Liquid-Liquid Extraction
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Lecture - 19
Liquid-Liquid Extraction

Let us continue with the Liquid- Liquid Extraction. Generally, in Liquid- Liquid Extraction we use a solvent to extract the desired solute. But there are situations where you would like to use water instead of an organic solvent. For example; if you want to recover proteins. Proteins may denature in organic solvents. So, you would like to use water there would be situations where you may like to transfer from 1 water phase to another water phase. So, again you are talking about ((Refer Time: 00:40)) so, there are certain different strategies where you adopt when water is also present either as a solvent or as a both the heavies as well as the lights.

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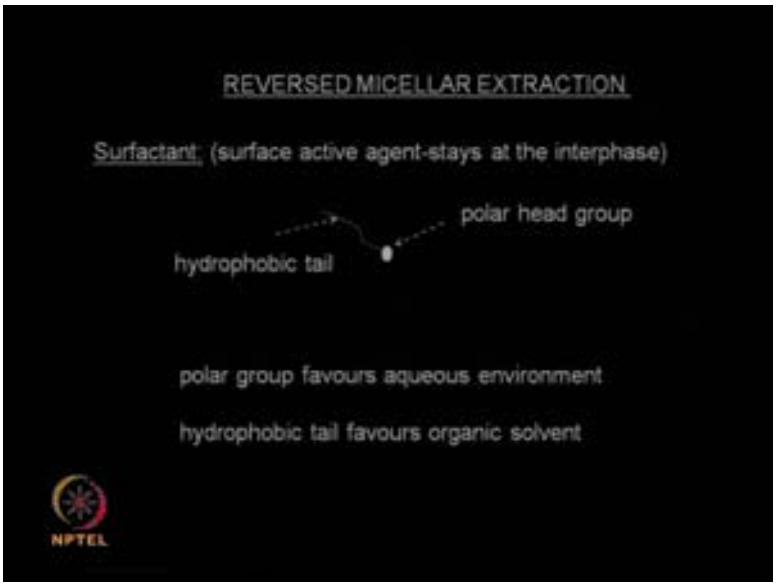

One of the important Liquid- Liquid Extraction techniques is called the revered micellar extraction. Let us look at what is a micelle? Before that we will look at what is a surfactant or a surface active agent? Surface active agent it always stays at the inter phase because you have a water layer and you have a solvent layer. So, the inter phase is between the water and the solvent layer. The surfactant has a polar head group. A polar head group could be a oh or it could be a nitrogen or could be some sort of an ion and

then it will have a hydrophobic tail. So, hydrophobic tail could be a hydro carbon type of a small c 8, hydrocarbon it could c 12, it could be even c 18. There are surfactants which have very very long a hydrophobic tails.

So, hydrophobic groups like prefer solvent layer and the hydrophilic or the polar groups prefer the water layer. So, if you have a inter phase, that means; if you have an organic solvent and water and you put in surfactant the polar head group will always go towards the water layer, whereas; the hydrophobic tail as we call it because it is a long chain hydrocarbon we call it a tail this will always go the organic a layer, that is; how a surfactant partitions at the inter phase.

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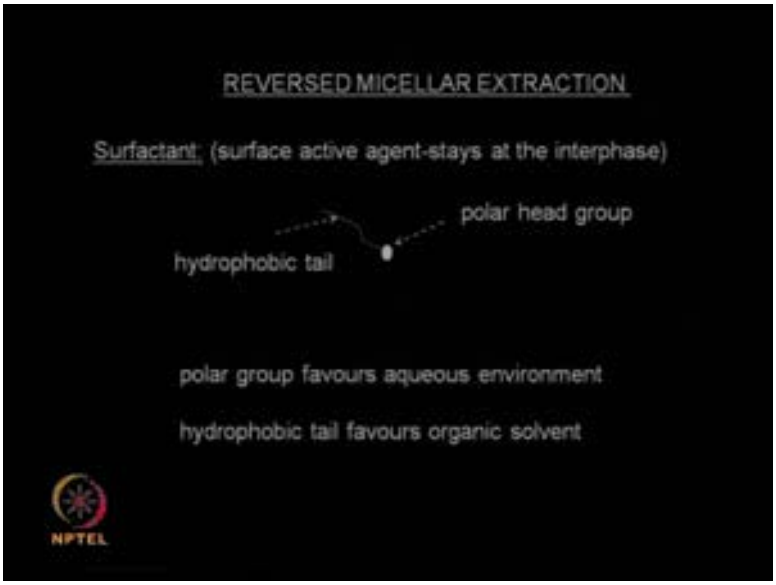

Now, what happens? Suppose I have a water and protein and there is surfactant. Now, I add a solvent what will happen? The surfactants will spontaneously aggregate in the organic solvent to form something called reverse micelle. What is a reverse micelle? If you look here you have all the polar head groups inside, that is; if they are away from the solvent which is outside. And, all the hydrocarbon portion is in the solvent region. So, inside region is like a small cage which is highly hydrophilic, that means; there will be water inside and if there are any water soluble solutes, proteins all of them will be inside. Because it is a hydrophilic region inside outside is hydrophobic that is why it is called a reverse micelle?

Here, the polar head groups are inside buried inside whereas; the hydrocarbon chains are outside in the solvent layer. So, this is what is called a reverse micelle? So, where do you use reverse micelle? Reverse micelles are very very important. If we have a protein which is water soluble and we want to barrier it or we want it to be kept away from an organic solvent. Because organic solvents may denature you are protein.

So, you want to keep it away from an organic solvent then we may use a concept of reverse micelle. Now, what happens? You have the solvent outside, you have the surfactant which is forming a reverse micelle, you have a polar region inside, where you may have water you may have buffer, that means; salts present and you are protein also present inside. So, that is what is a reverse micelle? So, we can use this concept of reverse micelle in extraction especially; for solvents sensitive proteins, that means; proteins which may get denatured because of the solvent.

