Proteomics : Priniciples And Techniques
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Module No. # 10
Lecture No. # 10
Sample preparation for proteomics applications: Serum and bacterial proteome

Welcome to the proteomics course.

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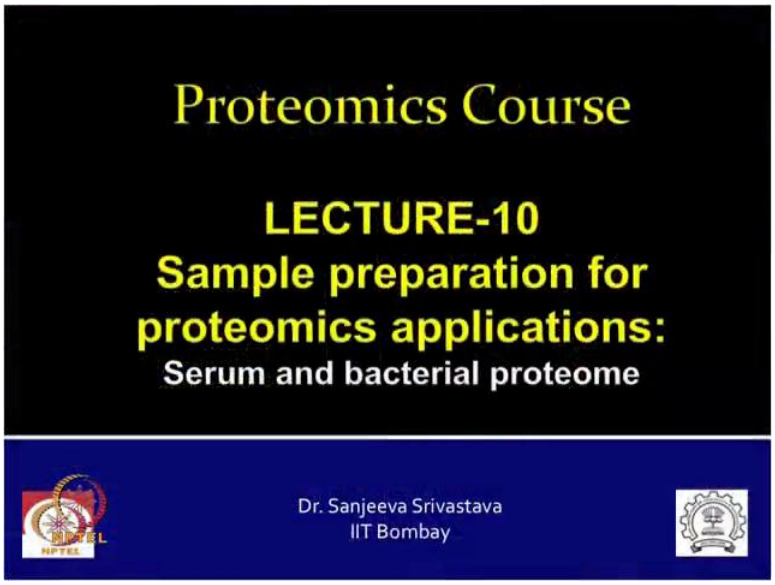

In today's lecture, we will talk about Sample Preparation for various Proteomic Applications. I will give you some specific examples for serum and bacterial proteome analysis.In the previous lectures, we were discussing about the workflow, how to make a good protein sample preparation, I described cell disruption or lysis, protection by applying different proteus inhibitor during the proteolysis step, how to fractionate the samples and reduce the complexity of the proteome, how to extract and solubilize protein.

So, in the previous lecture, we tried to give you different type of commonly used methods available for each of these steps.And as I recommended, none of these methods you can directly follow in your own protocol; because you have to think about your biological question which you want to ask and then, depending upon that, you can apply a combination of these techniques or these methods.

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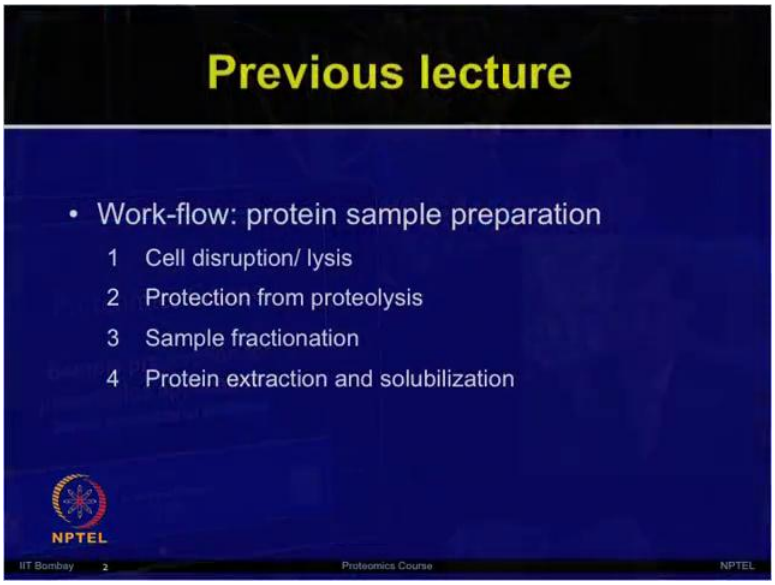

So, let us look back the last lecture.In the previous lecture, I described how to disrupt the cell or lysis a cell I gave you different methods, how to protect the cell, the protein component during the proteolysis; and different type of proteus inhibitors were described, the sample prefractionation methods were discussed and then, we started talking about protein extraction and solubilization.

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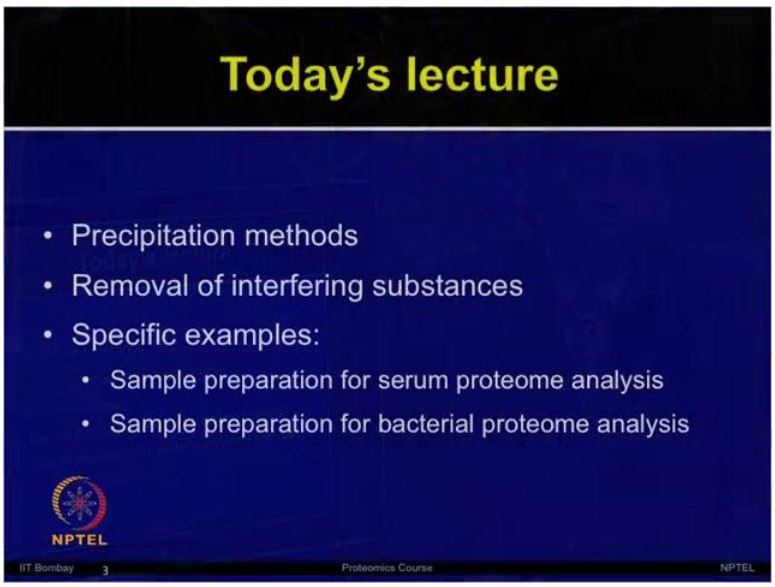

In today's lecture, I will first talk about few precipitation methods, which are commonly used for different type of protein sample preparation.Then, we will talk about the removal

of interfering substances.These small substances interfere during the proteomic applications, whether these are salt or nucleic acid or other type of contaminants; often they totally waste the whole experiment, if you do not remove them in the beginning. So, I will talk to you about some of the commonly interfering compounds, and how one can try to get rid of them.

Then, we will move on to specific examples.The sample preparation for the serum proteome analysis, and sample preparation for bacterial proteome analysis. So then, you will realize that, we need to integrate lot of concepts, which we have talked in the previous lecture and today's lecture.And then, we need to apply that for performing the proteome analysis of serum and bacteria.We will continue our specific examples in the following lecture and then, we will talk about the protein quantification.

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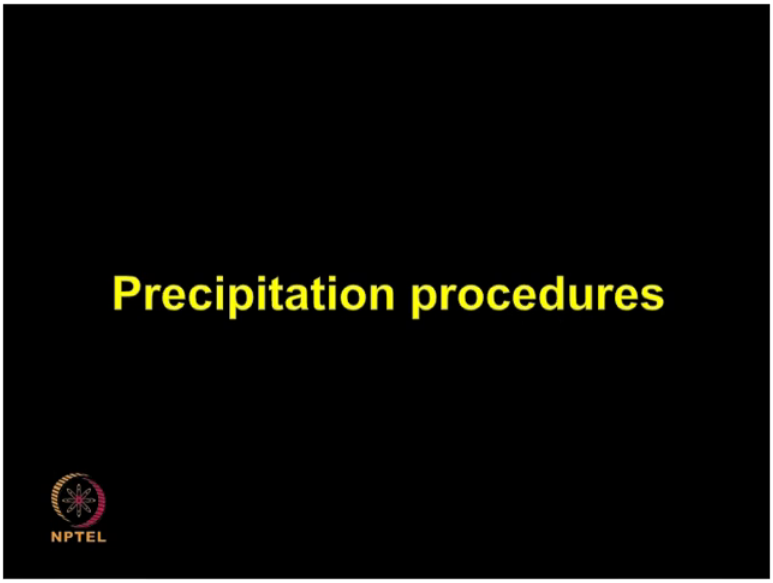

So, let us first talk about the precipitation procedures.There are different types of precipitation methods available, I will go through one by one and then, we can give you some recommendation about which ones can be more commonly used.

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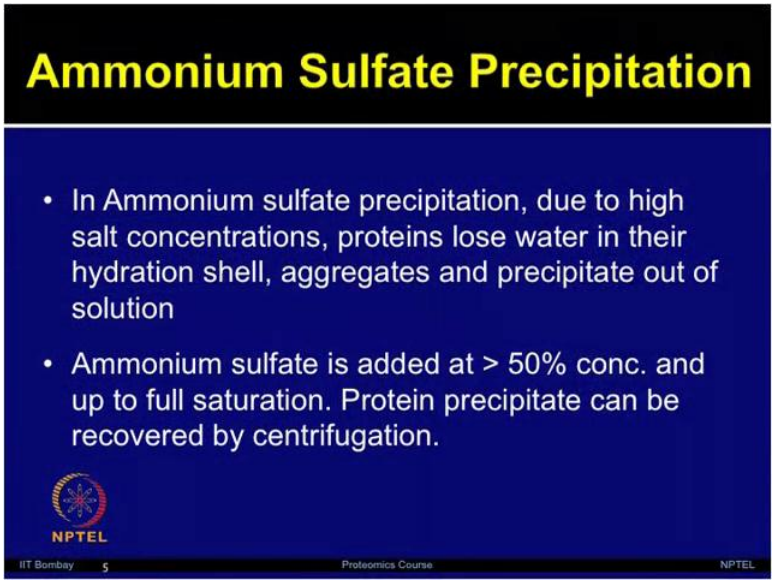

So, let us talk about ammonium sulfate precipitation, which is one of the most previously used methods, from the classical way of performing experiments.The ammonium sulfate precipitation was used,although its uses is not so common, when you are preparing samples for the proteomic application, but this one still remains a good choice.

So, ammonium sulfate precipitation, due to the high salt concentration, the proteins lose water in the hydration shell, they aggregate ad precipitate out of the solution. So, if you add ammonium sulfate at greater than 50 percent of concentration and upto its full saturation, the protein precipitation will occur and then, by performing a centrifugation step, this can be recovered.

