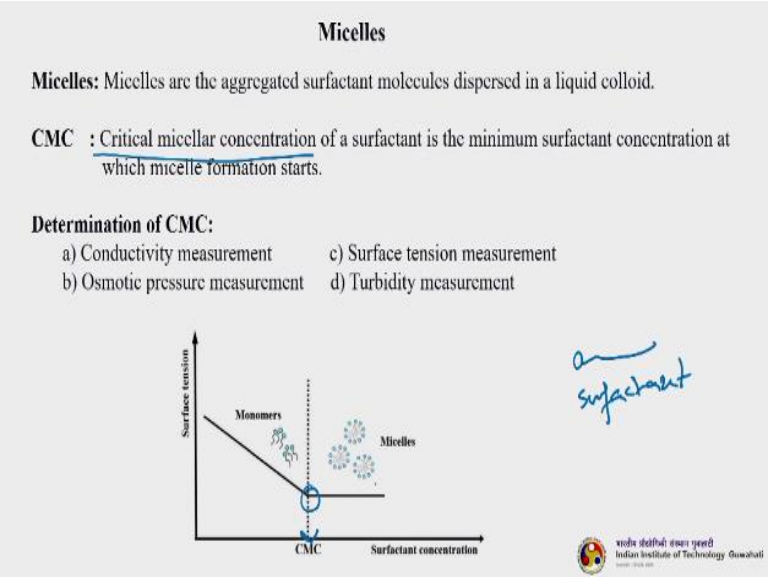
Membrane Technology
Prof. Kaustubha Mohanty
Department of Chemical Engineering
Indian Institute of Technology, Guwahati

Lecture-22
Micellar-enhanced UF, affinity UF, UF based bioseparation

Good morning students, today is lecture 22 under module 8. In today's lecture, we will discuss two most important applications. Or you can say types of ultrafiltration and of course applications, so the first one is called Micellar-enhanced ultrafiltration and the second one is affinity based ultrafiltration and then subsequently in today's lecture also will discuss about bioseparations.

Because you know bioseparation is one of the field in which the application of membrane technology has been successfully commercialized. And one of the foremost important of, you can say membrane technology application, long back it started with protein separation. So we will try to understand, what is the little basics of bioseparation? And then we will see how ultrafiltration is helping in bioseparation application.

(Refer Slide Time: 01:19)



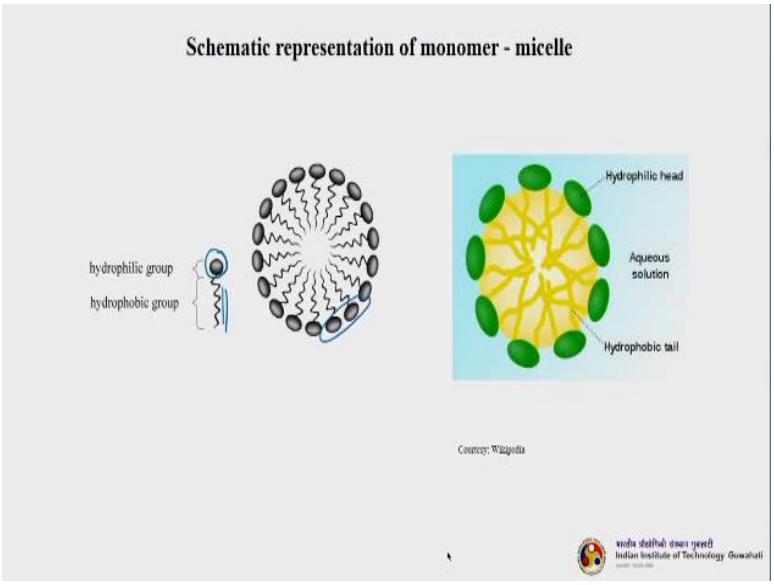
So let us understand what is actually Micellar-enhanced filtration? So before we discuss that we should understand, what is Micelles? So what are Micelles? The Micelles are actually the aggregates of the surfactant molecules, which are dispersed in a liquid colloid. So, basically a

surfactant molecule will be looking something like this. There is a head and there is a tail. So this is a typical surfactant molecule.

So when this surfactants, the dispersion aqueous media and beyond certain concentration, they will forms Micelles. And that concentration beyond which the surfactant forms Micelles are called CMC, critical Micellar concentration. And how do you determine CMC? So we can do it by measuring conductivity we can measure osmotic pressure, we can measure surface tension or we can measure turbidity.

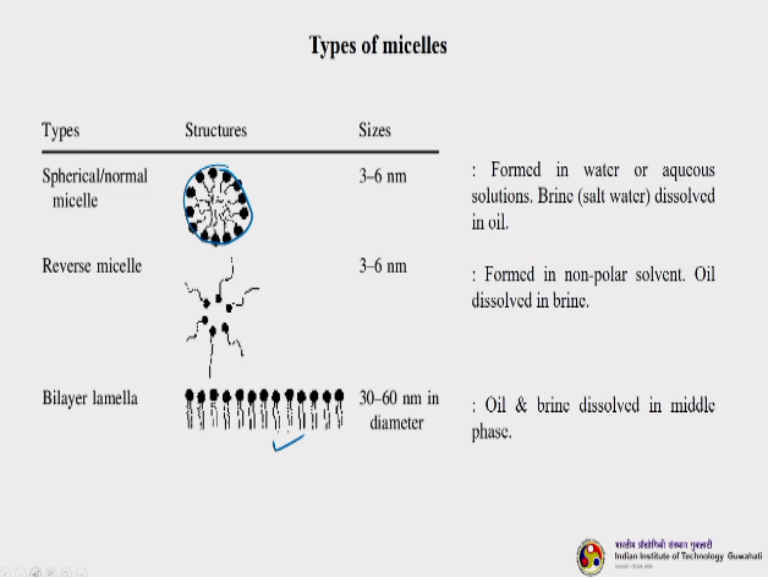
You can see this particular figure here, which is plotted between surface tension and surfactant concentration and you see these are the monomers which I was just showing. So, this is the CMC concentration here. Beyond this the Micelles are getting formed.

(Refer Slide Time: 02:28)



So this is schematic representation of monomer Micelle, how the Micelles are getting formed? Say this is the hydrophilic group or the head and this is the tail of the surfactant which is hydrophobic, this one and this is how the form in a aqueous media. This is another representation where the hydrophilic heads are exposed outside, so these are outside and their tails are getting joined and this is your aqueous solution.

(Refer Slide Time: 02:55)

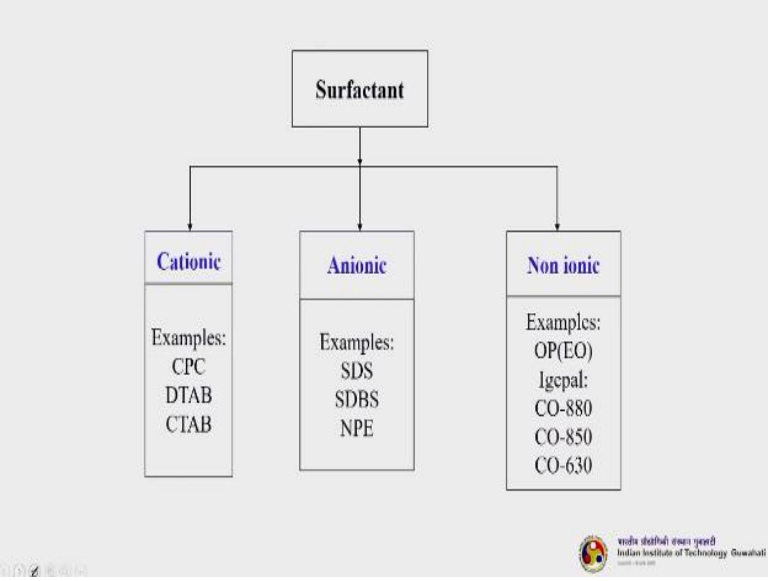


There are different types of Micelles, so we will not go into details about Micelles and different properties because that itself will take so much of time, and it is of not much of interest to us. We will just try to understand, what is Micelles? How they look like? How they function? And how it is helping in our ultrafiltration? So just quickly we will go through different types of Micelles. There are three different types of Micelles.

One is the spherical or normal Micelles usually 3 to 6 nanometres. So they are formed in water or in aqueous solutions. So brine or salt water dissolved in oil is one typical example. Then there are reverse Micelles, so you have seen here in the normal Micelles the tales are joining together and the head is exposed outside, the head is outer side here. But here it is reverse Micelle the head is inside and tails are outside.

So usually 3 to 6 nanometer, formed in non-polar solvent oil dissolved brine is an example. Then there are Bilayer lamellas, so usually 3 to 60 nanometer in diameter oil and brine both dissolved in middle phase will give us something like this, so you can have a structure, something like this.