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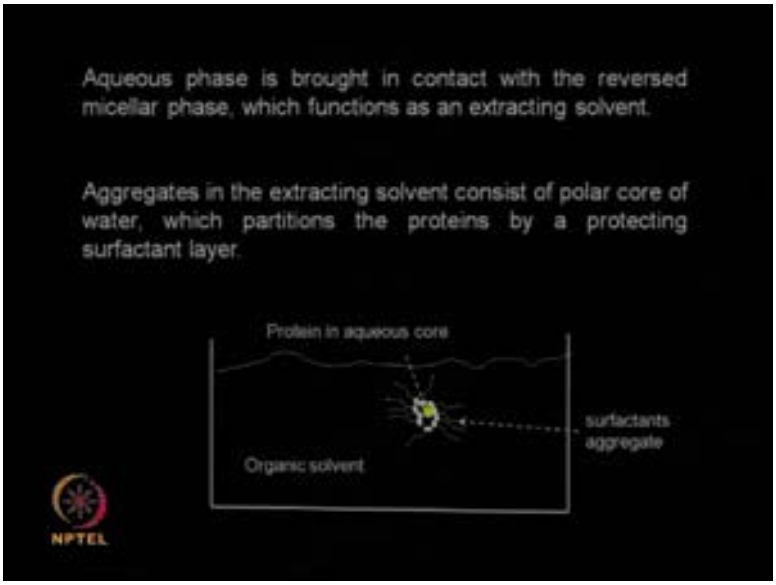

So, we have as I said inside before we have the organic solvent, we have the reverse surfactant aggregates the polar head groups are located inside and the hydro phobic tails are pointing outside. So, inside you may have a core which is the aqueous. So, the protein can be kept inside. So, you are protecting you are protein. So, you can have many surfactants aggregates like that present inside the organic solvent. And, each surfactant aggregate will have the required protein. So, all you need to do is remove the aggregate

from the solvent. And, then you make the surfactant a leach out the desired protein. So, this is how you recover proteins.

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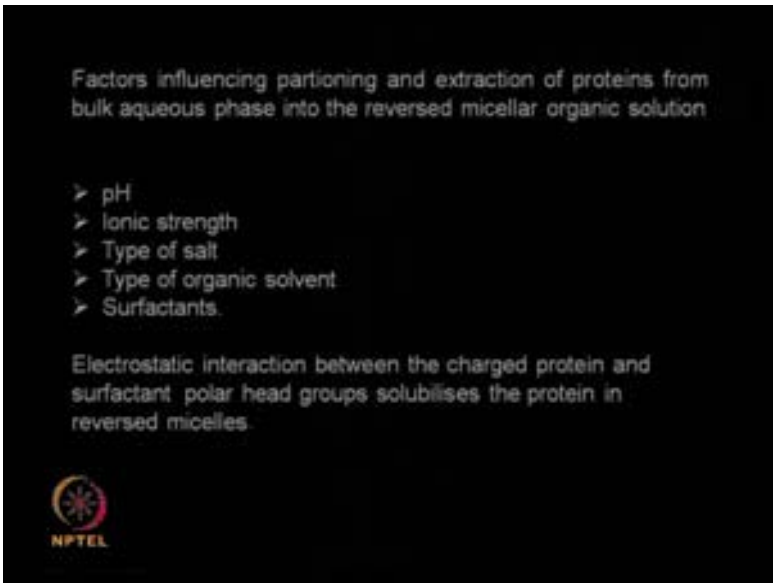

Now, what are the factors that effect? As you can realize it is an aqueous system, that is; where the reverse micelles are formed. So, there are many factors which effect reverse micelle. Most important thing is the pH the ionic strength the type of salt we use, type of organic solvent, type of surfactants we use. So, there could be surfactants which will form a very good a reverse micelle inner core. So, they may prevent the protein leaching out from the inner core. So, there is going to be lot of electrostatic interaction between the protein and the surfactant polar head groups which solubilises the protein in the reverse micelle inside.

So, if you have very strong forces between the protein and the surfactant you are going to dissolve more of the protein in the ((Refer Time: 06:18)) core. So, by selecting these conditions especially, the type of surfactants you take, the type of salt you add the changes in pH as well as the ionic strength. We will be able to solubilise more of the protein that way we will be able to extract things better.

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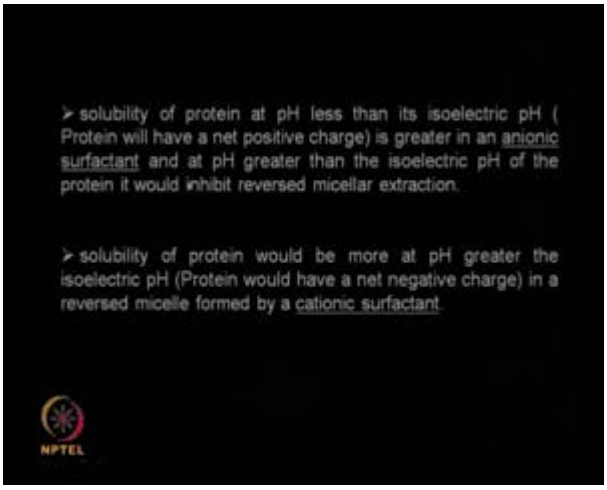

So, if you have you all know what an isoelectric pH that means is; isoelectric pH is the pH at which a protein will have net 0 charges. So, if you know what is the isoelectric pH of a protein? And, if you are operating at a pH less than the isoelectric point of the protein. The solubility of the protein at pH less than the isoelectric pH is greater in an anionic surfactant. And, at pH greater than the isoelectric pH of the protein it would inhibit reverse micelle extraction. So, if the pH is greater than the isoelectric pH then you are going to prevent the reverse micelle extraction. So, solubility of the protein would be more at pH greater than the isoelectric pH the protein has a net negative charge in a reverse micelle formed by a cationic surfactant.

So, depending upon the pH we can select the type of surfactant do I need an anionic surfactant or do I need a cationic surfactant. So, if you want to work at a pH which is greater than the isoelectric pH of the protein then ideally I should have a cationic surfactant. Otherwise, if I am going to work at lower pH lower than lower than its isoelectric pH then it will be good to have an anionic surfactant.

So, by selecting the surfactant depending on the pH at which you are going to operate you can increase the solubility of the protein or conversely. If I know what type of surfactant I am going to use then I will select the pH either greater than the isoelectric pH or less than the isoelectric Ph. By adjusting the pH based on the isoelectric point I can increase the solubility of the protein in that particular surfactant. So, you see there is a very close interaction between the type of surfactant I select anionic and cationic and the pH at which I operate with the respect to the isoelectric pH.