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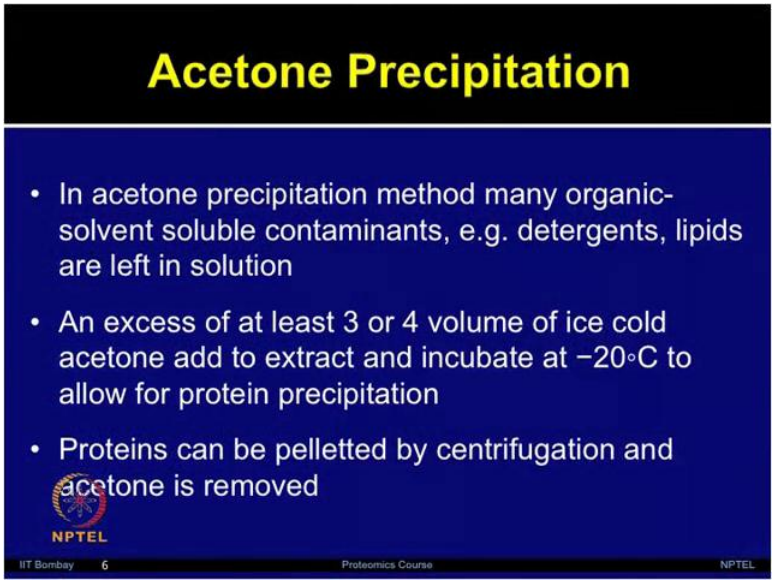

Now, let us talk about the acetone precipitation.In this method, many organic-solvent soluble contaminants such as detergents, lipids, they are left in solution, so it is very effective.If you add an excess of at least 3 or 4 volume of ice cold acetone in your extract incubate it in minus 20 degree for 1 to 2 hours and allow the proteins to precipitate by performing this step.The proteins can be pelleted down during the centrifugation step and then, subsequently you can remove the acetone and dry it out. So, acetone precipitation is very easily performed method and it is very effective.

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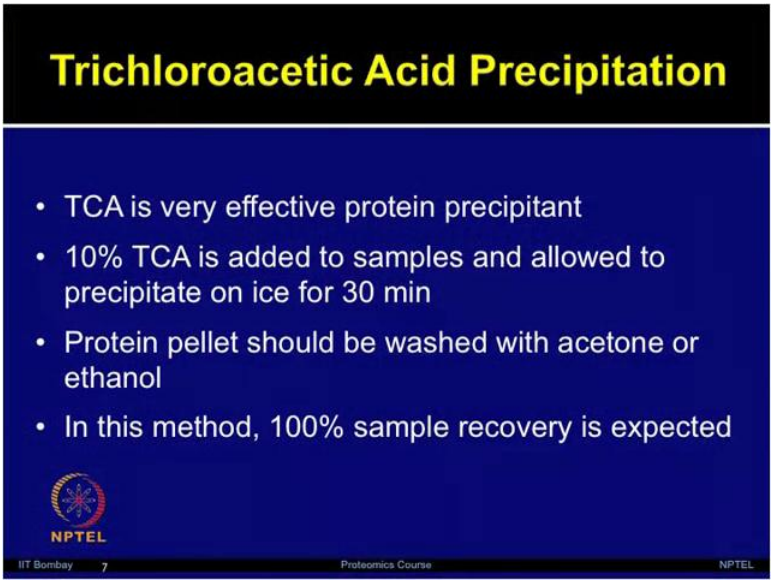

Now, let us talk about T C A or Trichloroacetic Acid precipitation. T C Ais one of the very effective proteins precipitant.One can use 10 to 20 percent of T C A usually 10 percent T C A is commonly added to the samples and then allowed to precipitate in the ice condition for almost half an hour to 1 hour.Protein pellet should be washed by adding acetone or other organic solvents such as ethanol.

This method is very effective for the sample recovery point of view almost 99 to 100 percent sample recovery can be expected in this method.Now, since both T C A and acetone alone are very effective, people have tried combining both the methods together, it means addition of both T C Aand acetone.

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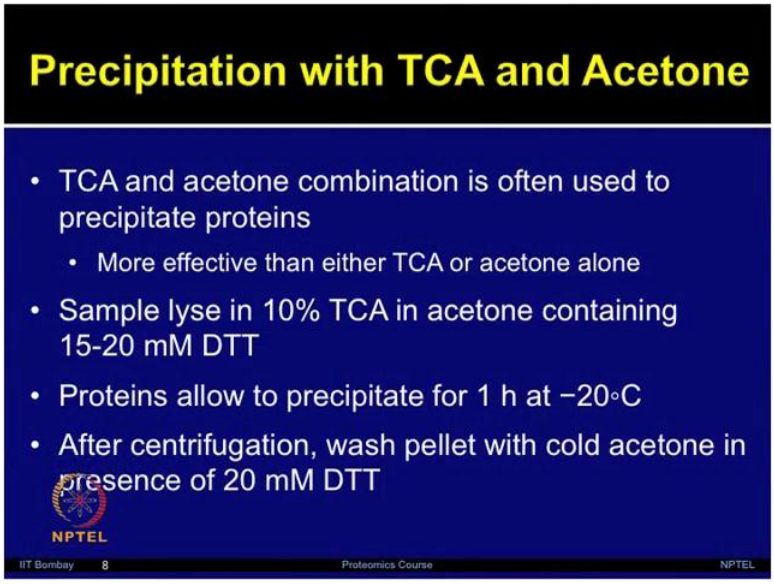

So, this combination has demonstrated that it can precipitate the proteins more efficiently, which could not be achieved either by using T C A alone or by using acetone alone. So, a recommended concentration one can try lyse the sample in 10 percent T C A made in acetone and also add 15 to 20 mill molar of D T T.

Now, allow the protein sample to precipitate for a hour or 2 hours at minus 20 degrees centrifuge and the pellet can be further washed with acetone alone, try performing the whole step in the cold condition, so that you can avoid the proteolysis degradation; even this step, the acetone with 20 milli molar D T T will be effective. So, this is very easy method to precipitate out the protein first add 10 percent T C A with acetone; and after centrifugation, wash the pellet to remove the T C A which could be present there and

then,further wash three of four times with acetone containing D T T, after that you need to dry out your pellet, so that any residual amount of acetone is not remaining in pellet.

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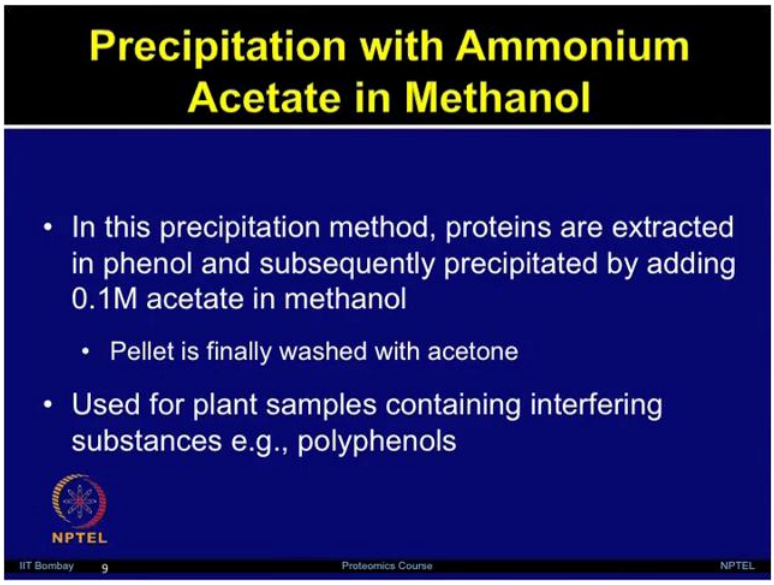

Now, let us talk about one of the less commonly used method, precipitation with ammonium acetate in methanol.This is more commonly used, when you are talking about some plant samples, which are rich in polyphenol and other interfering substances. So, by using this precipitation, proteins are extracted in phenol and subsequently precipitated by addition of 0.1 molar acetate in methanol; pellet can be finally washed by adding acetone. So as I mentioned, less commonly used method and for the specific application people try different type of precipitation and washing steps.

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So, now let us talk about how to remove the interfering substances, because as I mentioned the interfering substances are very detrimental for your any proteomic application whether you want to perform two dimensional gel electrophoresis or you want to go for directly l c m s based applications or you want to do surface plasmon resonance, label free based proteomic techniques or you want to apply on protein microarrays, in all of these methods different type of interfering substances will be very (( )).

So, let us talk about, what are these different type of interfering substances and how we can get rid of them.It is probably not possible to completely remove these interfering substances, but at least partially if you can remove them, that will ensure the success of your further experiment.

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So, we need to remove the contaminants and these contaminants include salt, small ionic component, polysaccharide, nucleic acid, lipids and many other small interfering components. So, if your aim is to perform two dimensional electrophoresis experiments, please ensure that, you have removed salt very efficiently otherwise, it is going to interfere in the isoelectric focusing step.

Now, there are different type of contaminants, which may also affect the quality of your proteomic experiment such as polysaccharides, lipids, nucleic acids, these type of components can form complexes along with the proteins by electrostatic interactions; and when you are separating the proteins by using gels, they can form clog on the gel. So, in the gel base proteomic methods, these types of components, these artifacts are going to affect the quality of the experiments very much.

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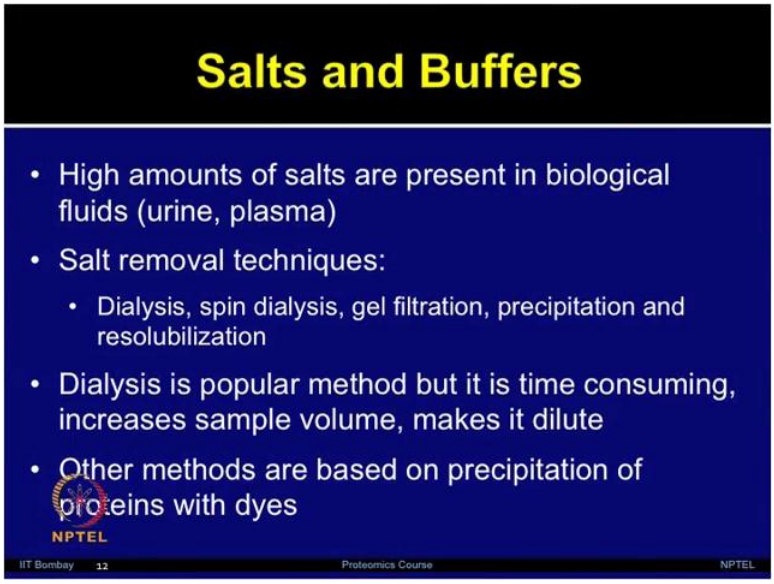

Let us first talk about salts and buffers, during your entire processing you use different type of buffers and residual buffers are always there, which could affect the overall sample preparation.Now, salt is also present due to the sample type, the kind or the nature of the sample itself.For example, if you are talking about biological fluid such as urine, plasma (( )), these sample are already very much rich in the salt content.

Similarly, there are different type of plant cells, which are quite rich in the salt contents. So, if you want to remove this salt, you have to follow different type salt removal methods, these methods could be dialysis, spin dialysis, gel filtration method, precipitation, and resolubilization. Dialysis is one of the most commonly used method, in which in a dialysis membrane you can add your sample the protein along with salt or other interfering components and in the water or different buffer condition, slowly the salt can be eliminated out; only problem here is, your sample volume can be very dilute and it can become very much.

So, if your application requires concentrated solution and with a small quantity, then this may not be the very popular choice for doing application for the proteomics.There are other methods based on the precipitation of proteins with dyes, those are also commonly used depending upon your proteomic application.

