins, which are very sensitive to pH and temperature.

Then I can remove those impurity protein by changing the temperature or pH. They where as the protein of my interest, which is very stable will un alter. So, that is called selective denaturation, so denaturation of protein is due to destruction of the tertiarylaquaternery structure resulting in the formation of random coiled polypeptide chain.

So, it is a no more a protein it is a polypeptide chine. So, what happens this changes aggregate one they aggregate, they are less soluble. So, the easily come out or easily precipitate out of the solution.

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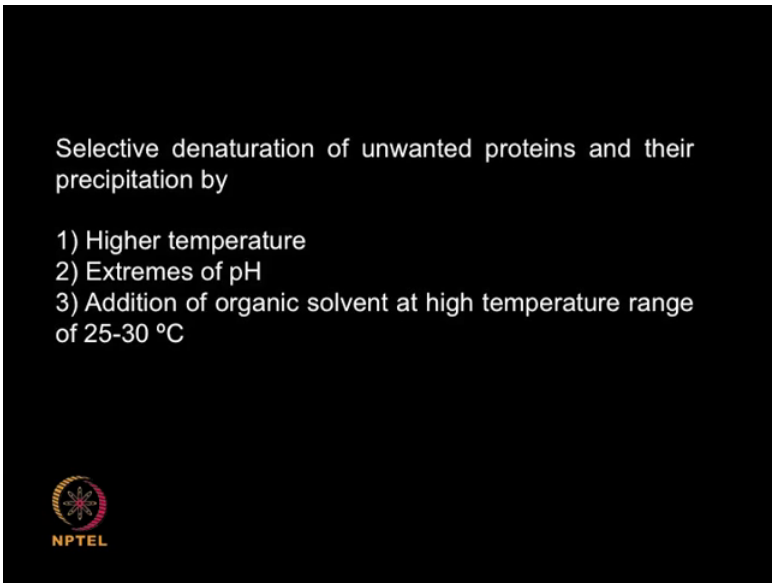

So, how do I denature? I can use higher temperature, so as I increase temperature the activity of the protein will go down. It will form, it will follow nurange relation. So, the once the activity goes down it is become like a polypeptide and it will aggregate. It will precipitate out, I can use extreme pH as you all know proteins have stable only at a range of pH when I change the pH beyond this. Protein will get denature addition of organic solvent at high temperature range at 25 to 30, so I can add solvent which will denature proteins.

So, there are certain solvents which may remove the water of water surrounding this protein it may disturb the coiled structure. The cashmere or quaternary structure there by the protein get denature, so all the techniques can be resorted to, but you are to be very care full in using this techniques bit, because they may affect the protein of your interest also.

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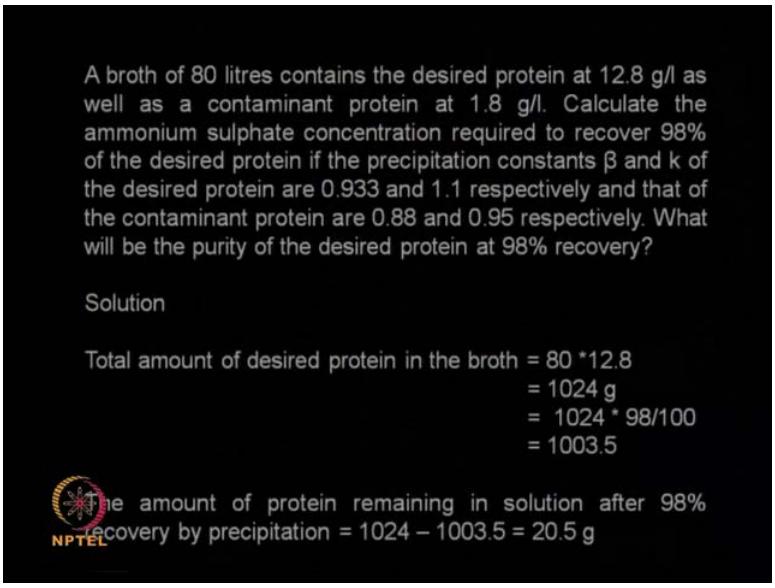

For example, let us look at a simple problem a broth 80 liters. It contains the desired protein at 12.8 grams per liter as well as another protein. This protein I am not interest, but it is present as 1.8 grams per liter. Now, calculate the ammonium sulfate concentration required to recover 98 percent of the desired protein. That is this protein, if the precipitation constants beta and K of the desired protein are 0.9333 and 1.1 respectively and that of the contaminant protein are 0.88 and 0.95 respectively.

What will be the purity of the desired protein at 98 percent recover, now total amount of the desired protein in the broth 80 liters into 12.8 correct? 10 24 I am interested in recovering in 98 percent; that means 98 by 100 into 10 24 that gives you 1003.5. So, what is amount of protein remaining in the solution 10 24 minus 1003.5 that gives you 20.5 grams, so this much amount of proteins left behind, correct?