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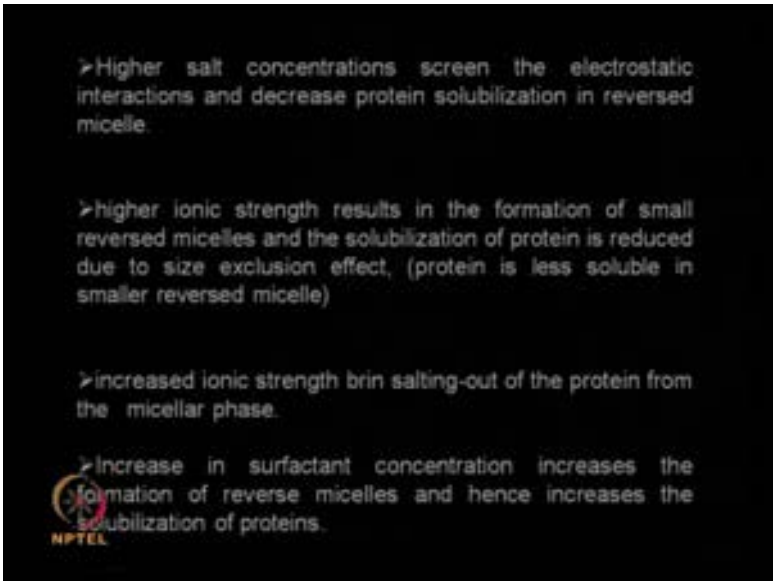

So, there are many conditions by choosing we can alter the solubility of the protein in a reverse micelle extraction. So, if I have higher salt concentrations then it is going to screen the electrostatic interaction that means; the electrostatic interaction between the protein and the polar head group is going to be barred or screened. So, I am going to decrease the protein solubilisation. So, if the salt concentration is increased then if there is going to be very strong electrostatic interaction between the protein and the polar head group is going to get weakened.

Similarly, if I have higher ionic this results in the formation of small reverse micelle. So, when I am forming very small reverse micelle then my solubilisation of the protein is reduced. Because of the size of the core of the reverse micelle hydrophilic region, whereas; if I lower ionic strength then I am able to make larger reverse micelle, that means; I will be able to solubilise more of the protein because of the size.

Increased ionic strength of course, it brings out a salting out of the protein from the miceller phase. So, if I am going to have a very high ionic strength you know what is a salting out the solubility of this ion will reduce the solubility of the protein. So, the protein solubilisation decreases actually. Increase in the surfactant concentration is going to increase the formation of reverse micelles.

So, I am going to increase the solubilisation of the protein. But of course; you need to consider the economics of the entire process do I need to put in lot of surfactant or can I

afford to waste lot of surfactant. So, you need to consider all this points actually. So, if you have very high electrostatic interaction between the protein and the hydrophilic head group or polar head group. If I put salts I am going to create a barrier number 1. If I am going to have very ionic strength then the size of the reverse micelle is going to go down. So, solubilisation of the protein also goes down if I have too much salt then I may be creating a salting out effect so you need to be very careful about the whole thing.

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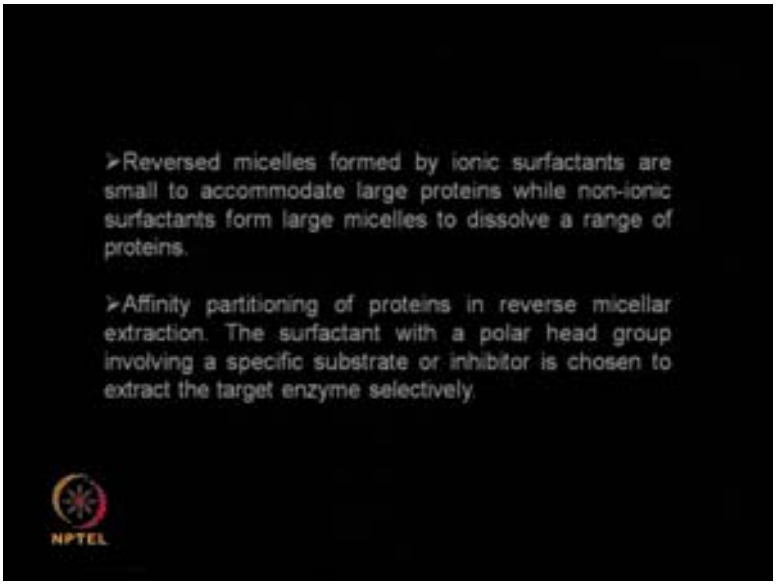

So, if I have reverse micelle formed by ionic surfactants they are small to accommodate large proteins while non-ionic surfactants they will form large micelles to dissolve a range of proteins. Now, this point is very very important. If you are comparing an ionic surfactant verses non-ionic surfactant. So, if I use a non-ionic surfactant I may be able to form larger micelle and compare to ionic surfactant which may form a smaller micelle. So, obviously; as I said before when you have very small core of a reverse micelle the amount of protein that is dissolved also goes down actually. In addition to reverse micelle I may be able to incorporate something called affinity partitioning.

So, I can have a polar head group of the surfactant which is inhibitor for a particular enzyme, so that, particular enzyme may be selectively getting trapped inside the aqueous core. So, this is very intelligent way of improving the selectivity of the protein which you are extracting. So, if the polar head group if I have certain inhibitors attached to the polar

head groups then the enzymes or proteins may be selectively captured inside the inner core. So, that is called affinity partitioning in reverse miceller extraction.