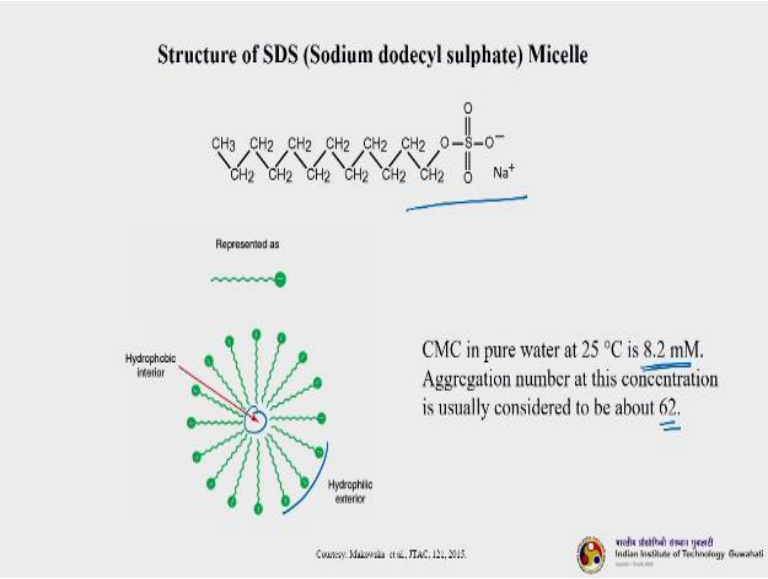
(Refer Slide Time: 04:05)



So let us understand different types of surfactants, basically we have cationic, anionic and non-ionic. So in cationic we have so many different types, CPC is one of the most widely used surfactants which is used in Cetyl- pyrdinium chloride. It is used in mouthwash, the toothpaste and some other applications. Then in anionic, we have SDS, one of the most important surfactants, sodium dodecyl sulfate.

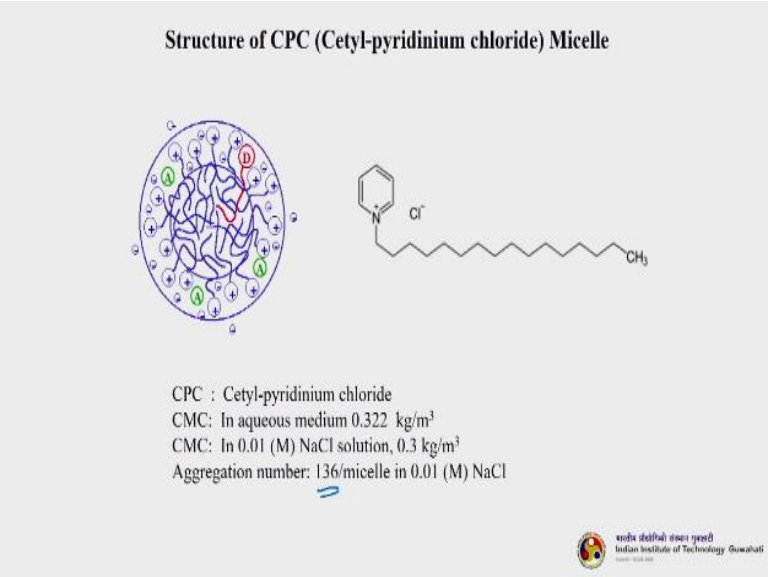
So this is used in most of the detergents powders and it has other applications also then we have different non-ionic surfactants.

(Refer Slide Time: 04:38)



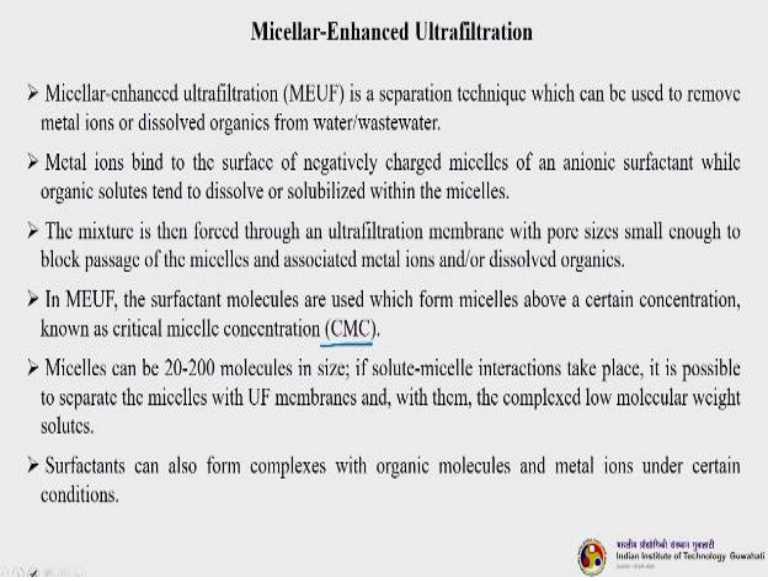
So we will see how the structure of SDS looks like. So this is your SDS structure can be represented as a tail and head. So when a CMC in the critical Micellar concentration of SDS in pure water at 25 degree centigrade is 8.2 mill molars. So beyond this 8.2 mill molar they will form Micelles and the aggregation number is about 62 you can see how the SDS will form Micelles. The heads are outer side and the tails are joined here, so this is your hydrophobic interior when this is the hydrophilic exterior.

(Refer Slide Time: 05:17)



Similarly this is CPC, Cetyl- pyrdinium chloride, Micelles, this is the curve structure and this is how they are forming Micelles, so you can see here heads are outside and tails are inside. So CMC is about 0.322 kilo per meter cube and CMC is in aqueous medium and 0.1 mole sodium chloride solution, in if you are using a brine solution of 0.1 sodium chloride solution, then the CMC is 0.3 kg per meter cube and aggregation number is almost 136 Micelles in 0.01 M sodium chloride.

(Refer Slide Time: 05:55)



Now let us discuss the Micellar enhancement ultrafiltration, how these Micelles are helping in ultrafiltration operation to remove certain compounds. Now MEUF as popularly known is a separation technique which can be used to remove metal ions or dissolved organics from water and wastewater. So, metal ions bind to the surface of negatively charged Micelles of an anionic surfactant while organic solutes tend to dissolve or solubilized within the Micelles.

So ions will try to bind to the head actually of the anionic surfactant and while most of the organic solutes will try to dissolve insides in the core of the Micelles are solubilized inside the core of the Micelles. The mixture is then forced through an ultrafiltration membrane with pore size small enough to block passage of the Micelles and associated metal ions and/or dissolved organics.

Now what is happening when the metal ion binding to the heads of the Micelles or the organic compounds which are dissolving themselves or solubilizing themselves inside the core of the Micelles will be retained. By, the ultrafiltration membrane. Why this is Micellar-enhanced ultrafiltration because in normal ultrafiltration if you are not able to separate this, heavy metals or organic compounds.

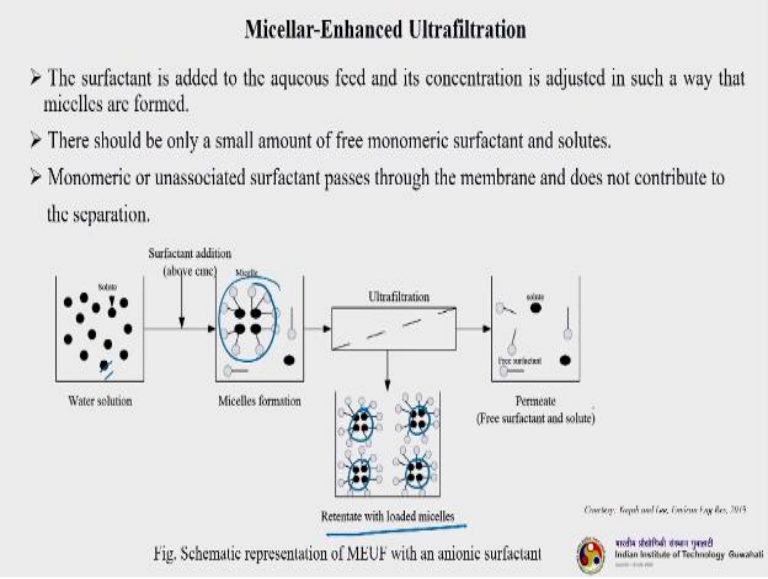
Due to their sizes and the size of the pore and there are other factors also using Micelles what is happening is that the size is so big that it will be retain on the ultrafiltration membrane, of course,

you have to choose a particular membrane cut off again, so that membrane will definitely retain all these Micelles and the Micelles are retaining the heavy metal bound to its surface or organics, dissolved in it.

So the surfactant molecules are used which for Micelles above a certain concentration that we have discussed, of course, it is known as CMC. So Micelles can be 20 to 200 molecules in size; if solute Micelles interaction takes place, it is possible to separate Micelles themselves with ultrafiltration membranes and with them, the complexed low molecular weight solutes. So now you can understand that the solute which are very small size and it is difficult to even separate using ultrafiltration membrane also.

So by using Micelles the Micelles will retain them on their surface ok and will be rejected by the membranes. So surfactants can also form complexes with organic molecules and metal ions under certain conditions.

(Refer Slide Time: 08:14)

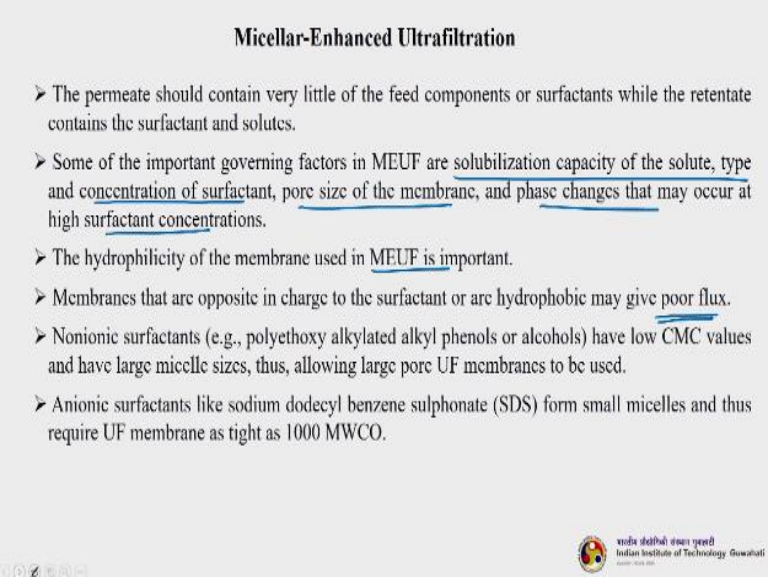


This is how the schematic actually how the ultrafiltration is happening, using the Micelles so surfactant is added to the aqueous feed and its concentration is adjusted in such a way that Micelles are formed. That means you have to add it beyond its CMC, so that should be only small amount of free monomeric surfactants and solutes, as less as possible. Then monomeric or unassociated surfactant passes through the membrane and does not contribute to the separation.