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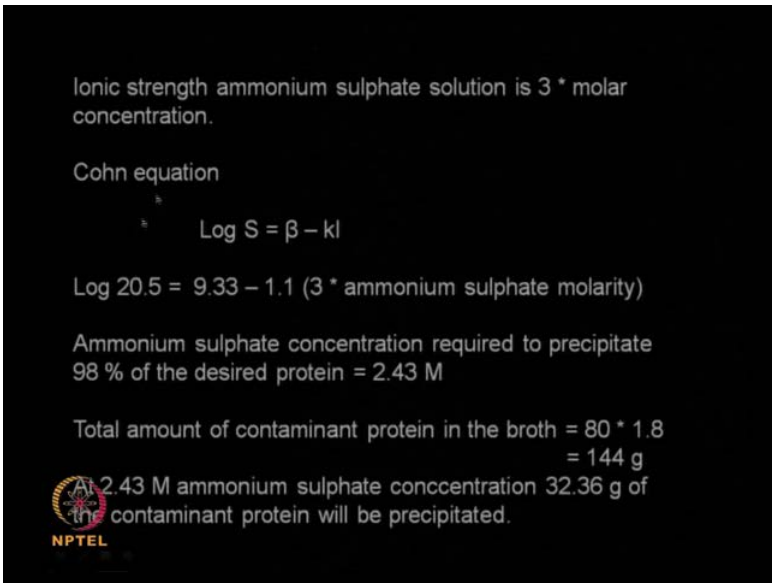

Now, ionic strength of ammonium sulfate solution is 3 molar concentration. Now, the Cohn equation, there is an equation called Cohn equation, which relates there solubility's and the ionic strength in this fashion. Log S equal to beta minus K into I beta is a constant, K is a constant, S is the solubility and I is the ionic strength.

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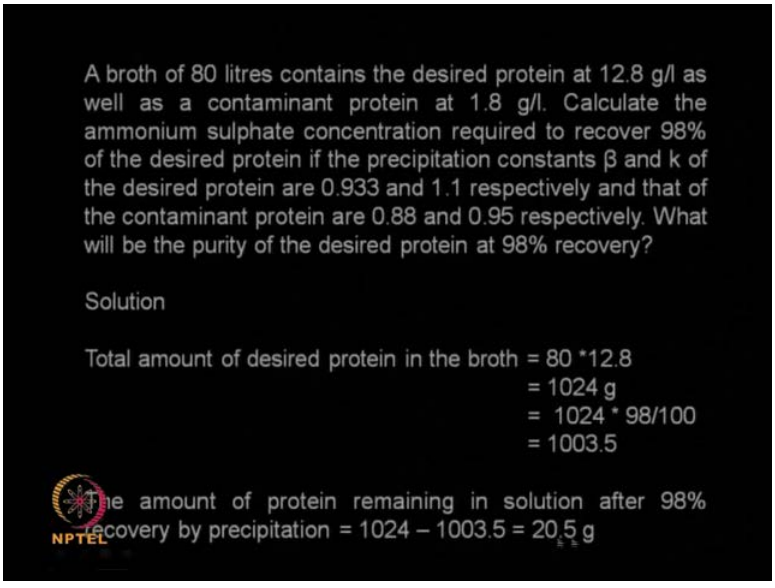

Now, I put 20.5 that is the amount of protein that is left behind in the solution, I am recovery 98 percent, so 2 percent is left behind.

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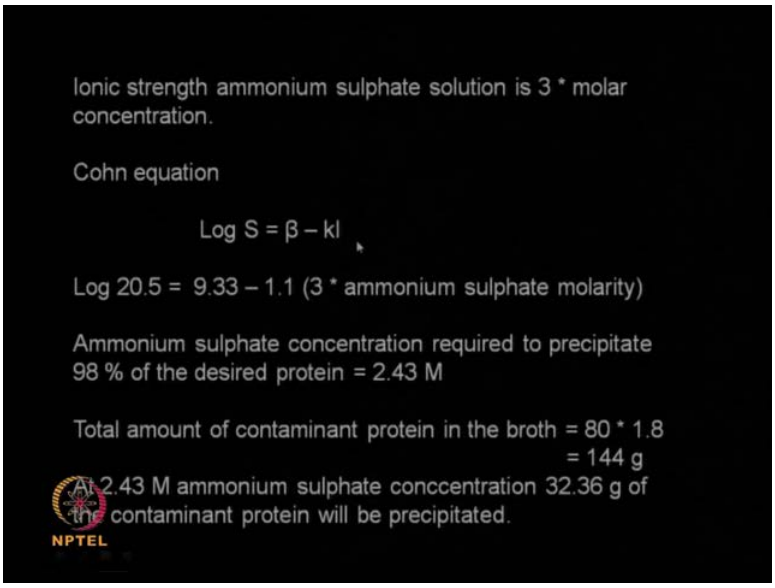

I is the ionic strength of a ammonium sulfate, which I am adding that is about 3 molar concentration, okay? So, ammonium sulfate 3 the K is 1.1 and the concentration of ammonium sulfate. So, I can calculate so ammonium sulfate concentration required to precipitate 98 percent of the desired protein will be 2.43 molar. Now, total amount of contaminant protein in the broth, it is how much? 1.8 eight grams per liter and I am starting with 80, so 80 into 1.8 is 144 grams. At 2.43 molar ammonium sulfate concentration 32.36 gram of contaminant protein also will precipitate out, okay?