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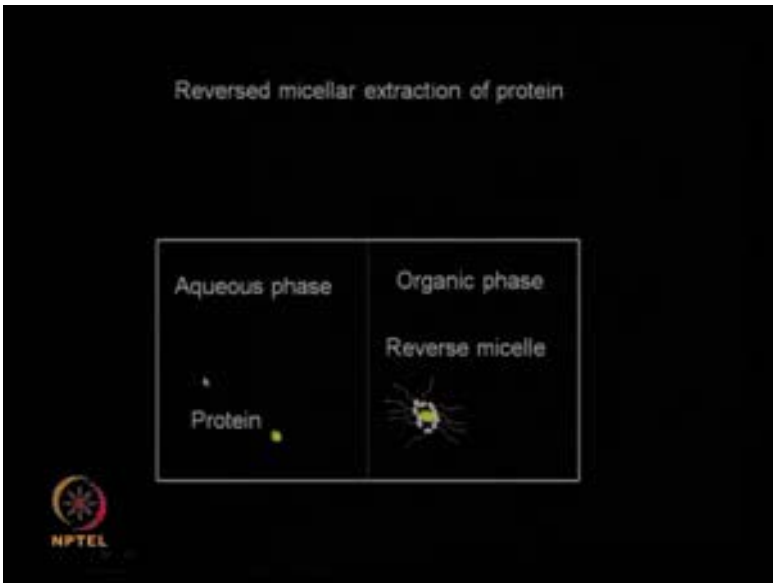

So, typically that is what happens? You have the aqueous phase, you have a protein. So, when I put in organic phase what happens? The reverse micelle is formed. So, the all the hydrophobic tails are pointing out hydrophilic polar groups are inside buried and your protein also is inside buried. Now, I have lots of micelles with just the protein. So, I can nicely separate them. So, it is a very very intelligent way of a separating protein.

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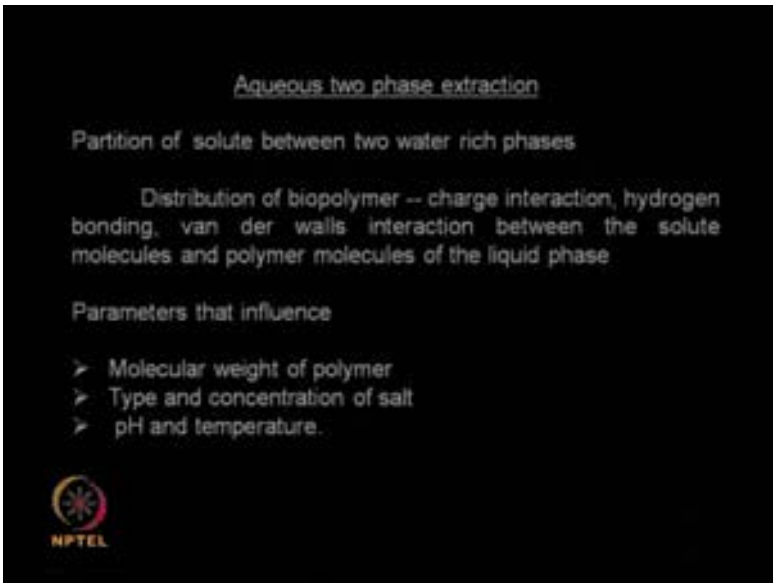

As I said suppose, I have water in which I have certain proteins which I like to extract again, with water itself. Because as I said before proteins are very sensitive in a solvent environment and they may get denatured. So, you would like to use water itself as an extracting medium. So, how do you do that? So, we add something called a bio polymer. So, the bio polymer in aqueous medium may interact with the protein of a very interest these are called non bonded interactions.

So, the interactions could be because of charge, it could be hydrogen bonding; it could be van der wall interaction between the solute molecule or protein and the polymer molecule in the liquid phase. So, what happens? So, I may have a system where a protein is present in an aqueous medium, I add another aqueous medium as a solvent where I may have a bio polymer. So, there could be a interaction between the bio polymer and the protein. And, the protein gets extracted into the solvent which is again water actually.

So, what are the parameters that influence in this particular situation the molecular weight of protein? So I am going to show you some interesting results where molecular weight of the protein affects the extraction efficiency very drastically. Mostly we use biopolymers like PEG, dextran, citrosan so, all these are biopolymers. And, they will not have any adverse effect on the protein. So, that means; the protein will not get denatured by using these biopolymers. The type of concentration of salt we use and of course; the pH the temperature all these factors affect these aqueous two phase extraction system.

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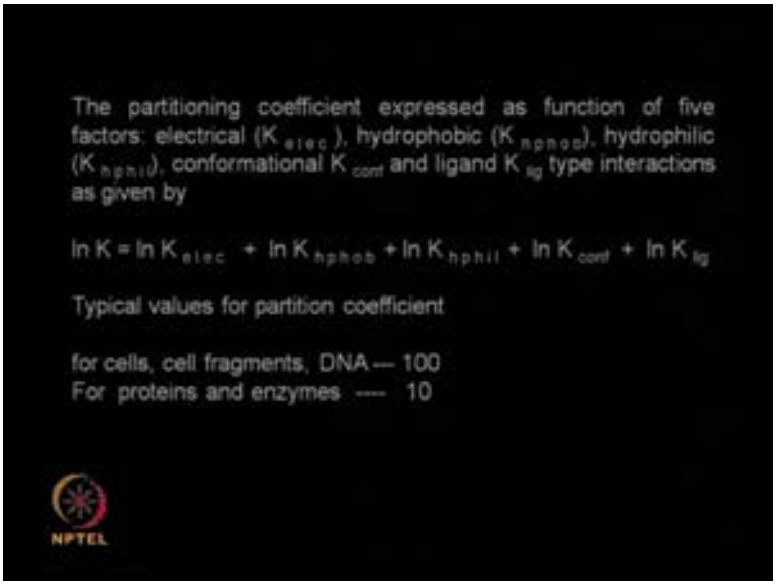

So, the partition coefficient of the protein into the water containing the biopolymer will be function of many parameters as I said they are all non-bounded interactions. So, the interactions could be electrostatic in nature, it could hydrophobic in nature, it could be hydrophilic in nature conformational bind ligand binding and so on. So, we could have large combination of partition coefficients all getting added up to give you the overall partition coefficient for the protein.

So, typically if so if you are talking about cells, cell fragments, D N A the K value will be the order of 100. If it is protein and enzyme the K value will be the order of 10. The type of polymer which you use as well as the molecular rate of the polymer which you use for extracting the protein will have very strong effect on the extraction efficiency this particular example tell you the molecular weight of PEG is effecting the extraction very dramatically.