So in the water solution, these are the solutes which we have to separate this black things. Then I add surfactant here beyond the CMC, so now they form the Micelles. Micelles formation and the solute binding to the Micelles. Then we are going for ultrafiltration, so you see that this is your rejected or retentate with loaded Micelles. Now you can see the Micelles are taking the solute inside its core.

This is a schematic representation it can be outside also, inside also depending upon what type of micelle it is, what is the surfactant using? And what is the solute? And then whatever you are getting in the permeated is little free surfactant and some solute of course which are not bound to the Micelles will come out. This is how Micellar-enhanced ultrafiltration happens.

(Refer Slide Time: 09:25)



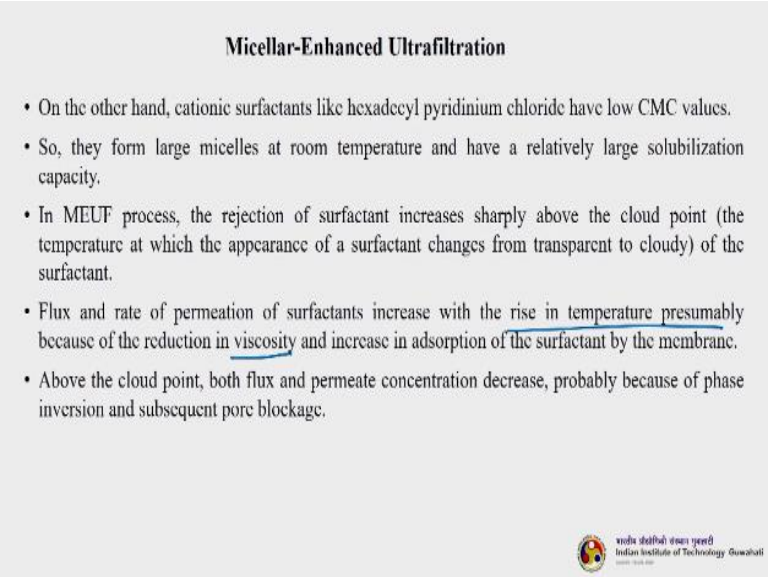
So the permeates should contain very little of the feed components of surfactants while the retentate contain the surfactant and solutes. So some of the important governing factors in MEFU are solubilization capacity of the solute, type and concentration of surfactant pore size of the membrane and phase changes that may occur. Usually in most of the cases phase changes is not happening, but in certain specific application it happens.

So the hydrophilicity of the membrane used is very important. Membranes that are opposite in charge to the surfactant and are hydrophobic may give poor flux. So this is what we do not want,

non-ionic surfactants such as polyethoxy alkylated alkyl phenols or alcohols have low CMC values and have large micelle sizes, thus, allowing large pore ultrafiltration membranes to be used.

So anionic surfactant, like Sodium dodecyl sulfonate forms small micelles and thus require ultrafiltration membrane as tight as 1000 electron molecular weight cut off, actually.

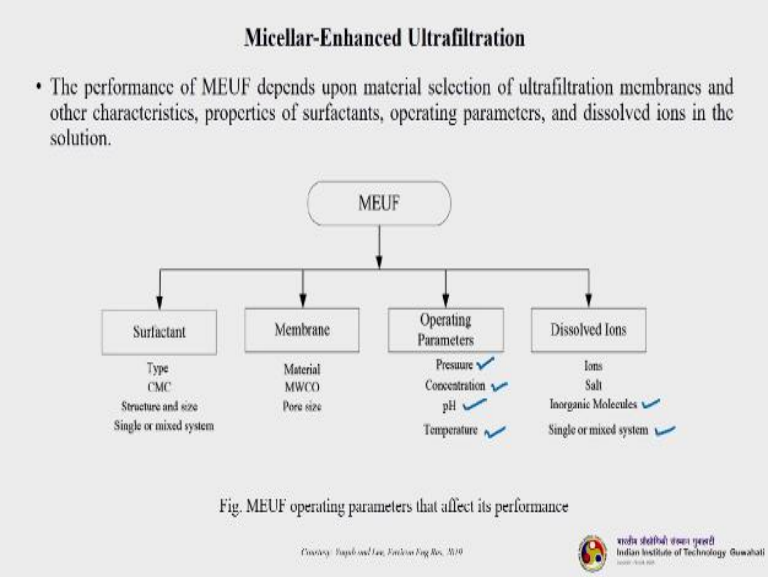
(Refer Slide Time: 10:21)



So on the other cationic surfactants like hexadecyl pyridinium chloride have low CMC values. So they form large Micelles at room temperature and have a relatively large solubilization capacity. Now in MEUF process, the rejection of surfactant increases sharply above the cloud point and the temperature at which the appearance of the surfactant changes from transparent to cloudy of the surfactant.

So flux and rate of permeation of surfactants increase with the rise in temperature, presumably because of the reduction in viscosity. So as you know, when you increase the temperature the viscosity decreases. So, that is how the rate of permission of surfactants increasing, in case you are using high temperature. Above the cloud point both flux and permeate concentration decrease, probably because of phase inversion and subsequent pore blockage.

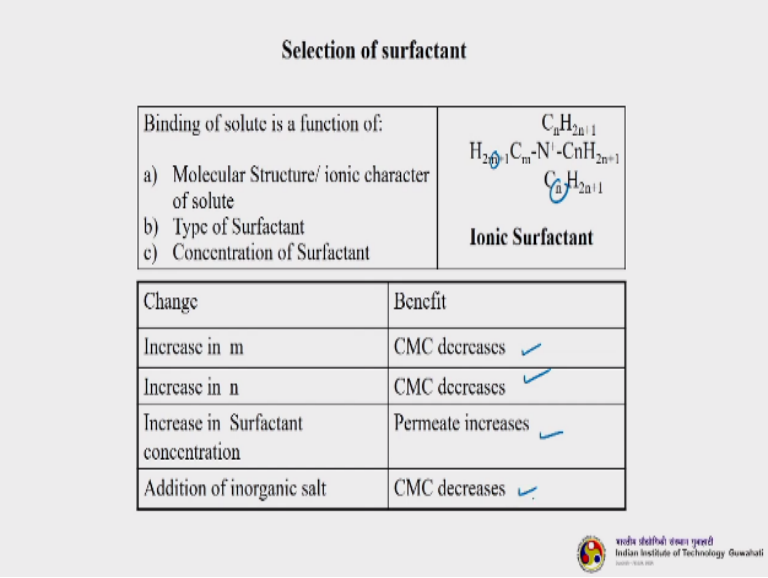
(Refer Slide Time: 11:12)



So the performance of MEUF depends upon material selection of ultrafiltration membranes and other characteristics properties of surfactant, operating parameters and dissolved ions. So you can see, so these are the parameters that are affecting the MEUF performance. First is what surfactant? So that surfactant type, the CMC value, structure and size, whether it is a single surfactant or a mixed surfactant.

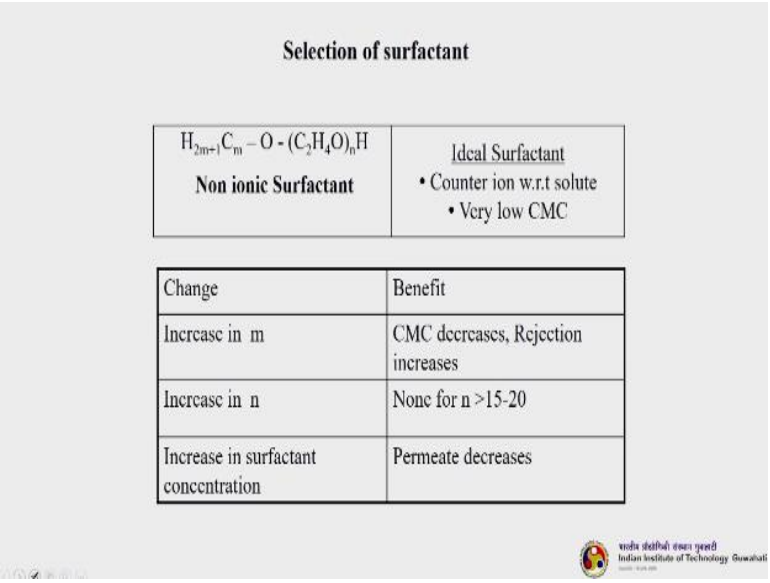
Then membrane; so in membrane, what is the membrane type; material, polymeric ceramic or what it is, its molecular weight cut off, its pore size. Then operating parameters; so the transmembrane, pressure, concentration, pH and temperature. Then we have ions, dissolved ions; so what types of ions? What type of salt are present? Whether they are inorganic or organic whether the single or mixed system. So all these things affect the MEUF performance.

(Refer Slide Time: 12:01)



So, the selection of surfactants; so if this is one of the most important parameter. So binding of solute is a function of molecular structure or ionic character of solute, type of surfactant and concentration of surfactant. This is one typical ionic surfactant example. So you can see that when I increase m, this m, the CMC decreases; even I increase n also CMC decreases. So increasing surfactant concentration permeate increases and addition of inorganic salt CMC decreases.