So, the product desired protein when it precipitate out; that means desired protein so much 1003.5 grams is precipitating out, I will also have about 32.36 grams of the contaminant protein also precipitating out. So, as I originally said protein precipitating out is not going to give you a very, very pure solid product. What you said very good method to the sought to at the early stages of downstream.

Press crystallization can give you a very pure solid product. So, this particular problem tells you something. There is equation called Cohn's equation, which gives you some idea between the solubility and the ionic strength. This is used for salting out technique and these are constants the beta and K, so log S is directly related to the ionic strength. So, log S and ionic strength are linearly related. If once I know beta and K if I, if I know the ionic strength, I can calculate what is the amount of protein is left behind after

precipitation or if I know what is the amount of protein left behind after precipitation, I can calculate the ionic strength.

So, I can do both these way or that way, so if I have the details for beta and K for radius proteins. I can calculate how much concentration of the salt needed to precipitate out 98 or 99 percent of the protein that is present originally.

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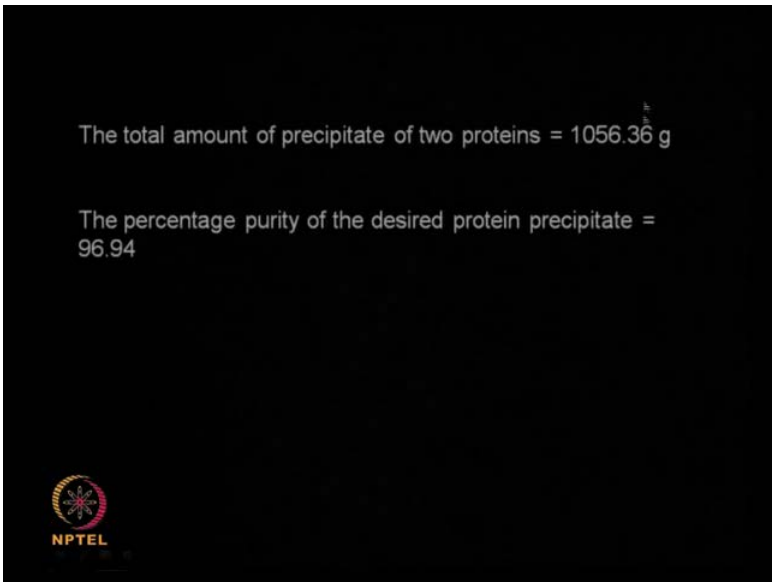

So, the total amount of precipitate of both the proteins, so I need to add the initial protein amount the second protein amount to get the total amount. In the percentage of the purity of the desired protein in the precipitate, obviously it is having certain in purity 32.36 grams the desired protein is 1003.5 grams, so that will give you the percentage purity. So, kind a simple problem, but this particular relationship is necessary these values beta and K R also necessary for you to calculate the concentration of this ammonium sulfate requirement.

So, this problem gives you nice idea about concentration of salt that is required to precipitate 1 protein. When I say one protein if there is another protein, you cannot that the guarantee that the second protein will not precipitate out, because second protein also will be losing it is some of solubility when the first protein loses its solubility. When that happens the second protein also will be partially precipitating out and hence you will always have little bit of impurity in the solid that is coming out as the precipitate out.

Now, one can do here solubilization and removal of the un wanted protein and so on. So, that is further downstream, but when you do salting out you cannot expect the 100 percent pure protein. If you have mixture of proteins present in your original prop, but if you have only 1 protein, then you will be ending a big one single solid protein; that is sure. So, the precipitation as you can see is a quit a simple method, we can use it in the early stages of downstream.

We can change the properties of the solution, we can change the properties of the protein. How do we change the properties of the solution? We can do things like changing the isoelectric pH, we can change the dielectric constant, we can change the ionic strength and so many different approaches.

By doing this we are doing the precipitation or we can change the attractive forces between the protein by adding metal ions or we can add polyelectrolytes. So, this disturbs the electrostatic forces and those type of methods or also very good in precipitating out your protein the another method, where if you are not interested in the protein, but you want to remove it as in purity. We can denature the protein, so how do you denature the protein drastically changing its p h or drastically changing temperature or adding a solvent so it will denature the protein?

Once the protein becomes denature loses, its structure it becomes a polypeptide. It will agglomerate and just come out as a precipitate. So, all these methods can used for protein precipitation and one important point you need to keep in mind is during precipitation. You do not to want protein to lose its activity that means, you do not want the protein to lose its 3 dimensional structure. The folded structure, which is most important parameter, which mentions it activity, so that particular point need to be kept in mind, when one is designing in a protein precipitation technique.