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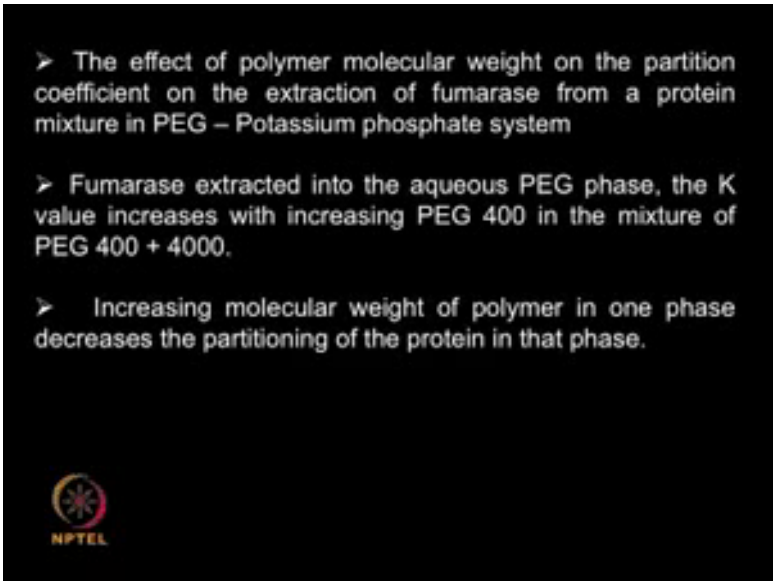

For example; you are trying to extraction of ((Refer Time: 16:47)) from a protein mixture using PEG potassium phosphate system. So, potassium phosphate is a buffer PEG is the bio polymer which you are using. So, the K value increases, that is; K is the partition coefficient value increase with increasing the molecular weight of PEG. So, if when I change from 400 as I keep on moving right up to 4000. So, increasing molecular weight of polymer in 1 phase decreases the partition of the protein in that phase. So, if I move from 400 to 4000 in 1 phase thus the partitioning of the protein in that particular

phase decreases. So, all I have to do is I keep on adding higher molecular weight PEG. So, the solubility of the protein in that phase decreases and it moves to another phase this is how you enhance the partition and separation of the protein from 1 phase to another.

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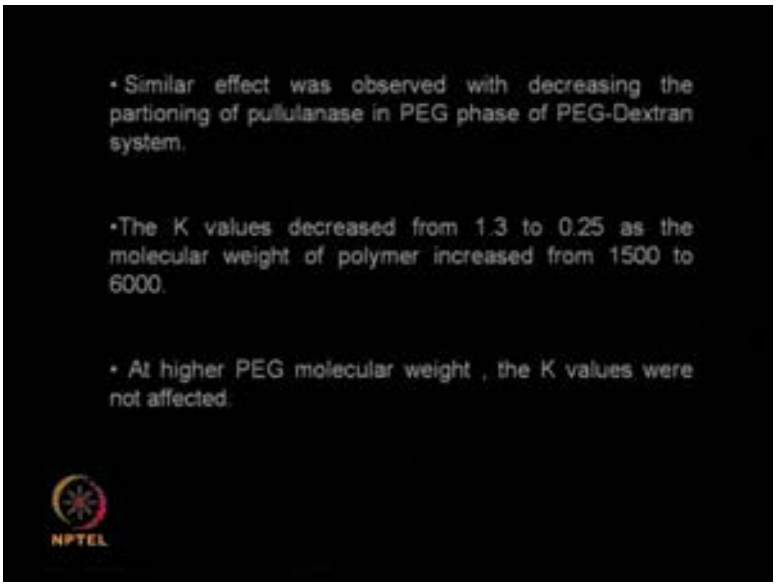

Another example; suppose, you want to partition pullulanase this is a enzyme in PEG phase containing PEG dextran. So, the K value decreases from 1.3 to 0.25 as the molecular weight of the dextran is increased from 1500 to 6000. So, at higher PEG value or here PEG molecular weight, the K value decreases. So, that means; I can reduce the solubility of the enzyme in that phase by adding higher molecular weight biopolymer.

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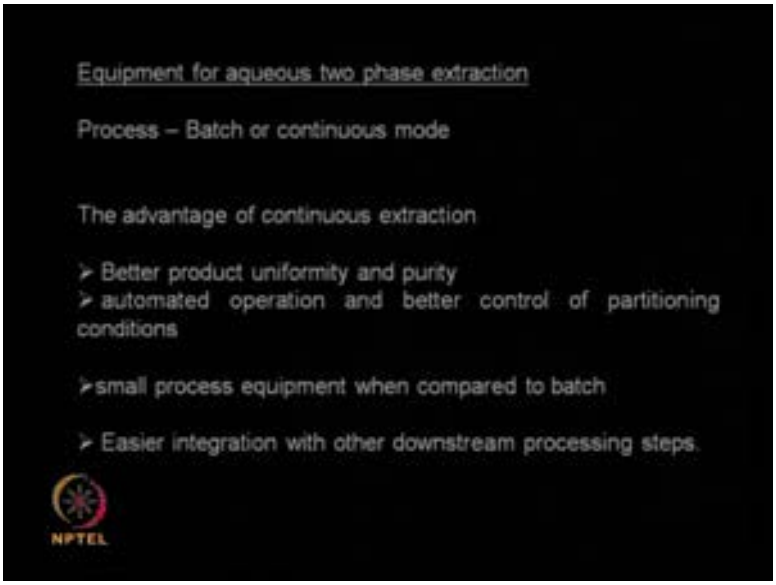

So, why does that happen? It is almost like salting out effect. So, you are adding a larger molecular weight material. So, the protein k leaves the system and goes to another phase. So, what type of equipments do you use in a two phase extraction aqueous phase to phase extraction? We can use batch, we can use continuous just like we are using solvent based assistance.

So, continuous has lot of advantages when compared to batch. But the advantage of batch is you just use a very simple tank or you mix the 2systems and let the two phases to separate and then just do separated to gravity whereas, in continuous system you need to have many equipments, many controls and so on. But then there are advantage are tremendous you get better product uniformity, you get better purity you can go into automated operations, we have better control of partitioning conditions. So, that is the one of the main advantage.

We can even go for smaller process equipment when compared to batch. You may require a very very large tank whereas, in a continuous system we may do the whole job in one fourth or one fifth the size of this large tank. And, it is easier to integrate with the downstream processing. So, I may have a continuous Liquid- Liquid Extraction. Then I may take it to a chromatography which may be operating continuously, I may go to a filter which may be operating continuously and so on. So, we can nicely integrate continuous operation whereas, batch operation you are going to have a product. And, for

some time there would not be any product coming out and then again there will be a product and so on. So, it is going to be start stop, start stop so that will be the main difference between a batch and a continuous operation.