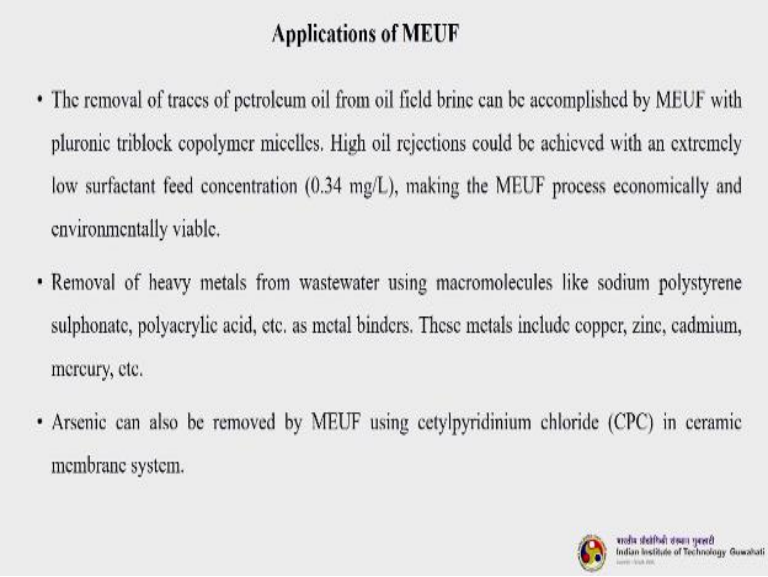
(Refer Slide Time: 12:30)



So another non ionic surfactant we will see; so this is counter ions and with respect to solute, if it is ideal surfactant and has a very low CMC. So when you increase the m, your CMC decreases,

rejection increases and if you are increasing n, for n greater than almost 15 to 20. So an increase in surfactant concentration permeate decreases.

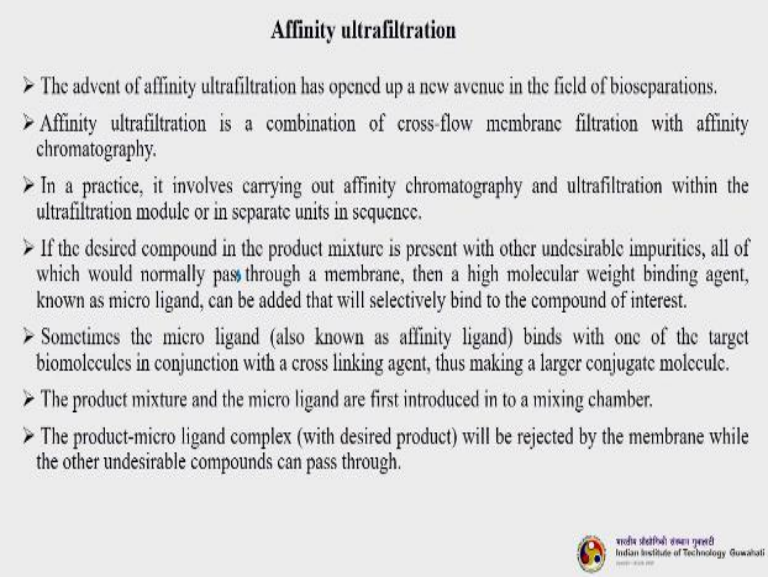
(Refer Slide Time: 12:55)



So applications of MEUF mostly, there are many applications but mostly restricted to remove low molecular weight solutes; heavy metals and some organic components. So a removal of traces of petroleum oil from oil field brine can be accomplished by MEUF. This is also one typical application where the concentration is very small, so MEUF actually placed a good role. So high oil rejections could be achieved with an extremely low surfactant feed concentration just 0.34 milligrams per litre.

Making the MEUF process economically and environmentally viable. Then removal of heavy metals from wastewater using macromolecules like sodium polystyrene sulfonate, polyacrylic acid etc as metal binders. So these molecules will bind to the metals and then they will form Micelles and they will get rejected. So some of the metals includes copper, zinc, cadmium, mercury etc. Arsenic can also be removed by MUEF using Cetyl-pyridinium chloride, CPC using in, ceramic membrane system. it is well established.

(Refer Slide Time: 13:59)



So, the next application of ultrafiltration or a type of ultrafiltration, we can say is affinity ultrafiltration. Now the advent of affinity ultrafiltration has opened a new avenue in the field of bioseparations, because it was initially designed or used for the separation of proteins. So affinity ultrafiltration is a combination of cross flow membrane filtration with affinity chromatography. So in practice, it involves carrying out affinity chromatography and ultrafiltration within the same ultrafiltration module or in separate units in sequence.

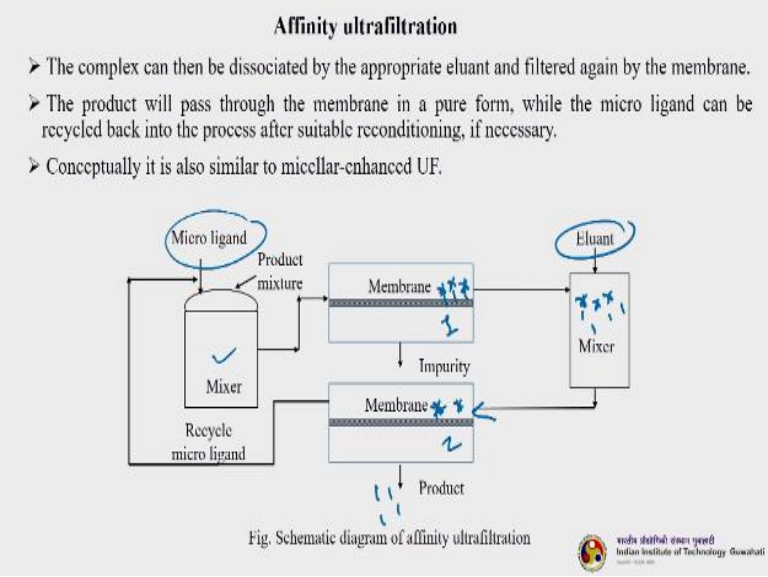
So either in the same module, UF module in which your affinity chromatography as well as separate ultrafiltration both are taking place or you can have a separate ultrafiltration module and a separate affinity chromatography module. So it actually depends on what type of application, you are going to use it in what type of applications, and what is the intended solute of rejection? So if the desired component in the product mixture is present with other undesirable impurities, all of which would normally pass through a membrane.

Then a high molecular weight binding agent, known as micro ligand, can be added that will selectively bind to the compound of interest. Suppose my compound of interest, let us say a particular protein is present with the several other solutes, some are higher molecular weights; some are low molecular weight then that particular protein. So in this case what is necessary is that, so you can have some sort of ligands or micro ligands, so what and what are they all?

So you choose the ligand in such a way that these ligands will bind to your protein of interest, selectively, thereby not binding to other solute which are present then it can be separated. So these ligands are called affinity ligands, so binds with one of the target biomolecules in conjunction with a cross linking agent thus making larger conjugate molecule, so when the binding takes place the overall size of this binded molecules becomes bigger, so they can be easily retent by the ultrafiltration membrane.

The product mixture and the micro ligand are first introduced into a mixing chamber. The product micro ligand complex will be rejected by the membrane while other can pass through, let us see the;

(Refer Slide Time: 16:19)



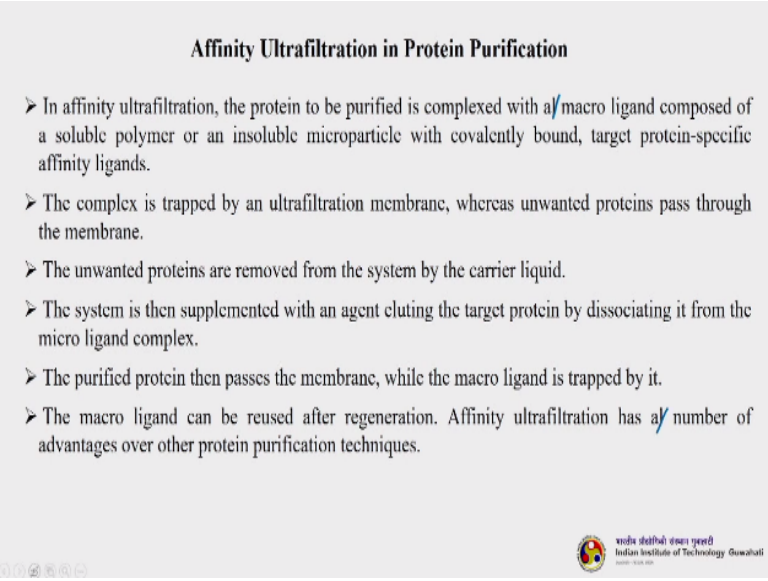
Schematic actually how it happens, so the product mixture that means in which your product is there and that you will mix with the micro ligand in a mixer. Then that goes to the membrane 1 and membrane 2. So in which you are retaining your, this one what is this is called product of interest because that is getting bound to the micro ligand. So your micro ligand and the product mixture will be retain on the surface of the membrane.

Now that goes to a mixture here so here I am adding an eluant, what this eluant will do? So this eluant will separate the ligand from the product they are separated. Now, let us say so this. So let this, here it is coming now, so it is separated into different sizes. It is done. Let us say now

membrane here. Now this membrane will separate these ligands which will be retent here and this is a product of interest that will come here, ligands will be separated.