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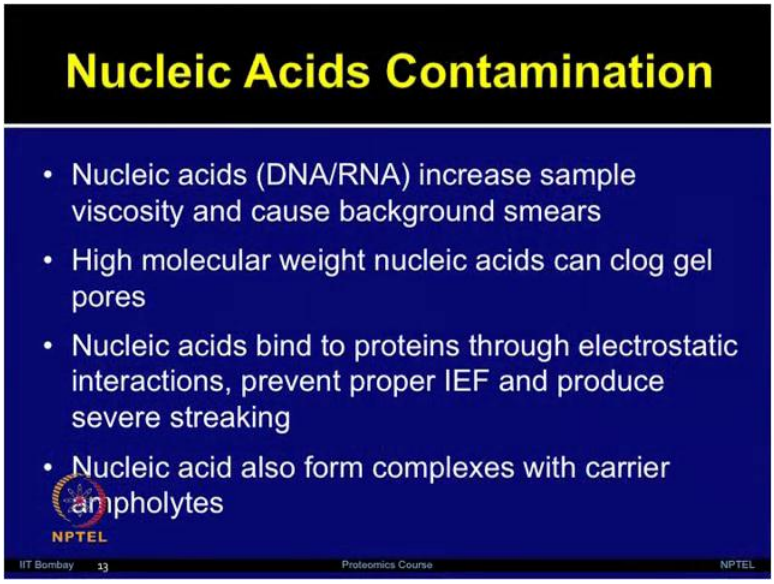

Then there are nucleic acid contaminations.Nucleic acids, if they are present as a trace amount or the more amount in the protein extract, they can increase a sample viscosity and later on, if you are separating the proteins on two dimensional electrophoresis gels, it is going to show background smear or different type streaking. The high molecular weight nucleic acids such as D N A or R N A, they can clog the gel pores, which will be used for gel based proteomic applications.

The nucleic acid can bind to the proteins through electrostatic interactions and it will interfere in the isoelectric focusing step and it may ultimately result into severe streaking.A nucleic acid can also from complexes with the carrier ampholytes, which are added during the isoelectric focusing step.

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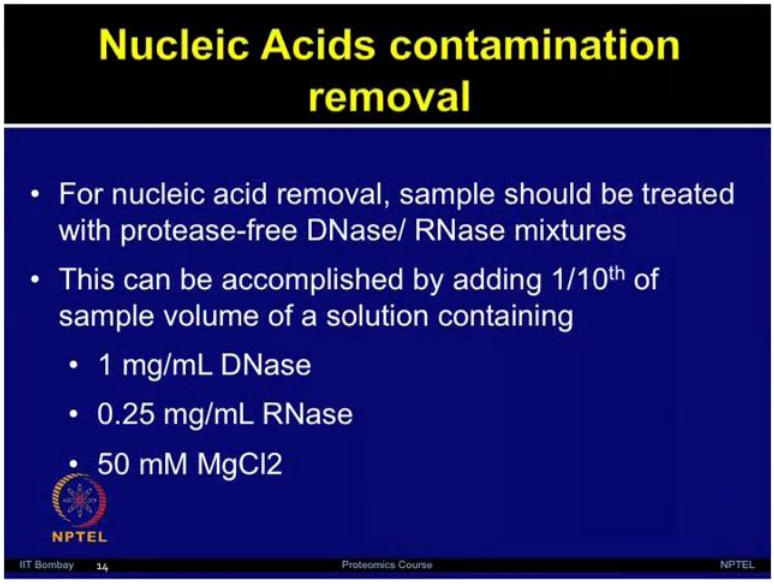

So, how to remove the nucleic acid contamination removal?To remove the nucleic acid contamination, your sample should be treated with protease-free DNase or RNase mixtures; and you can accomplish this by addition of one-tenth of the sample volume of the solution containing,1m g per m l DNase,0.25 milli gram per m l of RNase and 50 milli molar of magnesium chloride, please perform this steps in the cold conditions, try to keep this reaction in ice. So, that you are effectively performing the nucleic acid contamination removal.

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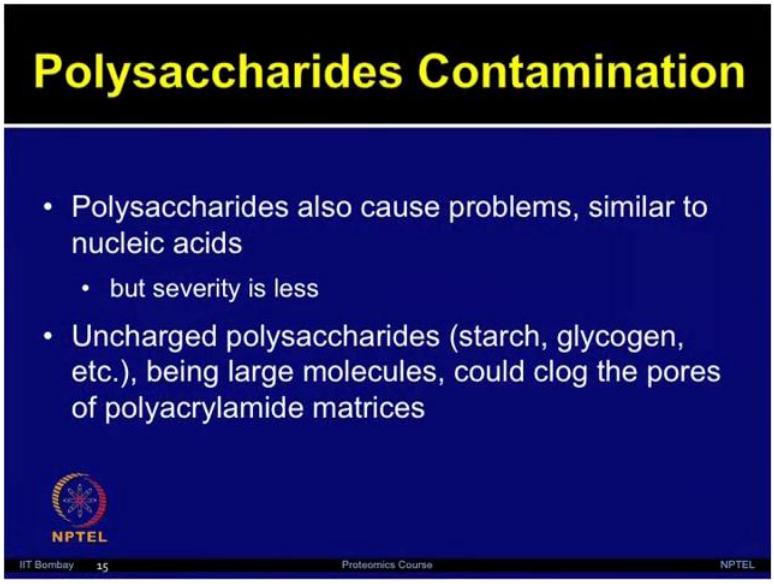

So now, let us talk about polysaccharide contamination.Similar to the nucleic acid, polysaccharide they may also cause problem; however, the severity will be less as compared to the nucleic acid contamination.There are different types of uncharged polysaccharides such as starch, glycogen and these are very large molecule. So, they can clog the pore of polyacrylamide matrices, similar what we talk for the nucleic acid.

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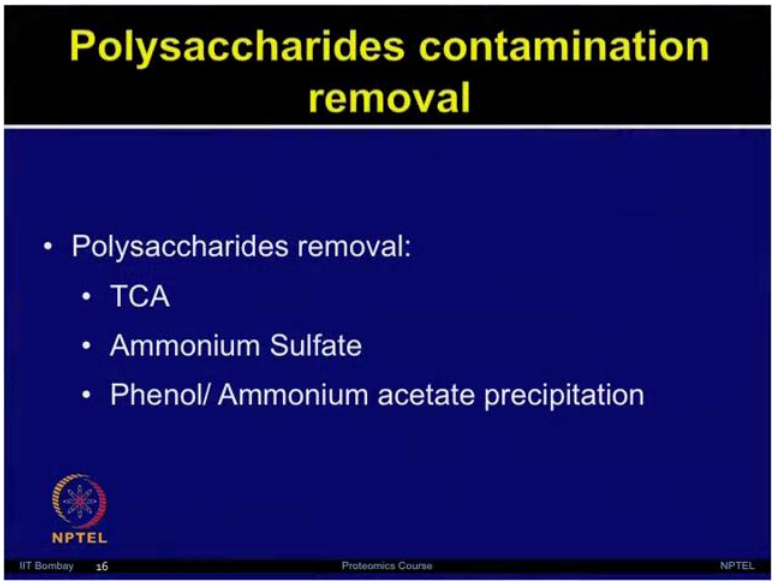

So, how to remove these type of polysaccharide contamination?During the precipitation step itself lot of polysaccharides, they get removed. So, T C ATrichloroacetate acid, ammonium sulfate of phenol or ammonium acetate precipitation, they are efficient ways of removing the polysaccharide contamination.

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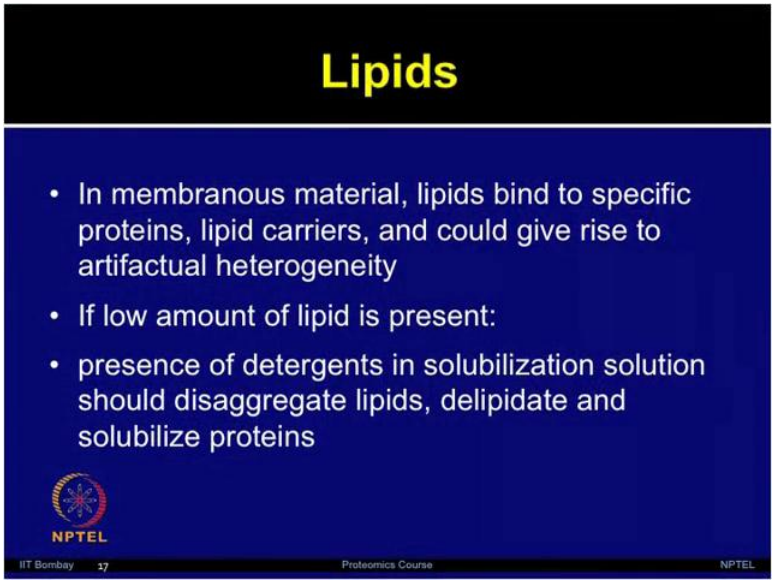

Lipids they are very important, they are used for various types of biological problems to probe, but if your context is to study about the proteins you would like to get rid of any lipids or any other nucleic acid or other interfering components, because you just want to analyze only proteins.Since we are talking about sample preparation for the proteins and proteomic applications, you would like to get rid of lipids.

In membranous material, the lipids bind to the specific proteins such as lipid carriers, and it could give rise to artifactual heterogeneity, if very low amount of lipid is present in your protein sample.The presence of detergents in solubilization solutions should disaggregate the lipids,delipidate and solubilize the proteins.

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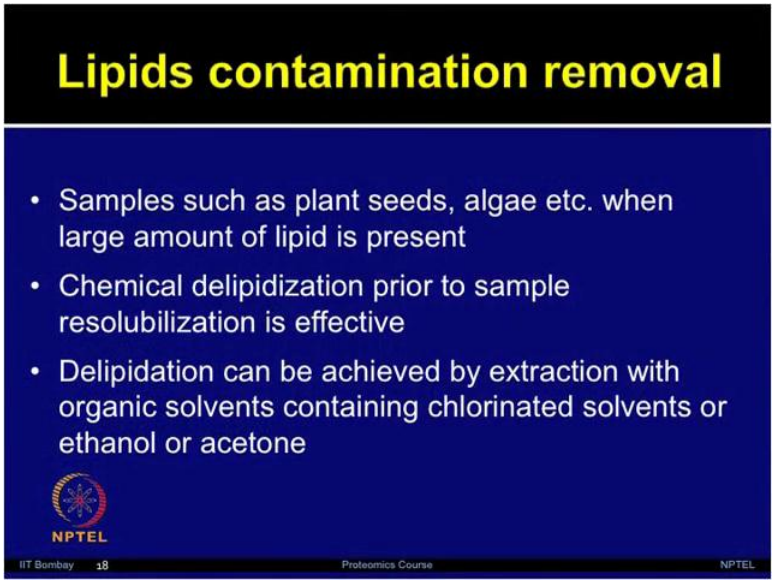

But, if your samples are very much rich in liquid contamination, few samples which are intrinsically rich in the lipids such as plant seeds or algae, so you need to treat the samples by using chemical delipidization prior to the sample resolubilization. This lipid is, this process of delipidation can be achieved by extraction with organic solvents containing chlorinated solvents or ethanol or acetone alone, but this step become very crucial, if you are analyzing the proteome of those samples, those biological samples which are very rich in lipid component.