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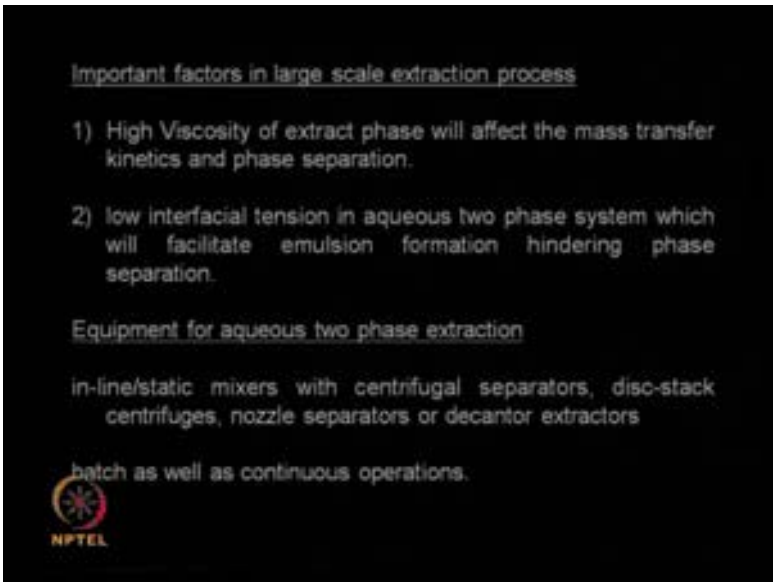

So, we need to think about large scale operations. Because whatever is done in the lab needs to be scaled up and taken up to kilo grams, kilo litres, meter cubes and so on. So, very large scale operation. So, when we talk about very large scale operations there are couple of very important parameters we need to consider one is the viscosity, other is the interfacial tension.

So, if you are handling very highly viscous material for example, fermentation broth is going to be very highly viscous. Because you are going to have dead cells, you are going to have cell debris, you are going to have an intracellular material, you are going to have D N A so many components. So, the viscosity is going to be highly non Estonian and the values are also going to be very high. So, when you are going to have very highly viscous material.

Now, you are adding a solvent to extract a protein or a metabolite. Because of the high viscosity there are going to be mass transfer limitations both during the extraction as well as in the phase separation. Because after you do the extraction you are going to allow both the phases to separate because the phases are highly viscous the separation also is going to take a very long. Not only the extraction is going to be mass transfer limited the

separation is also going to take a much longer time that is 1 problem. Problem number 2 if you are going to have low interfacial tension in aqueous two phase system you can have small emulsion droplets. So, when you are going to have emulsion droplets because you are having water, you are having solvent, you are having surfactant and interfacial tension is also very low.

So, what happens? You are going to have small emulsion droplets. So, the phase separation is not going to be very clear, you are going to have a liquid aqueous phase, you are going to have a liquid solvent phase, you may have in between emulsions formed. The emulsions may not separate out clearly. So, you may lose some of your product in the emulsion and so on actually.

So, low interfacial tension has this type of emulsion formation and it will affect the separation efficiency. So, all these 2 important points we need to consider when you are scaling up this type of aqueous 2 phase systems. So, people have considered in line and static mixers, that means; you will have long cubes filled with the certain static elements. And, when the liquid 2 liquids flow through it they get mixed. And, hence; during the mixing process extraction takes place this type of static mixers have been considered of once, you mix them.

You use centrifugal separators different types of centrifuges we talked about different types of centrifuges, you can disc-stack centrifuge, you can use nozzle separators or decant extractor and so on. So, you generally in a two phase extraction where you using aqueous as one of the phases and if the viscosities are very very high or the interfacial tension is very very low you go for a in-line mixing followed by a centrifugal separator. And, now a days you get very good Liquid- Liquid centrifugal separators, that means; they can separate two liquid phases very very nicely, just like; they can separate out a solid and a liquid. We can use both batch as well as continuous type of operation for a aqueous phase system.

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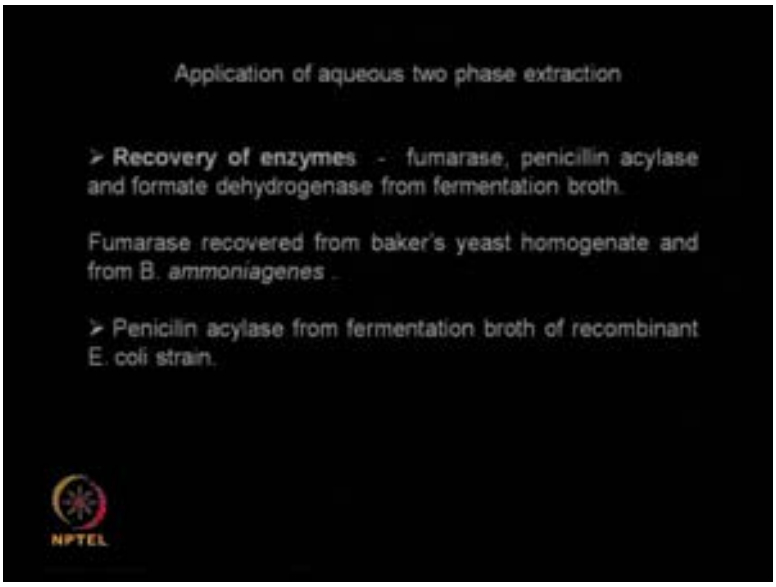

Where do you use aqueous phase extraction systems? I did tell that when you are handling enzymes or proteins they are going to get denatured. So, you do not like to use solvents and in such situations you will go for aqueous systems. So, if you are requiring enzymes like fumarase, penicillin, acylase, formate dehydrogenase all these enzymes from fermentation broth, fumarase recovered from baker's yeast, penicillin acylase from recombinant E.coli strain.

So, these are some situations where you will use a aqueous systems. If you are requiring bulk protein especially, valuable proteins you do not want the protein to get denatured because it is a very valuable protein. Then you may go for aqueous extraction systems like alkaline, protease for Licheniformis by aqueous two phase extraction using PEG and Reppal 200. Reppal is nothing but a partly hydrolysed hydroxide Reppal of starch, that means; you are using a bio polymer both are bio compatible. So, the protein does not get denatured during the extraction process.