Then ligands can be again recycled back, so this is conceptually similar to your Micellar-enhanced ultrafiltration, the only thing is that in here, the ligand will be bound to your product of interest and in a subsequent arrangement you are adding a buffer or a eluant which will release this products from the ligands. And then they are getting separated by another membrane system.

(Refer Slide Time: 17:56)

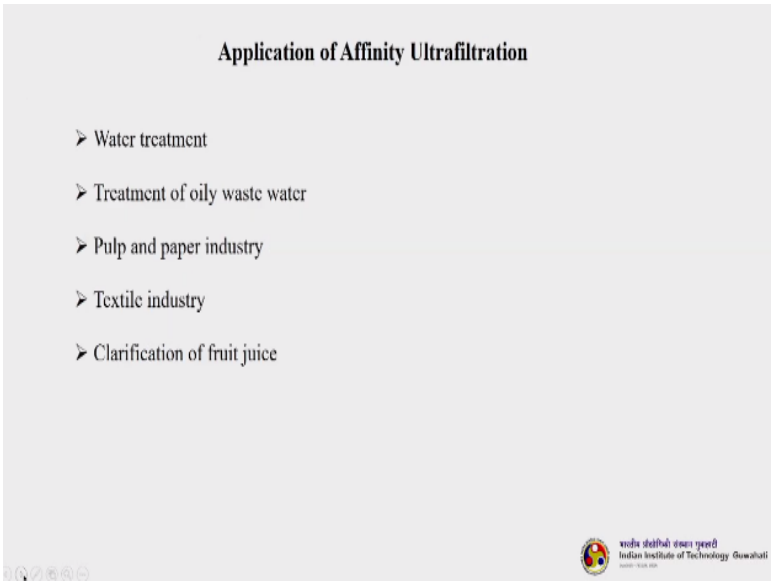


In affinity ultrafiltration, the protein to be purified is complexed with a macro ligand. composed of a soluble polymer or an insoluble microparticle which covalently bound, target protein specific affinity ligands. So we can have ligands with a very, very specific for a particular product or protein and it will be bound only to that proteins, so that is the beauty of this type of application actually.

So the complex is trapped by an ultrafiltration membrane, whereas unwanted proteins pass through the membrane. The unwanted proteins are removed from the system by a carrier liquid. The system is then supplemented with an agent eluting that target protein by dissociating it from the micro ligand complex. The purified protein then passes through the membrane while the micro ligand is trapped by it.

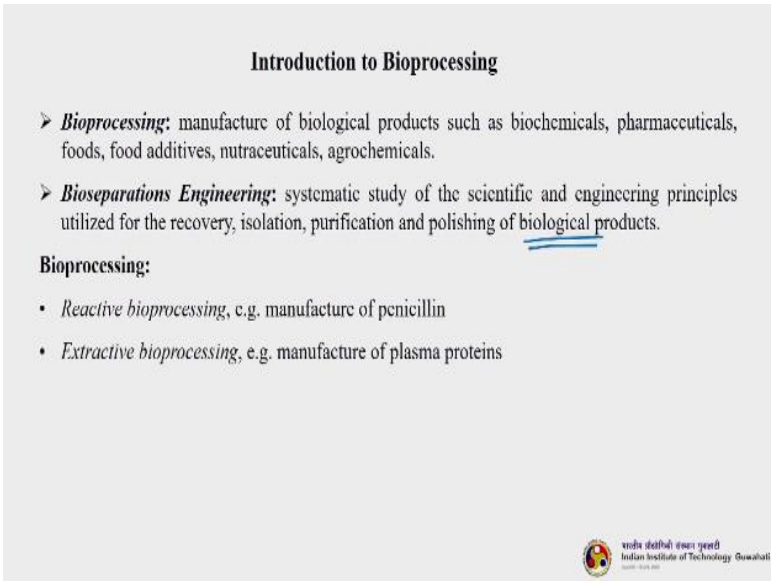
Now this micro ligand can be reused after some regeneration. The affinity ultrafiltration has a number of advantages over other protein purification techniques.

(Refer Slide Time: 18:52)



So the applications are; water treatment, treatment of oily waste water, pulp and paper industry, textile industry, and clarification of fruit juices.

(Refer Slide Time: 18:59)



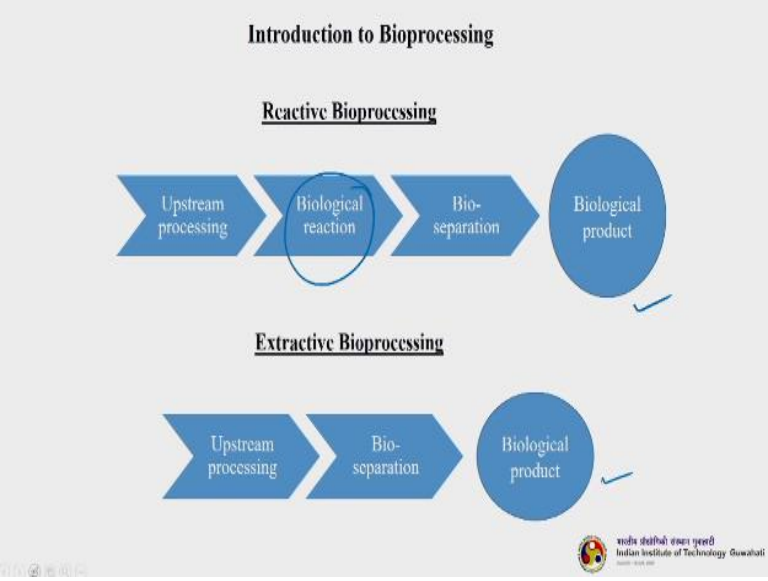
Now let us discuss bioprocessing; so you know in my introduction class I told you that membrane technology has a lot of application in bioprocessing industries, biotechnology industry, pharmaceutical industries, where we are dealing with various types of biological

macromolecules. So that is why it is very important to understand what are bioprocessing? What are the different types of bioprocessing, and what is bioseparations?

What are the different types of bioseparation technologies available? And how ultrafiltration is going to help us? So let us understand; so bioprocessing is the manufacture of biological products such as biochemical, pharmaceuticals, foods, food additives, neutraceuticals and agro chemicals, and what is bioseparation engineering? The systematic study of scientific and engineering principles utilized for the recovery, isolation, purification and polishing of biological products.

Now, please do not confuse bioseparation with separation of some waste things using a biological method, so those are biological wastewater treatments. So biosorption and these things,those are different do not confuse that. So there are two types of bioprocessing; one is reactive bioprocessing as per example a manufacturer of penicillin and we have extractive bioprocessing as per example plasma proteins.

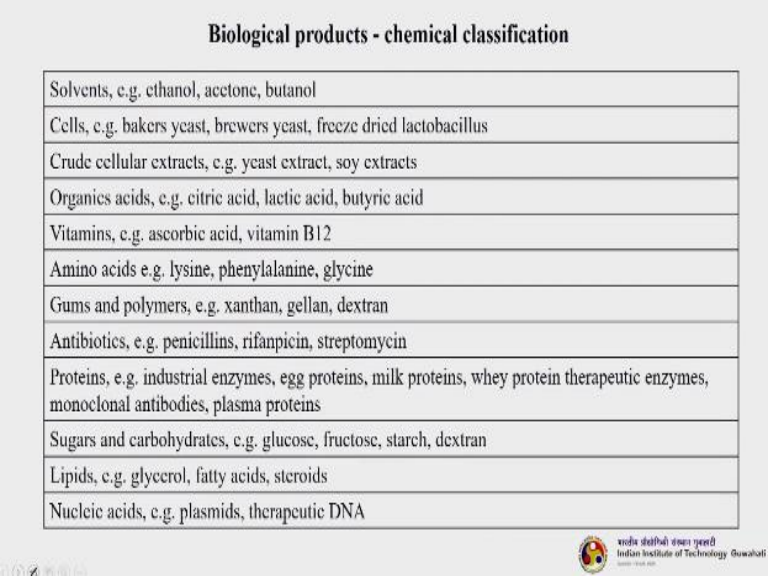
(Refer Slide Time: 20:19)



So in reactive bioprocessing the scheme is something like this, so there is an upstream processing then you go for a biological reaction. Some reaction is happening, during which the product is getting formed, then you undergo bioseparation and you get your product. So that reaction is not present in the extractive bioprocessing, so it is upstream processing bioseparation you get your,

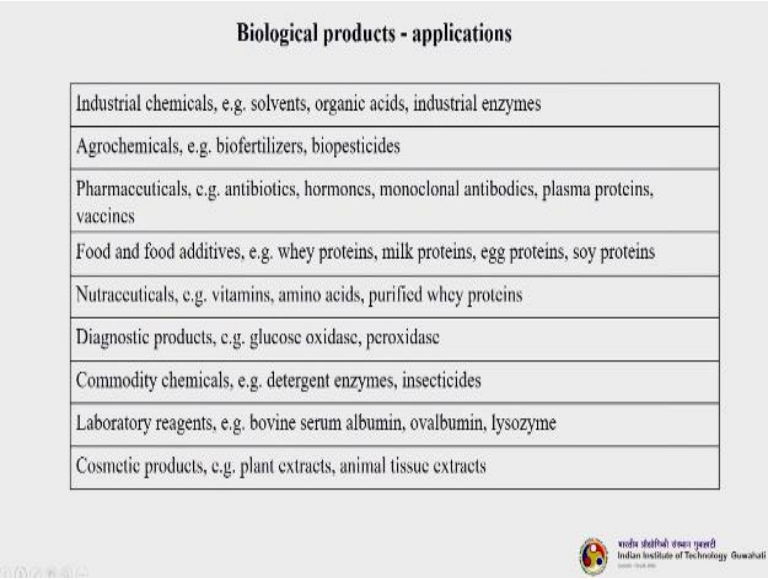
biological product. So the difference between these two is basically; the biological reaction that is happening in the reactive bioprocessing.