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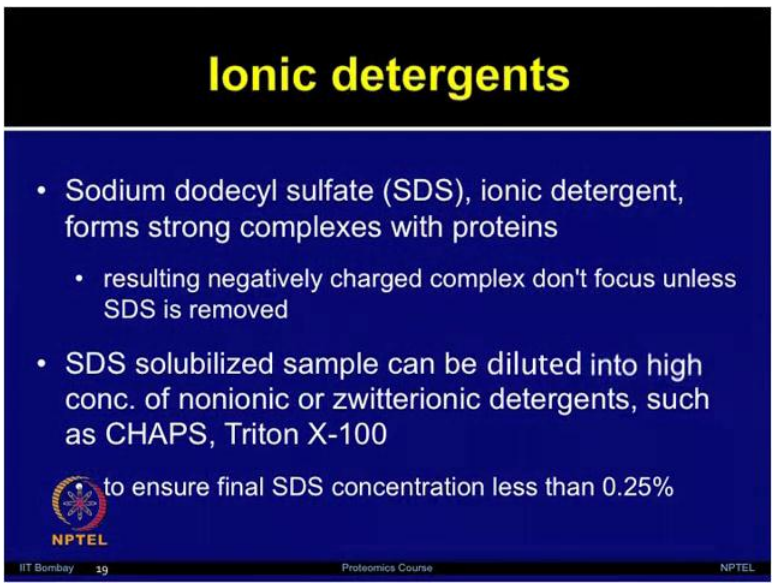

Now, let us talk about ionic detergent, S D S Sodium Dodecyl Sulfate, this is one of the anionic detergent which forms very strong complex with proteins, we will talk about S D S and how it can be used for gel electrophoresis such as S D S page. But, in this context when you are talking about protein preparation, S D S is one of the very efficient compound.

However, if you later on, your aim is to protein separation by using isoelectric focusing or other gel based methods, it is going to create some problems, because it will result into the S D S will bind to the proteins, which will result to the negative charge complex and that will not focus, unless the S D S is removed from the protein sample mixture.

S D S solubilized sample can be diluted by using high concentration of nonionic or zwitterionic detergents, which are CHAPS,Triton X-100 and we have talked about all different type of detergent in the last lecture. So, you can try different type of nonionic or zwitterionic detergent, this step will ensure that, the final S D S concentration is less than point 0.25 percent otherwise; your isoelectric focusing will be hampered by this excess of S D S molecule.

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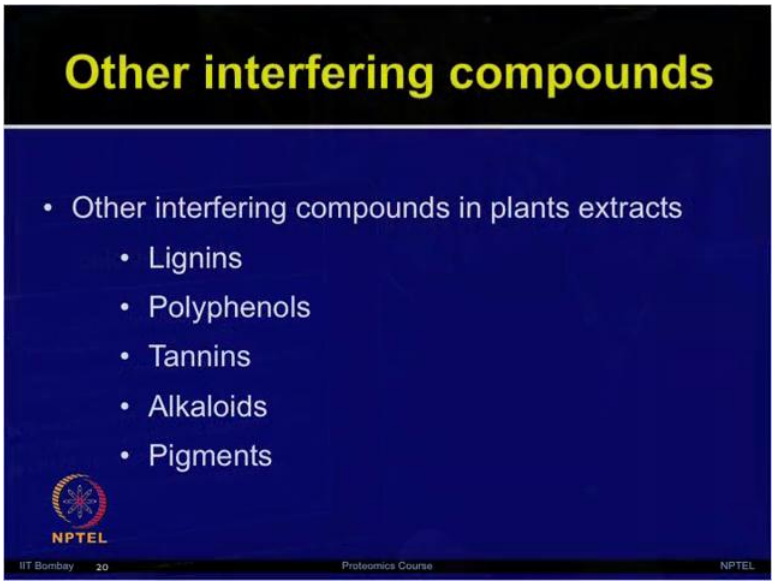

So, we have talked about different type of interfering components in the last (( )) I will just say that, this is not the end of the list.There are many other interfering compounds present and depending upon your unique biological sample, you may encounter more and more interfering compound and you may have to come up with new creative ways of

removing those interference, sothat your proteomic study can be performed with very high quality.

There are few interfering compound, which are also present in the plants extracts such as lignins, polyphenols, tannins, alkaloids and pigments, I will talk about some of these in more detail when I will talk in the next class about the case study, how to perform plant proteome analysis.

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So, we have so far covered different strategies of preparing a very good sample for proteomic application.Now, let us try to apply some of these knowledge for various specific application such as, I will show you these applications with serum proteome analysis and bacterial proteome analysis.

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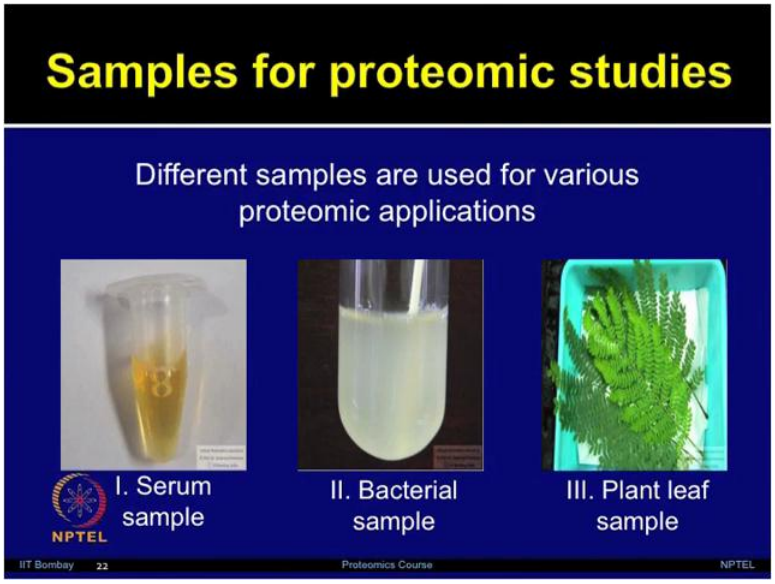

So, we will take examples from human serum, bacteria and plant leaf, this gives you the diversity of different type of biological samples, different type of challenges being imposed by each of these unique sample type.And during the process, we will try to see how we can integrate the knowledge which we have acquired during this lecture and the previous lecture of different type of sample preparation strategies.

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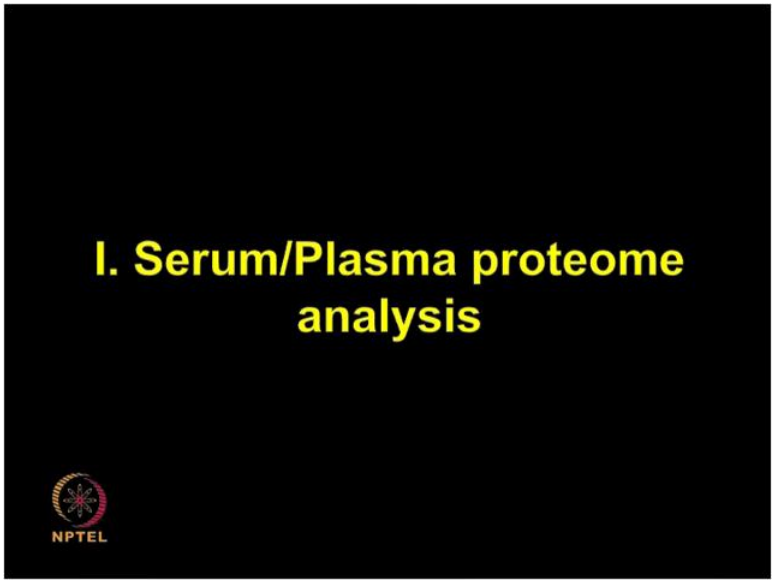

So, now let us move on to the first application, the serum or plasma proteome analysis.

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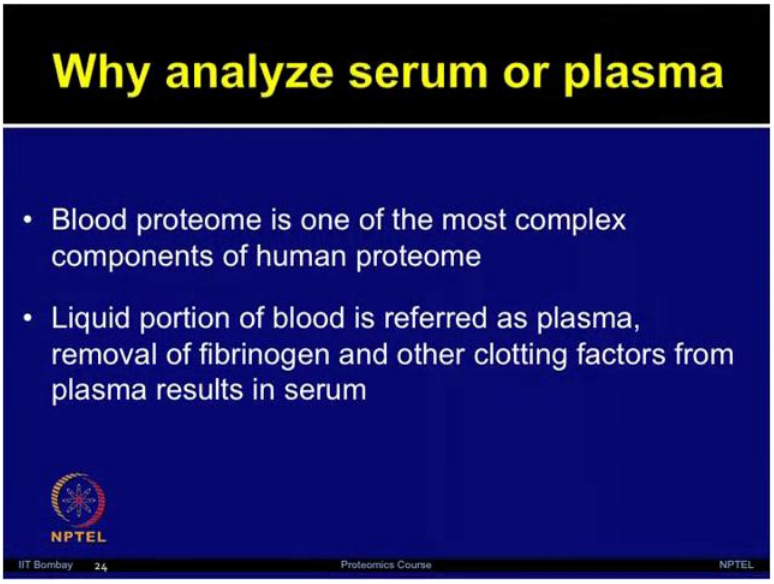

So, first of all you may ask why to analyze serum or plasma for any proteomic application? So, as you know, blood proteome is one of the most complicated components of the human proteome.The liquid portion of the blood is referred to as plasma, and removal of fibrinogen as well as, other clotting factors from the plasma result into serum.