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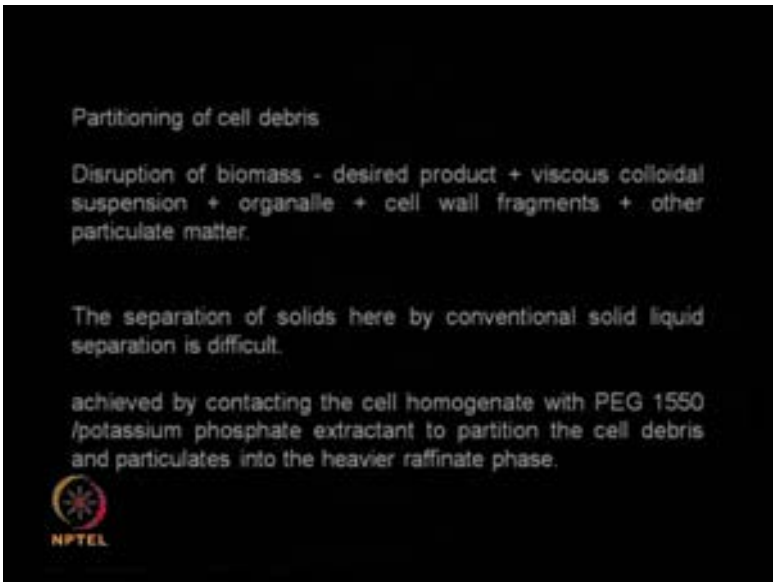

You can use this type of aqueous system for partitioning of cell debris as well. So, once I am disrupting a bio mass because my product is intracellular. So, when I disrupt a biomass you are going to have desired product, you are going to have viscous colloidal suspension, organelle cell wall fragments, other particulate, matter, salts and so on. General solid liquid separation may be difficult, that means; solid liquid separation if you recall we use a filter or we use a centrifuge that may be difficult. So, here we can achieve this separation by adding an aqueous system containing PEG potassium phosphate extractant. So, this will partition the cell debris and particulate into the heavier raffinate phase. So, by adding this aqueous bio polymer with salt I will be able to separate all the cell debris into the heavier raffinate phase. So that, way I will be able to recover my intracellular desired product.

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Affinity partition is it is based on molecular recognition it is like, affinity chromatography we will talk about chromatography later. So, you have your ligand which has an affinity for a protein. As, you know a ligand protein interaction is very very selective. So, if I have a ligand which is going to bind to a protein very selectively then I will be able to extract only that protein from a mixture of large number of proteins. So, that is what is called affinity partitioning.

So, I need to know what is the affinity ligand which is needed to selectively bind to the protein and then I will have that particular ligand present in my solvent. So, the protein will just selectively bind to the ligand there by I can separate out. So, how do you keep the ligand in your solution? So one of the polymers which you are using a bio polymer you can have the ligand covalently bound the bio polymer so that, you can reuse the whole system again and again.

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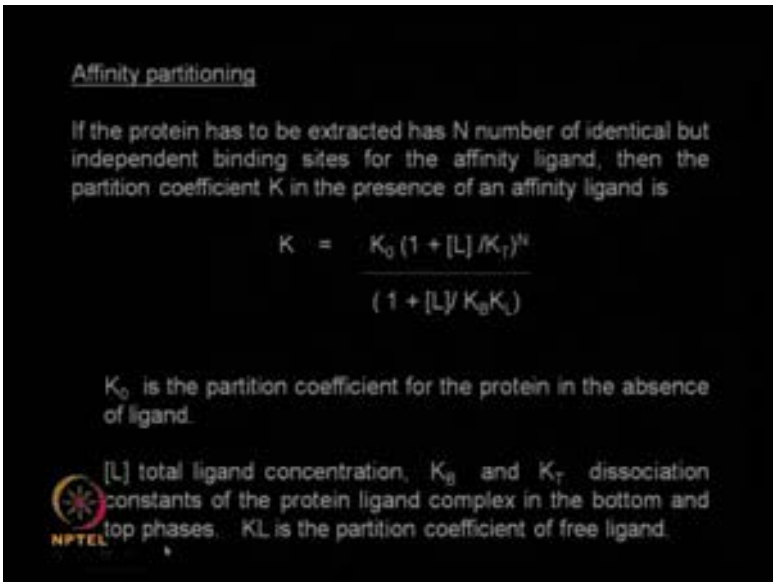

So, if I have an affinity ligand. So, if the protein has to be extracted and it has end number of independent binding sites for the affinity ligand then the partition coefficient K in the presence of the affinity ligand will be like this, K equals to K naught. Where K not is the partition coefficient of the protein in the absence of ligand. So, if I do not use the affinity ligand concept then my partition coefficient will be K naught but because I am adding an affinity ligand which may bind to the protein at end different locations. Then the K the partition coefficient gets modified like K naught into1 plus L divided KT raise to the power N divided by 1 plus L divided by KB and KL. What is L here? L is the total ligand concentration. So, as you can see if I have more ligand present obviously, my K will increase like this now, what is KB, KT and KL?

Now, KL is the partitioning coefficient of the pre ligand as you can understand the ligand also, can get portioned between the two phases. So, some amount of ligand is going to get lost. Because of the partition coefficient and then KB and KT are the dissociation constant of the protein ligand complex in the bottom and the top phases. Now, you have a protein ligand complex which may be present in the bottom that means; the heavies or the raffinate or it may be present in the lights or the top or the solvent phase. So, it can disassociate in this phase, that is; the heavy phase or it can disassociate in the light phase or it can disassociate in the bottoms or it can be disassociate in the top. And, the dissociation constants may be different so that is why you have KB dissociation constant in the bottom, KT dissociation constant in the top.

So, you see the partition coefficient for the protein varies and it depends on the concentration of the ligand I am using the number of sites available in the protein for the affinity ligand to bind the partition coefficient of your ligand. And, the disassociation constant of the protein ligand in the heavy phase as well as in the light phase. So, I can select a suitable ligand so that, I can enhance my partition coefficients tremendously. I will be able to enhance the extraction efficiency by a very large number by selecting suitable ligands and also by selecting suitable ligand concentration.