(Refer Slide Time: 20:47)



So there are different types of biological products, according to the chemical classification, they can be solvents, there may be cells also like yeast, there are crude cellular products like yeast extract, soy extract. Organic acids, like citric acid, lactic acid, they can be vitamins like ascorbic acid, vitamin B12. Amino acids, gums and polymers, antibiotics like penicillin. Proteins like milk protein, egg protein, and several plasma proteins. Sugars and carbohydrates as for example glucose, fructose there can be lipids also glycerol, nucleic acids.

(Refer Slide Time: 21:26)



So applications are; industrial chemicals, agrochemicals, pharmaceuticals, then food and food additives, nutraceuticals, then we have diagnostic products such as glucose oxidase, peroxidase and then we have commodity chemicals laboratory reagents and cosmetic products.

(Refer Slide Time: 21:43)

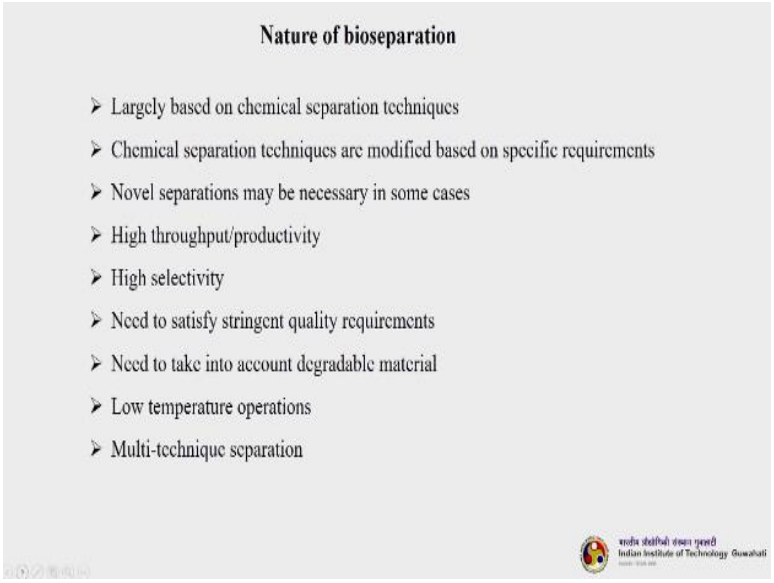
Cost of Bioseparation

Product	Bioseparation cost (%)
Solvents e.g. ethanol, acetone	15-20
Cells, e.g. bakers yeast, brewers yeast	20-25
Crude cellular extracts, e.g. yeast extract	20-25
Organics acids, e.g. citric acid, lactic acid	30-40
Vitamins and amino acids e.g. lysine, ascorbic acid	30-40
Gums and polymers, e.g. xanthan, gellan	40-50
Antibiotics, e.g. penicillins, rifanpicin	20-60
Industrial enzymes, e.g. Amyloglucosidase, glucose isomerase	40-65
Non-recombinant therapeutic proteins, e.g. pancreatin, papain	50-70
r-DNA products, e.g. recombinant insulin, recombinant streptokinase	60-80
Monoclonal antibodies	50-70
Nucleic acid based products	60-80
Plasma proteins, human albumin, human immunoglobulins	70-80

So, let us try and understand why I have given this. You will understand that what is the importance of bioseparation is based on the cost of bioseparation. You can see that the cost of bioseparation is huge for almost all the products. If for the solvents, it is 15 to 20%, for plasma proteins and human albumin it is 70 to 80%. For antibiotics, it is almost 20 to 60%. So the downstream processing cost is extremely high for most of the biological products.

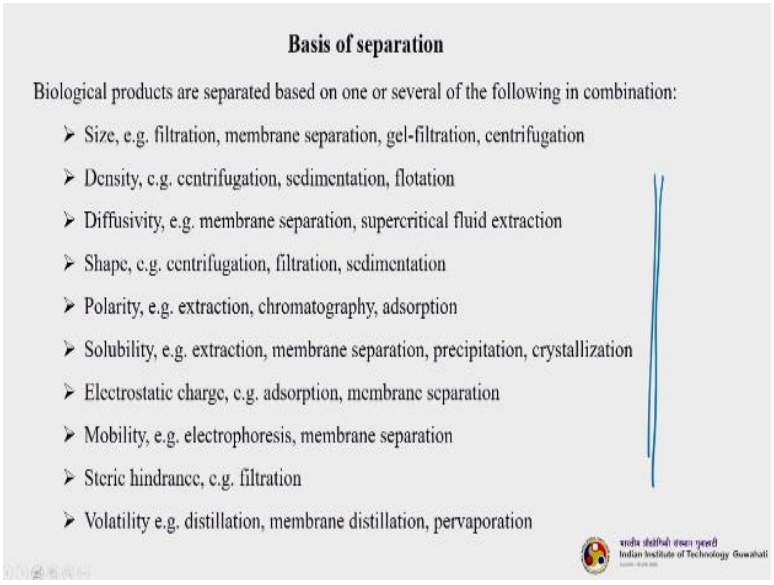
Now that is why and there are lot of scopes still available to do some research and to find out solutions are technologies which can reduce this cost of the bioseparation. So once this cost of bioseparation actually decreases to a significant level your cost of the final product will decrease.

(Refer Slide Time: 22:34)



So what we can do with bioseparation. So largely based on chemical separation techniques; chemical separation techniques are modified based on specific requirements. Novel separation may be necessary in some cases. High throughput and productivity, high selectivity, need to satisfy stringent quality requirements and need to take into account degradable material, low temperature operations and multi technique separation. So these are all the nature of bioseparation processes.

(Refer Slide Time: 23:01)

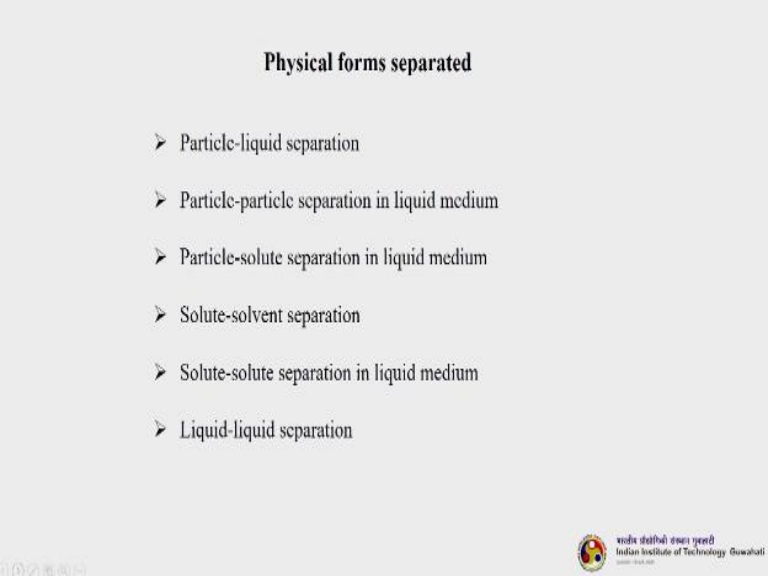


So the basis of separation is basically there are many, some of these we have already covered in our usual membrane separation. So the first one is size, of course, the second one is density, then diffusivity, then shape, polarity, solubility, electrostatic charge, mobility, steric hindrance, and

volatility. So all these parameters or based on this we can do separation. Like let us say, we are talking about size.

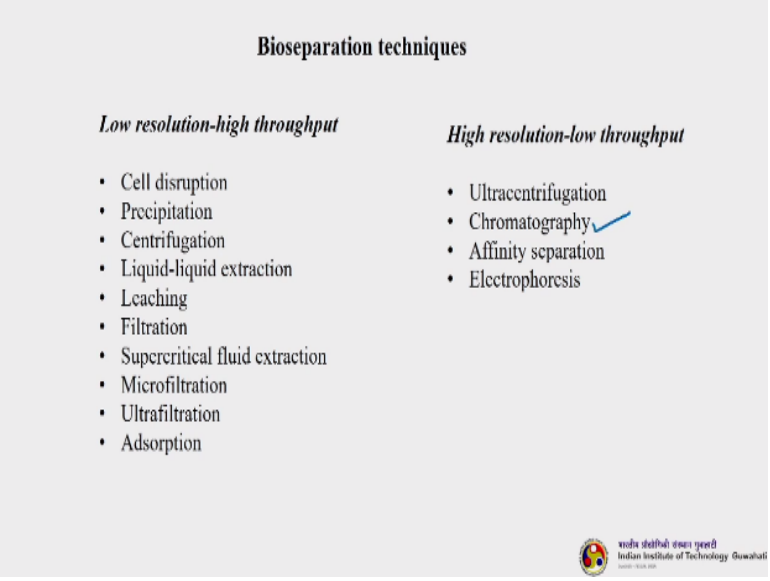
So we can go for a general filtration or even membrane separation. So centrifugation is a density based separation. So based on shape we can go for filtration, centrifugation, solubility plays a big role in crystallization. So steric hindrance in again filtration, volatility in distillation.

(Refer Slide Time: 23:49)



So these are the physical forms that can be separated during bioseparation; particle-liquid separation particle-particle separation in liquid medium, particle-solute separation in liquid medium, solute-solvent separation, solute-solute separation in liquid medium and liquid-liquid separation.