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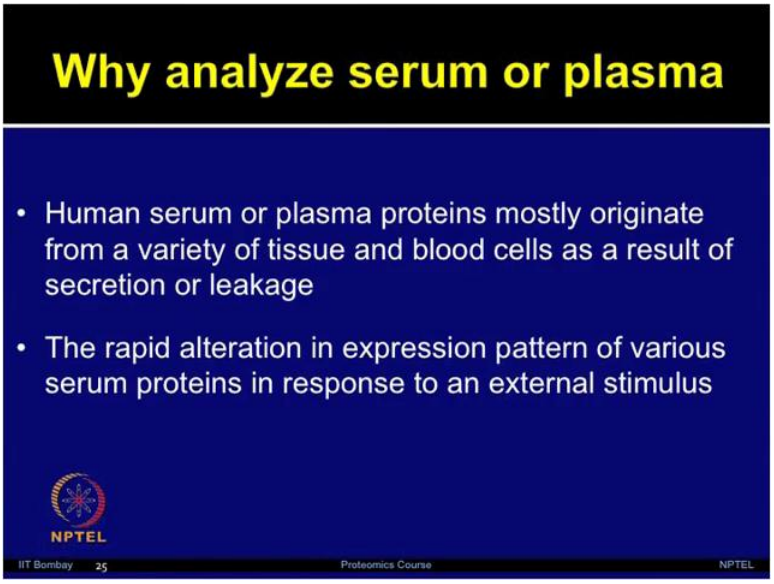

So, human serum or plasma proteins, they mostly originate from a variety of tissue and blood cells as a result of secretion or leakage from the neighboring tissue or the blood

cells.The rapid alteration in the expression pattern of various serum proteins, due to response of a disease condition or an external stimulus,yes through reflection of physiological changes occurring in an individual.So, to get a feel about what are all the physiological changes happening in a patient due to a disease, people analyze serum or plasma proteome very often, because blood removal for various type of test is being performed in clinic. So, blood is very easily accessible sample and performing the serum or plasma proteome becomes very easy as compared to (( )) out a tissue for further analysis.

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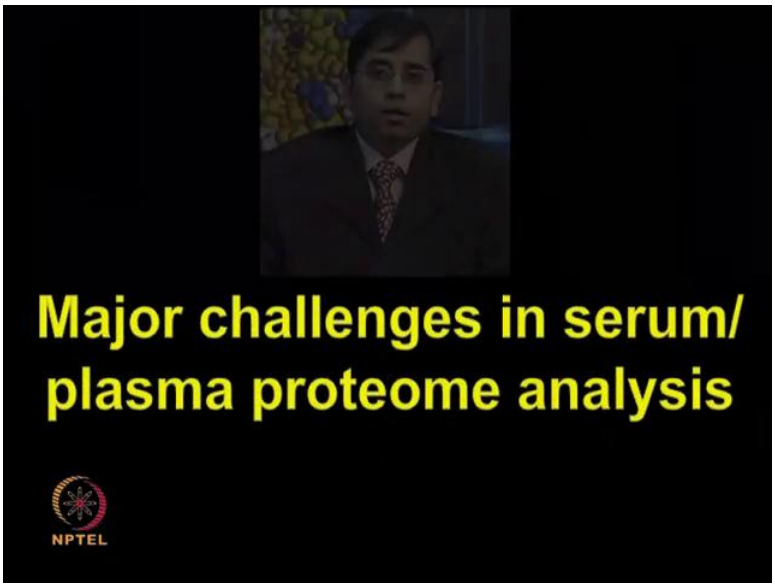

Although, sample removal is easy, but sample analysis the serum or proteome analysis is not so easy.There are major challenges in serum or plasma proteome analysis, let us talk about some of these challenges point wise.

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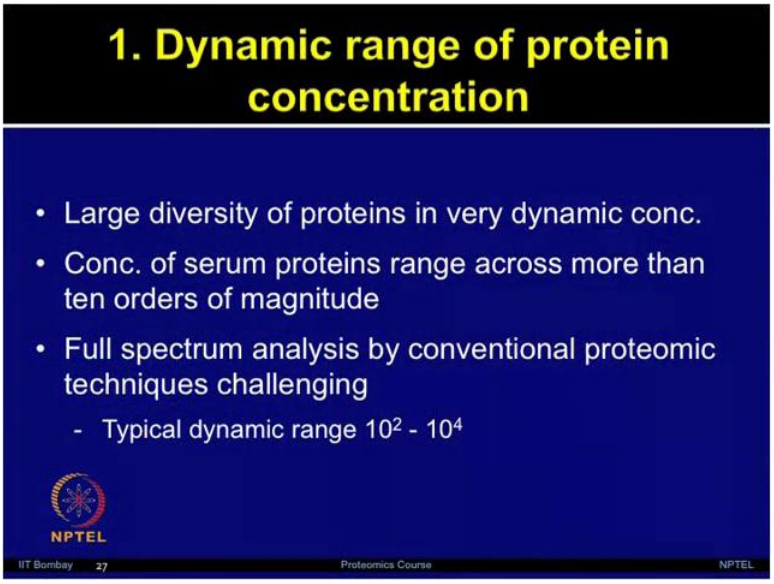

The first point the dynamic range of the protein concentration in serum, there is a large diversity of proteins, which provide a very dynamic environment of almost 10 to the power 10 magnitude. So, the concentrations of serum proteins range more than ten orders of magnitude.If you want to obtain full spectrum of serum or plasma by applying any of the conventional proteomic technique, it is very challenging because, the typical dynamic range for any of these techniques any these platforms will be much smaller between 10 to the power 2 to 10 to the power 4.

So, how to capture all the dynamic events which are happening in the serum, if the dynamic range of the proteins are very large and your detection techniques are not able to capture that, whole dynamic range? So, to avoid these issues, people try to remove some of the abundant proteins from very complex serum proteome, so that overall dynamic range can be reduced and minimized.

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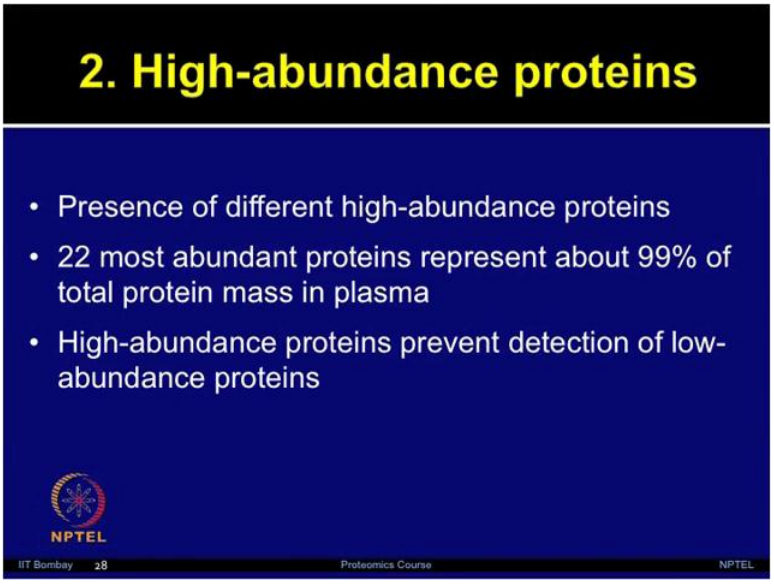

So, second point the high abundance proteins, there are different high abundant proteins which are present in serum and plasma, which makes its analysis very very complicated. There are almost 22 highly abundant proteins present in serum, which represent about 99percent of total protein mass of serum or plasma.These high abundance proteins prevent the detection of very low-abundant protein.

And often these, low-abundant proteins could be the target which you are probably looking for, as a part of bimolecular discovery.So, how to get rid of the high abundance proteins, I will describe some of the strategies, which can be use to remove high abundant proteins in next couple of slides, but first let us talk about which are other challenging factors for the serum or plasma analysis.

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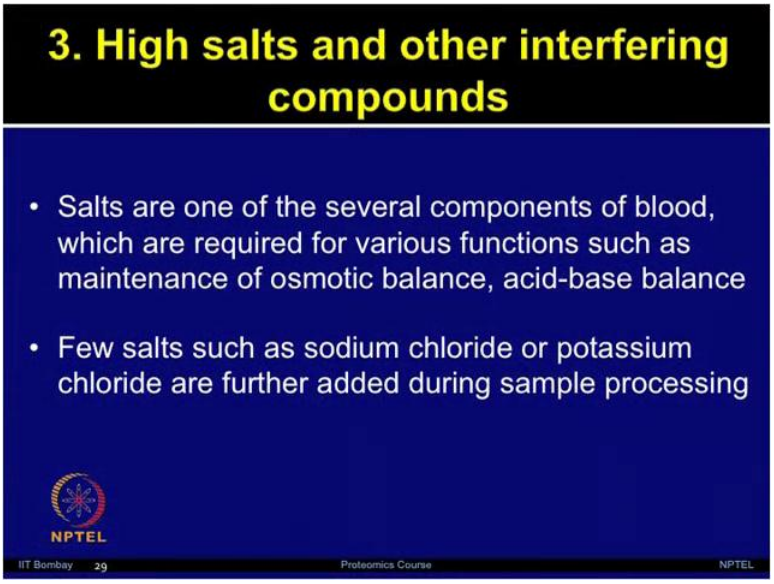

The third point the presence of high salt and other interfering compounds.As we have talked about different type of interfering compounds, so now you are familiar that, salt are one of the several component, which could be very much interfering during your proteomic application.Now, salts are present in the blood, which are required for various function such as the maintenance of osmotic balance,acid-base balance etcetera.

Few salts such as sodium chloride or potassium chloride are also added when you are processing the serum samples, during this whole sample manipulation due to the intrinsic salt present in the blood as well as, extrinsic salt added during the sample processing.The overall salt component becomes very high and that is and that creates problem for various type of proteomic application. So, high salt and interfering compounds should be removed.

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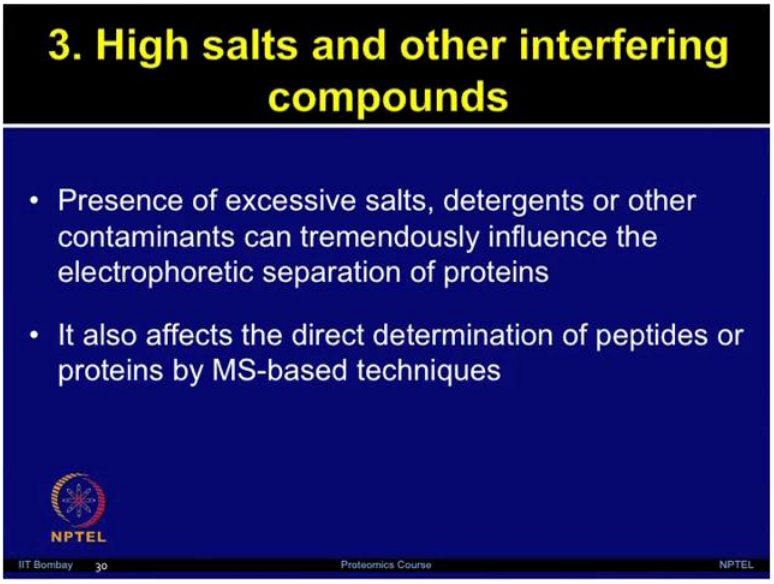

Now, these presence of excessive salt, detergent or other contaminant can tremendously influence the electrophoretic separation of proteins, if your target technology is two dimensional electrophoresis or other gel based method you have to really ensure that, salt is very low in the serum or plasma components.It also affects the direct determination of proteins or peptides by mass spectrometry based techniques. So, regardless of whether you use gel based or gel free method, you have to ensure the overall salt component is removed efficiently from the serum or the plasma.