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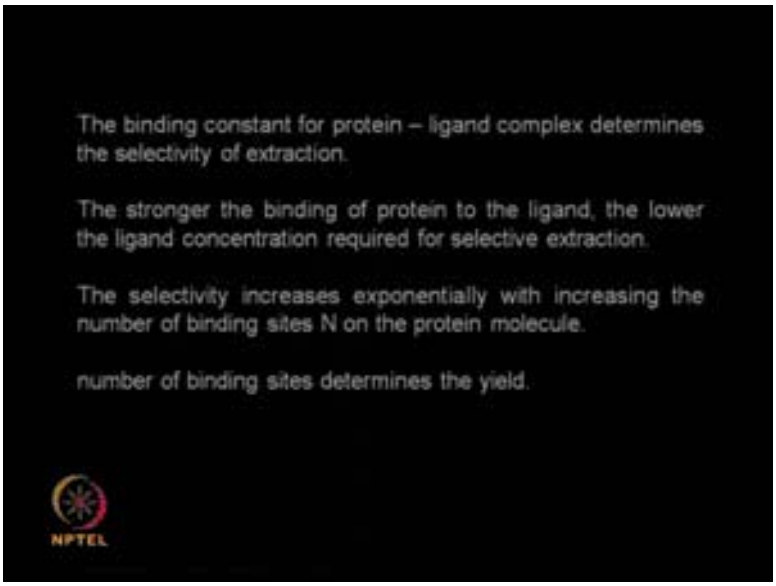

So, the binding constant of the protein ligand complex determines the selectivity of the extraction. So, the stronger the binding of protein to the ligand, the lower the ligand concentration required for selective extraction, that means; it is going to bind very strongly I can use less ligand amount. Now, also the selectivity increases exponentially depending upon the number of sites present. If the number of sites because the term end comes as an exponent, that is; why the selectivity increase depending upon the number of sites in an exponential manner. And, also the number of binding sites determines the yield. So, if I have more binding sites I am going to increase the yield of extraction.

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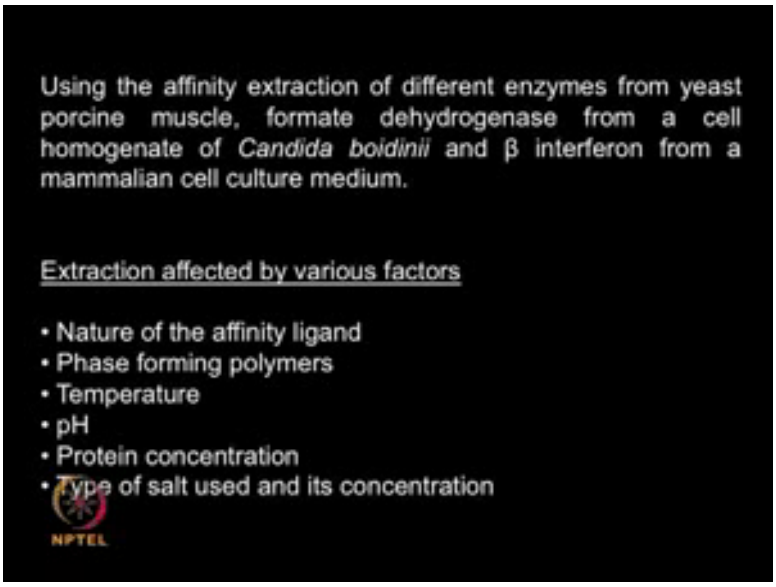

So, what are the advantages of affinity extraction we can use it for extracting different enzymes from yeast porcine muscle, we can extract formate dehydrogenase from cell of Candida boidini and Beta interferon from a mammalian cell culture medium and so on actually. So, we can very selectively extract a protein from a mixture of a large number of proteins. So, the extraction efficiency the selectivity or the efficiency is going to be affected by many factors the nature of affinity ligand I am using the phase forming polymer. Because you are affinity ligand is going to be immobilise on a polymer. We cannot just have the free affinity ligand floating around.

So, it is going on a foliber in a covalent manner. So, the phase forming behaviour of the polymer, the temperature at which you are studying the PH, the protein concentration the type of salts used and the concentration of the salts. So, these are some of these are very standard parameters which are going to effect in the aqueous system for the affinity, the nature of the affinity ligand and the nature of the polymers are 2 different parameters which needs to be considered as well while selecting a ligand. Now, let us go to the limitations of Liquid- Liquid Extraction process. We have looked at a large number of extraction approaches, we have looked at a mathematical relationships, we have looked at aqueous systems both as just the solvent, we have looked at the reverse phase approach, we have looked at a the affinity approach and so on.

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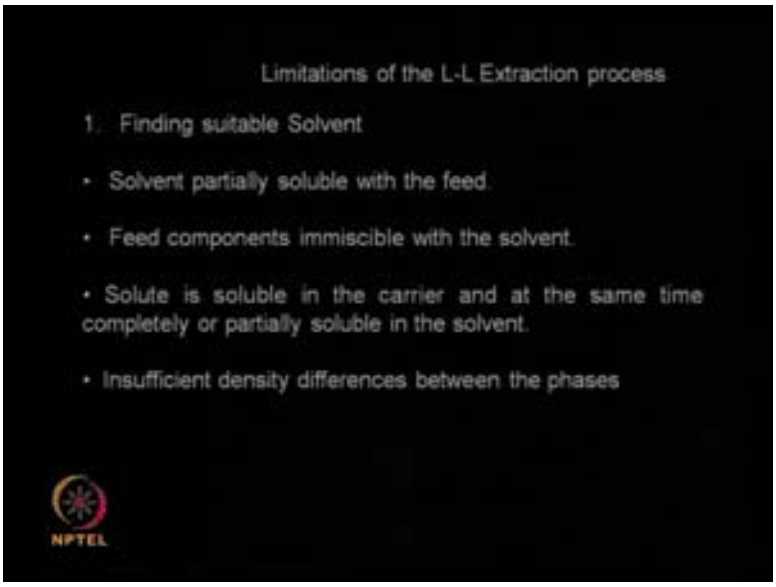

So, there are many limitations of the Liquid- Liquid Extraction it is not 100 per cent the best. We have to find a suitable solvent so that is one of the headaches. You want the solvent partially, soluble with the feed, you want the feed components invisible with the solvent, that means; we do not want other components to be dissolving in the solvent. So, solute is soluble in the carrier the same time it should be completely soluble in the solvent then only.