(Refer Slide Time: 24:04)



So bioseparation techniques are two types; one is low resolution high throughput and other is high resolution low throughput. So low resolution high throughput is, these are the techniques; cell disruption, precipitation, centrifugation, liquid-liquid extraction, leaching, filtration or supercritical fluid extraction, microfiltration, ultrafiltration and adsorption. And the high resolution low throughput is chromatography which is most widely used, then we have affinity separation, electrophoresis and ultracentrifugation.

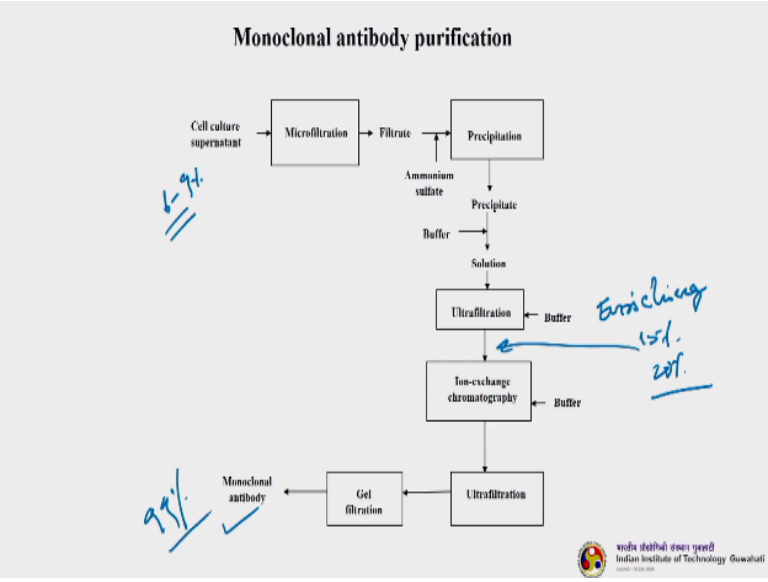
(Refer Slide Time: 24:34)

So what is the RIPP scheme; it is very famous in bioprocessing industries. So RIPP stands for recovery, isolation, purification and polishing. So it is a multi technique separation processes. So process design should take into consideration the following; the nature of the starting material,

the initial location of the target product, the volume of process stream, the relative abundance of the product in the starting material.

The susceptibility to degradation of the product, the desired physical form of the final product, the quality requirements and costing. So these are the things that should be taken into consideration when you design a RIPP scheme.

(Refer Slide Time: 25:13)

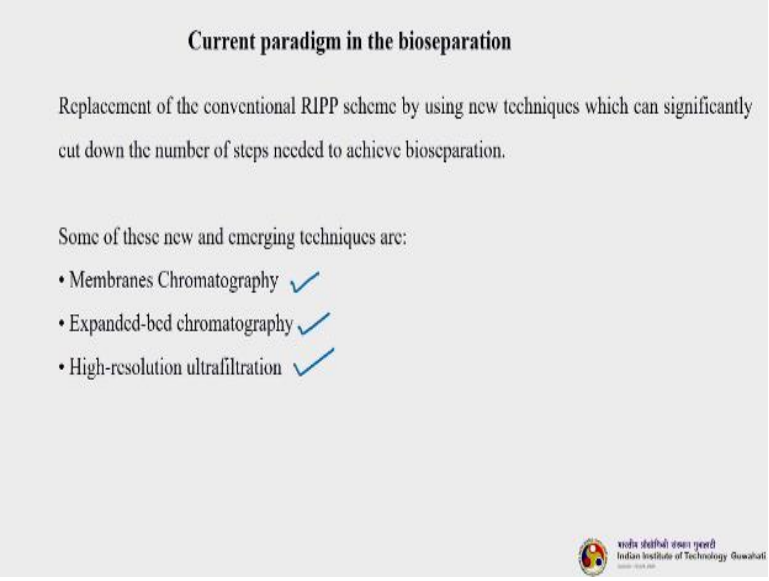


Let us understand how a monoclonal antibody which is a protein can be separated using a bioseparation technique; using of course membrane technology. So monoclonality antibodies are usually present in the cell culture supernatant. So, they are, this one is almost 6 to 9% initial purity in the cell culture supernatant. So initially, what will you do? You will pass through the microfiltration, so as to remove most of the other solutes which are of not interest.

Then it goes to a precipitation using an ammonium sulphate and that precipitate again you add some buffer and then you go for ultrafiltration. Now your ultrafiltration you remove your, what you are doing here, you are basically enriching. Enriching means what? Enriching means the 6 to 9% to certain %. Let us say 15%, 20%, something like this now then this goes to the ion exchange.

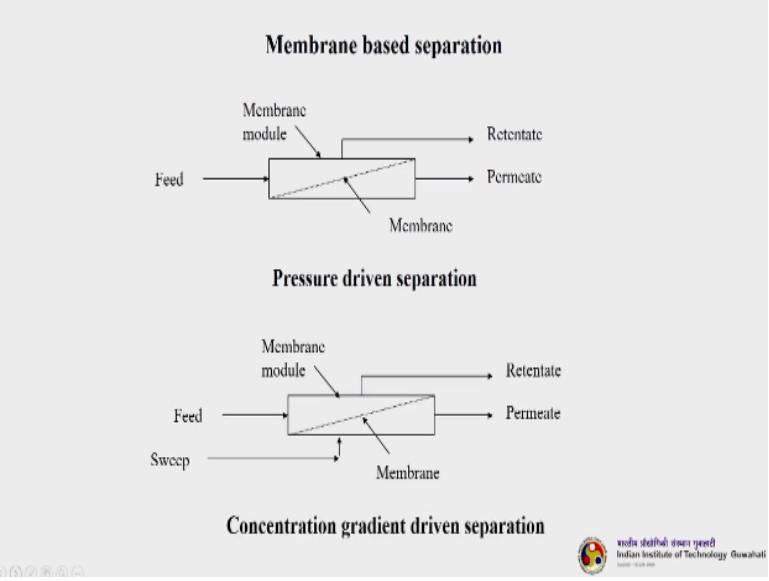
So this will finally, we will do because you know ion exchange is extremely selective. So it will actually give us very high purity, so you will get almost 98 or more than 90% pure of monoclonal antibody. Then a final ultrafiltration then we go for a gel filtration, you get final almost 99% pure monoclonal antibodies.

(Refer Slide Time: 26:35)



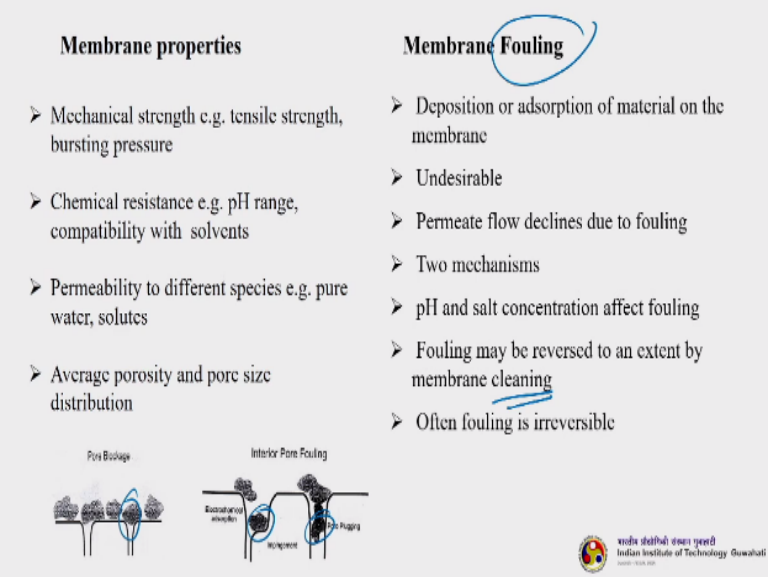
So let us understand the current paradigm in the bioseparation. So the replacement of the conventional RIPP scheme or RIPP scheme by using new techniques which can significantly cut down the number of steps or stages needed to achieve the bioseparation. Some of the new systems are; membrane chromatography, expanded-bed chromatography and high-resolution ultrafiltration. So you can see the membrane plays a big role in this.

(Refer Slide Time: 26:59)



So membrane based separation; we have already we are discussing, so either you have a membrane this one, pressure driven separation or you can have a concentration gradient driven separation.

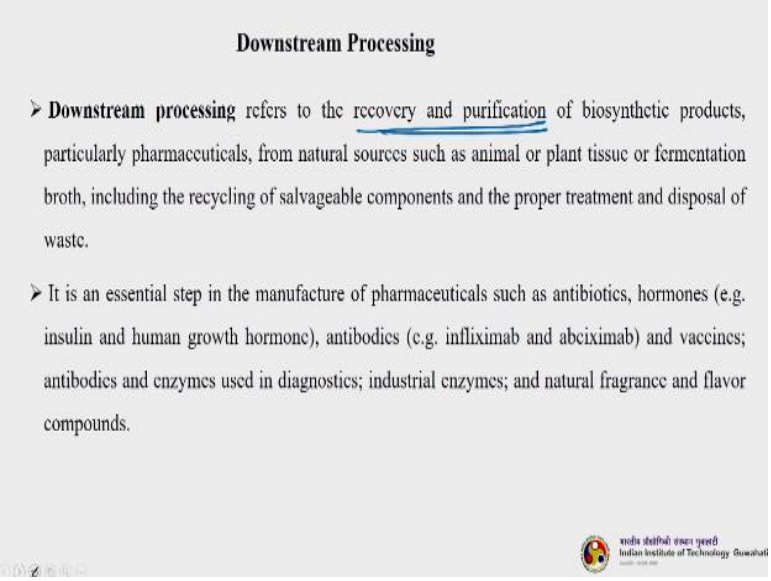
(Refer Slide Time: 27:15)



So membrane properties some mechanical strength like tensile strength, bursting pressure. Chemical resistance like a pH range and all. Then permeability to different species for example; pure water solutes and average porosity and pore size distribution. So as you know, fouling is a major problem in membrane system, any membrane. So deposition or adsorption of material on the surface which is highly undesirable.