Now, let us talk about forth point other challenge. So, variations among the individuals and lack of reproducibility, these are some of the very major issues in clinical studies where you have inter and intra individual variation.Intra individual variation is; obviously, more expected, but even within one person, due to diet due to different type of meditation the serum or plasma proteins can be changed.

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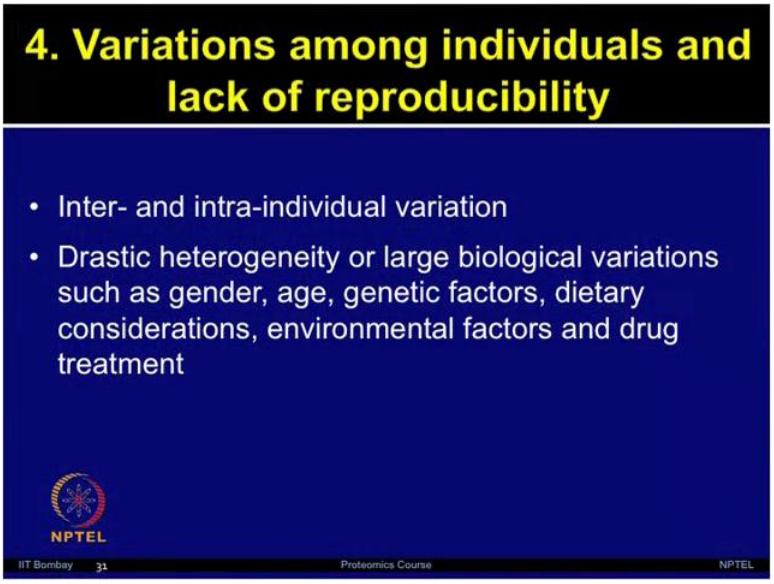

So, how to avoid these inter and intra individual variation and as if you refer to the previous lecture, when we talked about how to minimize different type of these factor which are going to ensure the success for clinical studies. Probably you will be able to keep an eye on different type of extrinsic and intrinsic influences, which one should try to remove as much as one can, during the clinical proteomic analysis.

So, the drastic heterogeneity or large biological variation such as gender, age, the genetic factors, dietary considerations, environmental factors and drug treatments are going to affect the reproducibility of your experiments. So, if you are careful in designing the experiment, one can at least minimize these variations and increase the reproducibility of proteome analysis.

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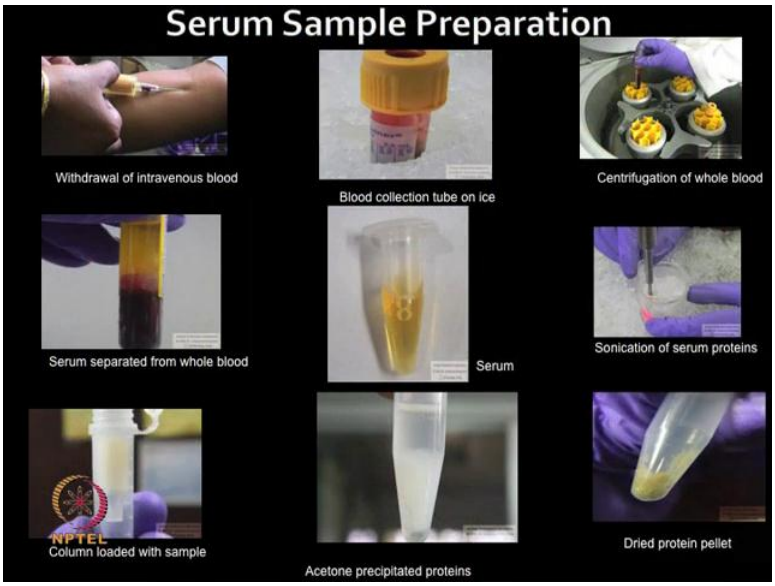

So now, giving you an overview here for the serum sample preparation, the various steps are shown in images such as withdrawal of intravenous blood,blood collection in the tube, centrifugation step of whole blood,how to remove the serum from the whole blood, serum can be transferred into the fresh tubes.

Now, for the serum proteome analysis, different type of modification can be performed such as sonication can be used for disrupting the high abundant proteins and it also helps in better resolution, then depletion strategies to remove the abundant protein, precipitation of these proteins by adding acetone.And then drying out the protein pallet and reconstituting for the in suitable buffer for further proteomic application.

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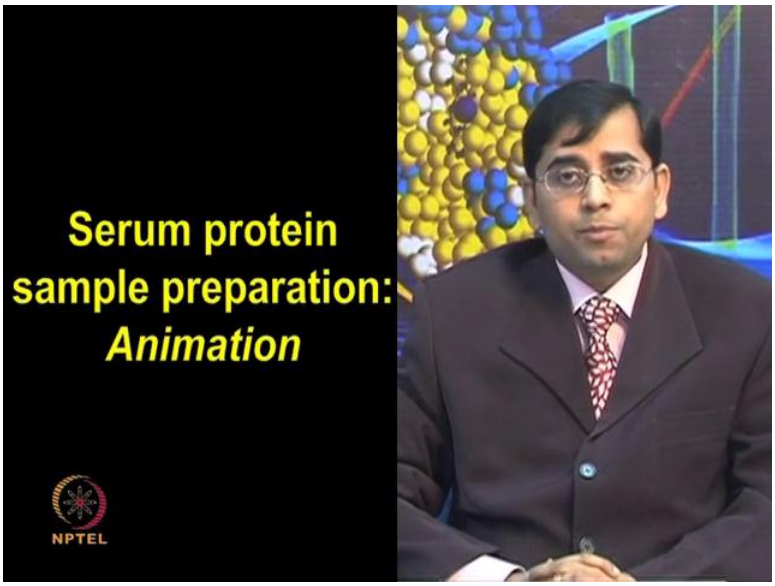

So, let let me show you the detail of serum protein sample preparation in this animation.Serum proteome analyses, in this interactive animation I will show you stepwise how to collect serum and how to perform the proteome sample preparation. So, first click on the syringe, so that blood can be collected.The blood proteome is one of the most complex component of the human proteome, it fluctuate depending on the physiological and pathological conditions of the patient.

So, collect almost 5 m l of blood from the (( )) of healthy or diseased participants using serum separation tube, immediately after the blood collection, the tube should be kept in ice for 30 minutes for clotting, after clotting the sample are centrifuged at 12,000 r p m at 20 degrees for 10 minutes, serum can be separated, and multiple aliquots can be made for further storage at minus 80 degrees.

So, during this animation for the sample preparation, I will describe one method which we are currently using in my laboratory, where we have tried combining different type of methods being available from different published protocols. So, the crude serum can be diluted five times with phosphate buffer p h 7.4ensure that, mixing is uniform and then vertex by clicking on this vertexes here, for 30 seconds, bisodication is performed by using a sonicated for six cycles of 5 second pulse with 30second gap in between at 20 percent of amplitude by sonication step in serum sample is found to be effective in improving the gel quality and resolution.

Now, you can start depletion strategies to remove the abundant proteins.There are commercially available depletion columns, the affinity binders or ligands are immobilized with solid support.The chromatographic medium and used to specifically bind abundant proteins from a complex protein solution. The depletion strategy effectively enhances the resolution and provides, the maximum number of spots on two dimensional gels, thereby a better coverage of the whole serum proteome.

The deputed serum is mixed with ice cold acetone containing 10 percent T C A and after, vortexing the mixture is incubated at minus 20 degrees for protein precipitation followed by centrifugation. After incubation, the tubes can be centrifuged at 12,000 r p m for 15 minutes at 4 degrees, the pellet can be dissolved in (( )) buffer containing 8 molar urea,2 molar thiourea,4 percent CHAPS, 2 percent I P G buffer,40milli molar of D T T; and trace amount of bromophenol blue you can then, again perform the vortexing step and keep this extract in minus 80 degrees until further use. So, I hope in the animation you are able to understand, how to go step by step to perform the serum proteome analysis.

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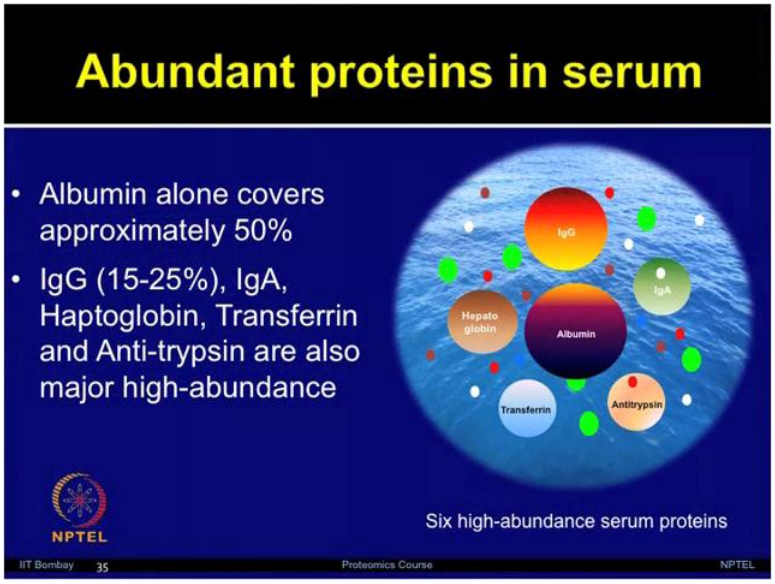

Now, as I promised previously I will talk to you about how to remove the high abundant proteins which are present in the serum. So, as we talked, there are more than 20abundant proteins which are present in the serum.And albumin alone covers approximately 50 percent of these abundant proteins.Immunoglobulin G contains 15 to

25 percent of the abundant proteins, then I g G, Haptoglobin, Transferrin and Anti-trypsin, these are also major high abundance proteins.

See, in the diagram I have shown you, the sixhigh-abundance serum proteins.The different ways people have tried to remove these abundant proteins, molecular weight cut off and different type of chromatography methods have been used. The affinity chromatography based methods are highly efficient for specific removal of these abundant proteins, which are present in the serum. Because, there is always at fear with the molecular weight cut off whether along with albumin and other high molecular weight proteins, you will also get rid of your various high molecular weight protein, which are non abundant.

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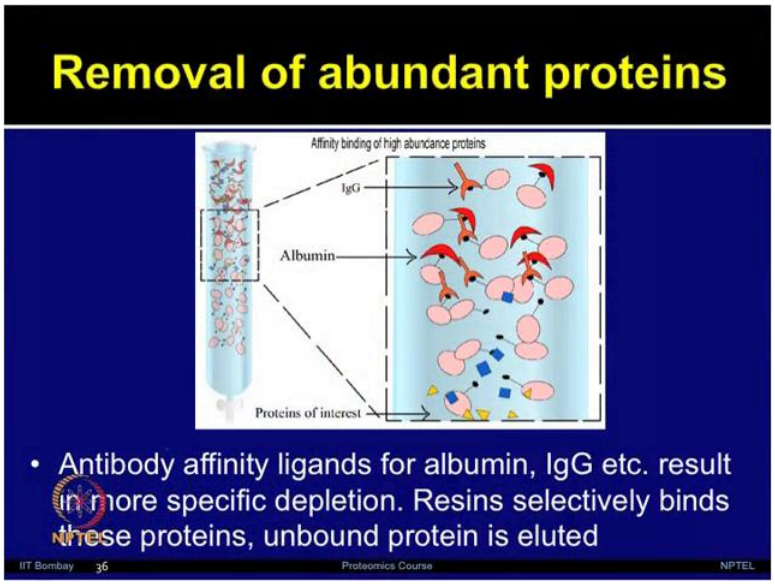

So, the affinity based methods ensure the specific targeting of albumin, I g G and other specific abundant proteins.The antibody affinity ligands are used for albumin, I g G and other abundant proteins and it resultinto various specific depletion.

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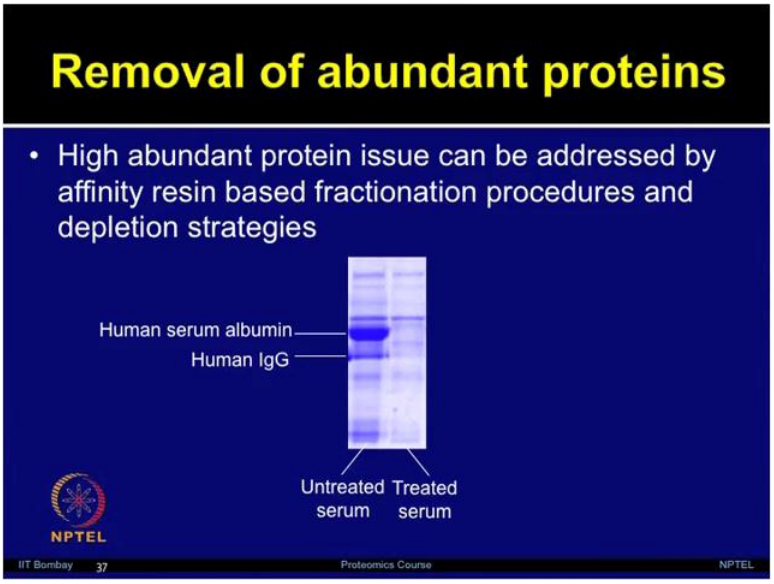

These resins can selectively bind to these proteins, and unbound proteins can be eluted in suitable buffer by applying the affinity resin based fractionation method and different type of depletion strategies.One can get rid of the high abundant proteins as shown in this image of S D S page gel, the left lane is loaded with the untreated serum and the right lane is loaded with the treated serum.As you can see, some of the very high abundant proteins such as albumin, I g G, those are efficiently removed and which allow for some low abundant proteins to appear on the gel.

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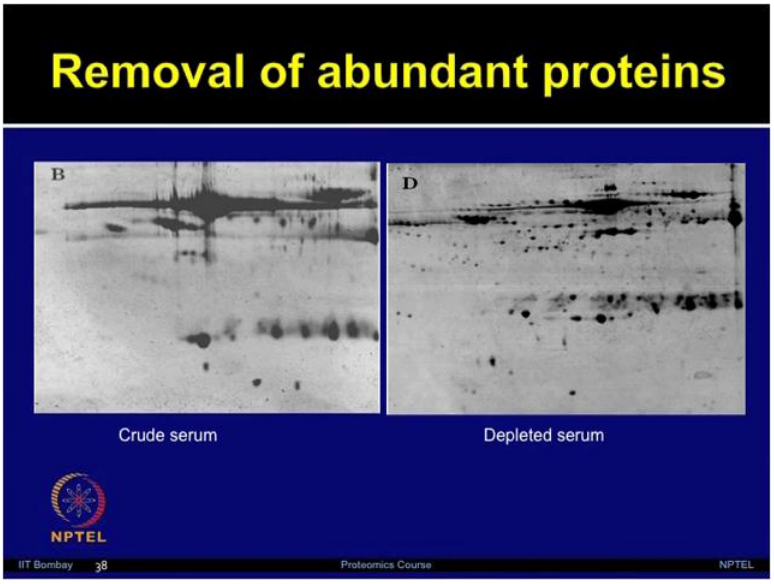

I am showing you image of two dimensional electrophoresis gel, which we will discuss in more detail in the next module of two dimensional electrophoresis, but in these gels from the left and right you can see, the left panel is shown for crude serum and the right one is shown for the depleted serum.In the crude serum, the albumin and other abundant proteins have resulted into very much masking of other proteins and the isoelectric focusing and the gel quality is also not very good. So, streaking and different type of artifacts can be seen on this gel whereas, the right hand depleted serum to the gel shows better pattern.

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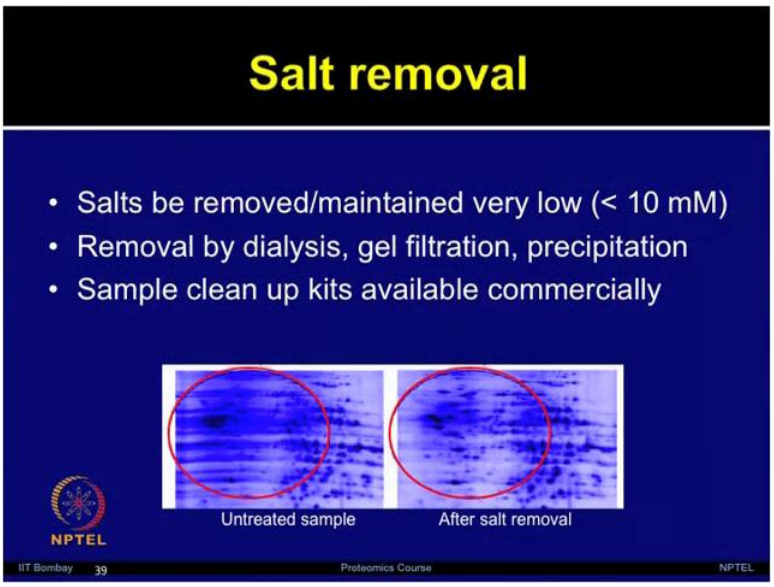

Salt removal that is definitely one of the very important step I hope by nowit is you are convinced that, you need to very efficiently get rid of all the salt components present in your serum or plasma samples. The salts can be removed by various type of dialysis and other resin based methods and you need to ensure that, the overall salt level is less than 10milli molar, if your aim is to perform isoelectric focusing.

There are different type of commercial clean up kits are also available and since they avoid making the samples very dilute and at the end you can obtain high concentrated sample, because you can elute your sample in a very less volume,these commercial methods are also very efficient.Now, I have shown you two gels from the untreated and treated salt removal gel and as you can see, this streaking is very high in the left panel, when there is a high amount of salt present, but in the right hand panel of the gel after

salt removal or desalting strip, one can see this streaking has been removed and different proteins can appear in the same region of the gel.

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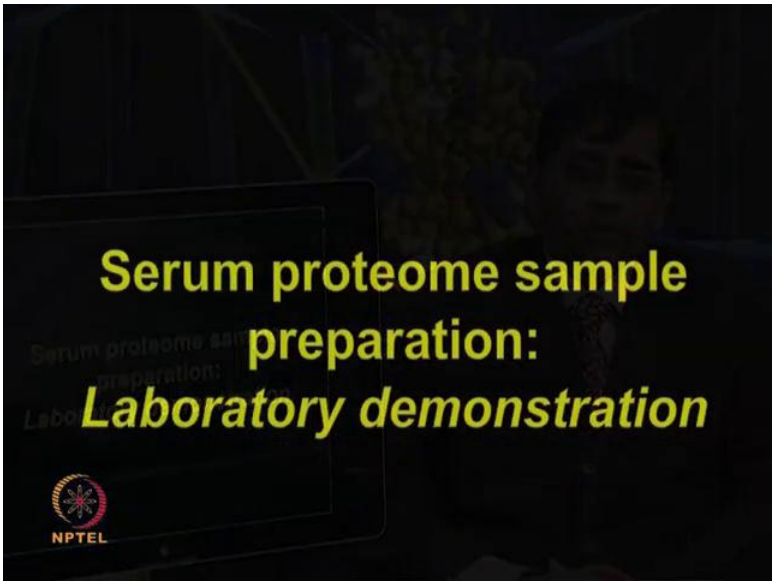

So, now let me show you the laboratory demonstration of serum proteome sample preparation, by watching this video you should be able to learn the very minute details about performing the serum sample preparation and how one can use that, for further serum proteome analysis.

Serum protein extraction, processing of serum involves blood collection and separation, sonication of serum, depletion of high abundance proteins and precipitation of proteins, carefully withdraw around 4 m l of intra-venous blood into a vacutainer tube, place the tube on ice for an hour immediately after collection to allow the blood to coagulate, centrifuge the tube for 10 minutes at 2500 r p m to separate the coagulated blood, the blood cells along with the clotting factors form the pellet while the serum containing proteins of interest forms a clear supernatant.

Transfer this supernatant containing serum into fresh tubes, these can be stored at minus 80 degree Celsius until required for further use, remove the serum sample from the freezer atleast 15 minutes before performing the experiment, place it on ice and allow it to thaw before use, then transfer the required amount of serum into a fresh clean tube.

Sonication, dilute the serum sample five times with phosphate buffer of p h 7.4, vortex the sample to allow complete and uniform mixing, place the tube on ice and sonicate the serum by providing six cycles of 5 second pulses at 20 percent amplitude with a 59 second gap in between each cycle.

Depletion of high abundance proteins, serum contains several proteins in a wide range of concentrations of these albumin and immunoglobulin G are found to be the most abundant, these proteins may interfere with the gel pattern and experimental analysis while trying to detect other proteins present at lower concentrations.It is therefore, preferred to remove these high abundance proteins before electrophoresis, depletion columns for removal of high abundance proteins are now available commercially, open the cap of the column, place it in a tube and centrifuge it after addition of phosphate buffer, discard the liquid that remains in the collection tube, then at the binding buffer provided with the kit to the column and centrifuge the tube.

Next add the serum sample to the column and incubate on ice for 5 minutes, this allows the high abundance proteins to bind to the affinity matrix of the column, while the rest of the proteins remain unbound.Again centrifuge the column and collect the serum that remains at bottom of the tube for further processing. Protein precipitation, transfer the serum depleted of high abundance proteins into a fresh tube, to this add a mixture of trichloroacetic acid and acetone and mix the contents well.

The solution gradually becomes turbid due to precipitation of proteins, place this tube at minus 20 degree Celsius for at least 4 hours before centrifugation to enable complete precipitation of proteins, then remove the tube and centrifuge the contents to allow the proteins to settle down as a pellet, carefully discard the supernatant and dry the pellet at room temperature, reconstitute this dried pellet with the rehydration buffer, vortex the tube to ensure uniform mixing, store the reconstituted protein solution at minus 80 degree celcius until performing the next step of protein quantification.So, I hope your video demo was effective for you to demonstrate the steps for serum proteome analysis.

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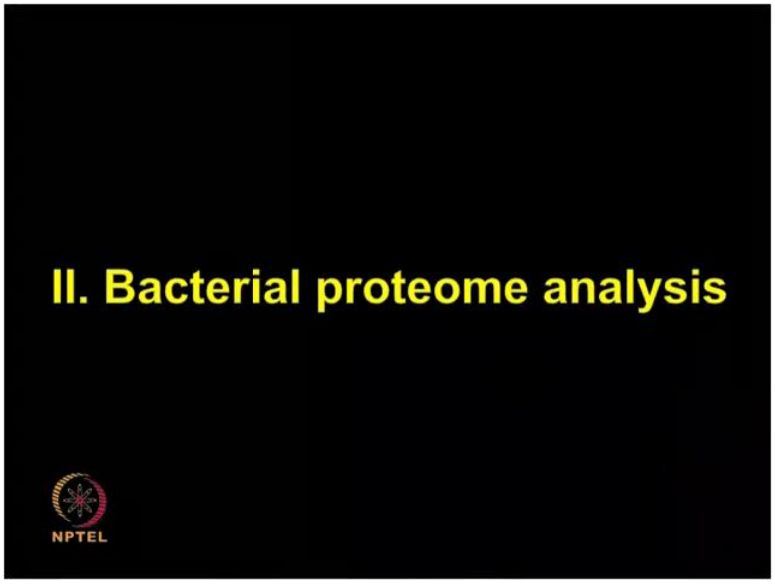

Now, let us move on to second example of how to perform bacterial proteome analysis.

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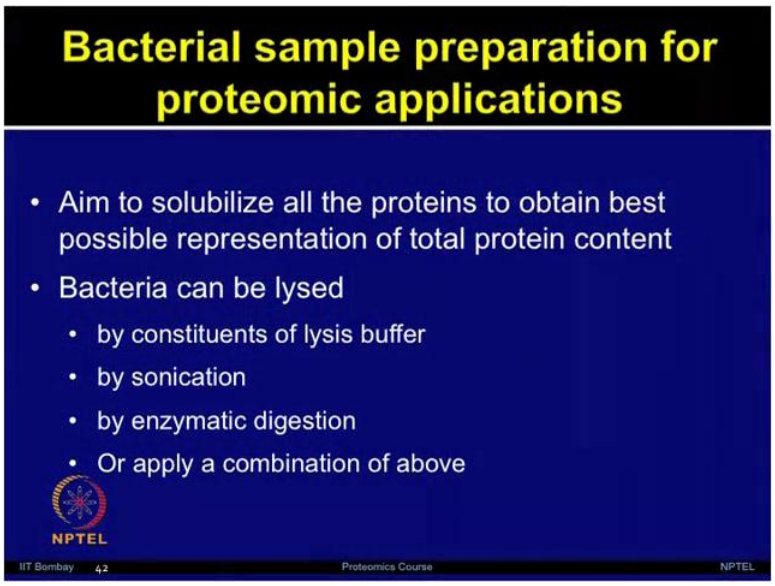

Let us talk about the sample preparation for bacterial proteome applications, similar to other samples aim is to solubilize all the proteins because, if you are aiming for a proteome level analysis, you would like to obtain all the proteins, so that you can represent all the possible proteins on gel map or by using a l c m s approach.

Now, bacterial lysis is often very challenging for different type of bacteria, but people have tried different type of lysis methods such as constituents of lysis buffer can be used

for lysis. Sonication is very efficient, people have also used enzymatic digestion methods as well as, sometime a combination of these methods have been applied for bacterial lysis.

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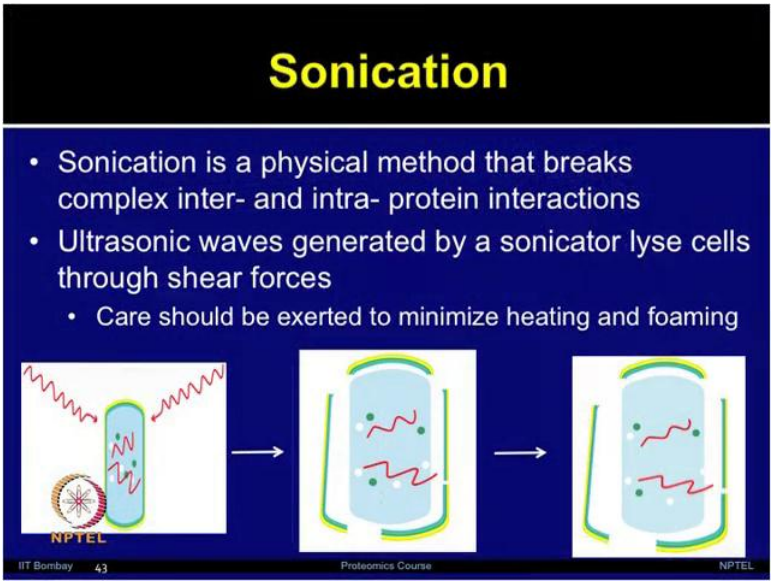

Let us, talk about sonication, which is very commonly used, if you are lysing the bacterial cells.Sonication is a physical method which breaks the very complex inter and intra protein interactions.The ultrasonic waves generated by a sonicator lyse the cells through shear forces, one has to take extreme care while performing these steps in the cold condition, so that the heating effect and foaming effects can be minimized.

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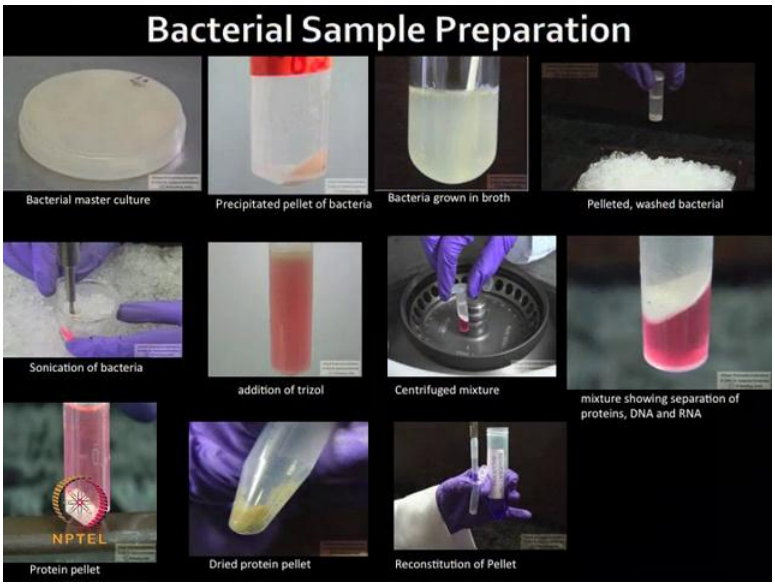

So now, I am giving you a pictorial overview of bacterial sample preparation.First you need to inoculate a culture, after obtaining the right colonies you need to grow the culture obtained the bacterial pellets, then you need to wash the bacterial pellet, sonicate bacterial pellet in the preferred different type of lysis buffers.

Then you add (( )) we are showing you one protocol, which is efficient for performing bacterial proteome analysis, but there are different type of protein extraction methods available for bacterial proteome analysis, after trizol addition, the centrifugation steps where one can see the layers of D N A, R N Aand proteins; and if you do not remove the correct layer, then you may result ultimately into some contaminations, coming either from phenol or from the interfering substances such as D N A or R N A. We will talk about all of this in much more detail in the next class.

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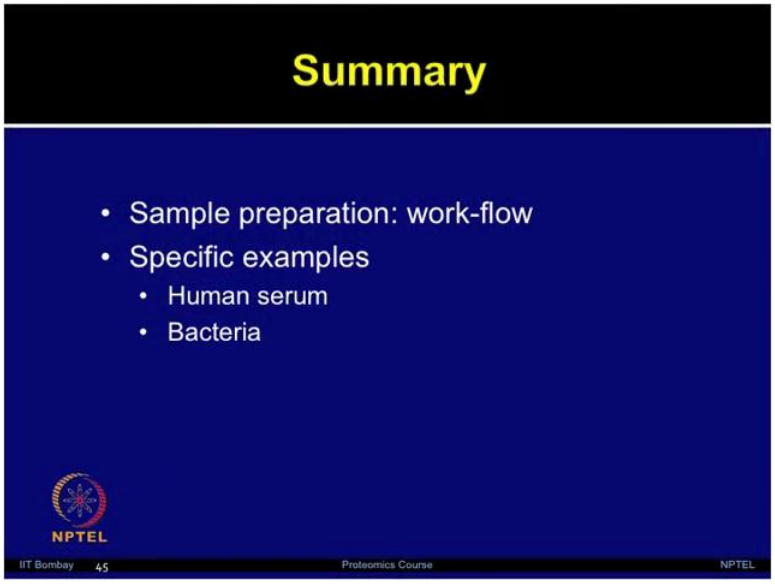

So, in summary today we have discussed about the sample preparation work-flow which was continued from the last lecture.We talked about different type of precipitation methods, which one can be used for different type of applications, we compare different methods, then we talked about removal of interfering components; and we also tried to look for what are the pros and cons for each of those components. Then, we moved on to looking at the specific examples I gave you demonstration for how to perform human serum proteome preparation and analysis, then we moved on to bacterial proteome sample preparation.

We will continue our lecture and in the next class, we will talk about the plant proteome analysis, followed by quantification.And once you have obtain a very good quality and quantity of the protein, then you are ready to perform the proteome experiment by using different type of techniques.Then, we will start discussing about two dimensional electrophoresis and other gel based proteomic technologies and then, subsequent lectures we will talk about mass spectrometry based methods, thank you.