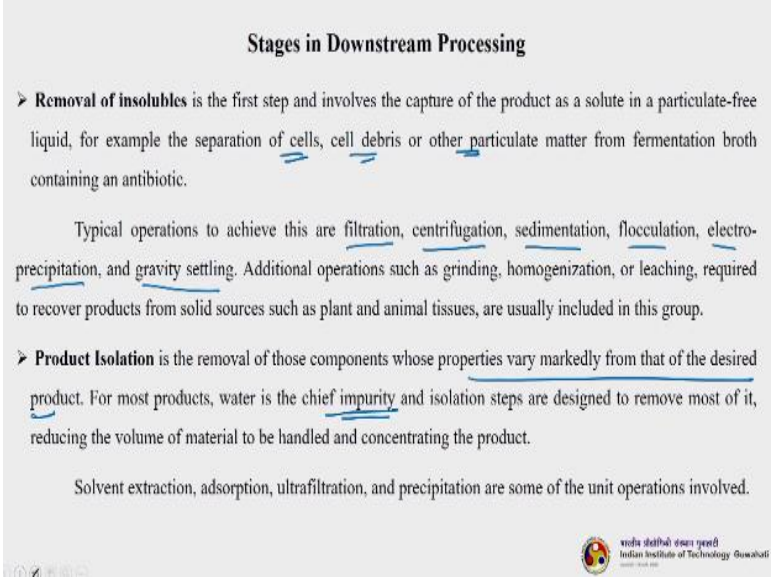
Permeate flow declines during due to fouling there are two mechanisms interior fouling, and exterior fouling. pH and salt concentration also affect fouling. Fouling may be reverse to an extent by membrane cleaning as we have discussed this in backflushing and other processes often fouling is irreversible. So you can see this, how the pore blockage is taking place here, this is and here interior fouling due to the different types of the structure of the pore.

(Refer Slide Time: 28:08)



Now downstream processing; downstream processing refers to the recovery and purification of the biosynthetic products, particularly pharmaceuticals from natural sources such as animal and plant issue or fermentation broth, including the recycling of salvageable components and proper treatment and disposal of waste. Now it is an essential step in the manufacture of pharmaceutical such as antibiotics, hormones, antibodies and vaccines and natural fragrance and flavor compounds.

(Refer Slide Time: 28:37)

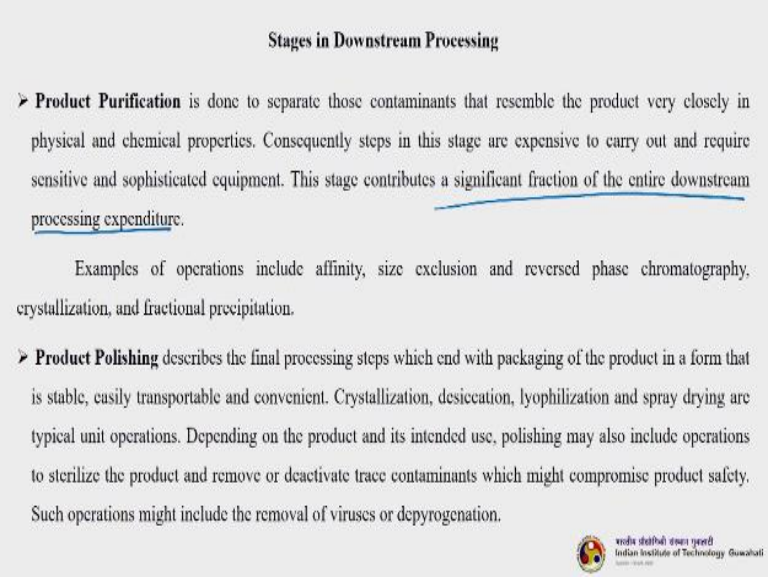


So removal of insolubles is the first downstream processing and involves the capture of the product as a solute in the particulate-free liquid, for example the separation of cells, cell debris and other particulate matter from the fermentation broth containing an antibiotic. Now typical operation to achieve the cell filtration, centrifugation, sedimentation, flocculation, electro-precipitation and gravity settling.

Additional operations such as grinding homogenization, leaching are required to recover products from solid sources such as plant and animal tissues, which are usually included in this group. Then the second is what after the removal of insolubles, is the product isolation. So if this is the removal of those components whose properties very markedly from that of the desired product.

For most products, water is the chief impurity and isolation steps are designed to remove most of the water reducing the volume of the material to be handled and concentrating the product. So solvent extraction, adsorption, ultrafiltration and precipitation are some of the operations.

(Refer Slide Time: 29:47)

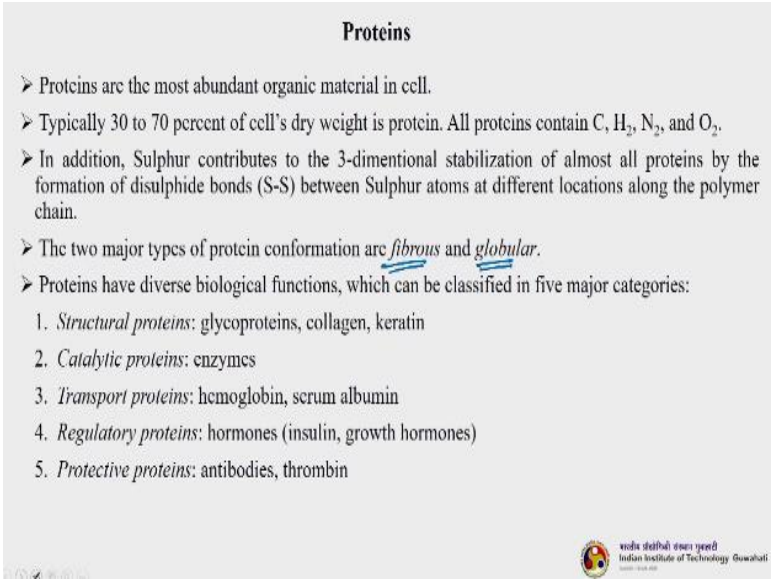


So the next is product purification. So this is done to separate those contaminants that resemble the product very closely in physical and chemical properties. Consequently steps in this stage are expensive to carry out and require sensitive and sophisticated equipment. Now this stage contributes a significant fraction of the entire downstream processing expenditure, so the purification one is the most contributing inside that downstream processing cost.

So examples are included by affinity by separation, size, exclusion and reverse phase chromatography crystallization and fractional precipitation. And then, last step is the product polishing; so this describes the final processing steps which end with packaging of the product in a form that is stable, easily transportable and convenient. Crystallization, desiccation, lyophilization and spray drying are the unit operations, which actually achieve this.

So depending on the product and its intended use, polishing may also include operations to sterilize the product and remove or deactivate trace contaminants which might compromise product safety. Such operations might include the removal of viruses or depyrogenation.

(Refer Slide Time: 30:58)

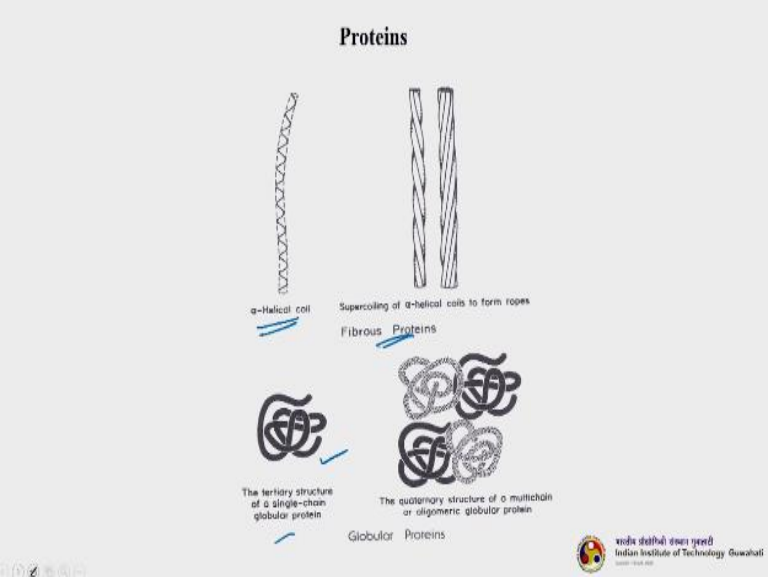


Now let us understand what are proteins because see some of you might end up in doing your job in some biotechnology or pharmaceutical industries or by processing industries and you will be dealing with proteins every day in your life. It is very important to understand what are proteins? We will just discuss little basics, the proteins are the most abundant organic material in the cell. Typically 30 to 70 % cells dry weight is protein.

So, all proteins contain carbon, hydrogen, nitrogen and oxygen. In addition, sulphur contributes to the 3- dimensional stabilization of almost all proteins by the formation of the disulphide bonds S-S bond between sulphur atoms at different locations along the polymeric chain. The two major types of protein conformations are fibrous types of proteins and globular types of proteins. Proteins have diverse biological functions which can be classified in 5 major categories.

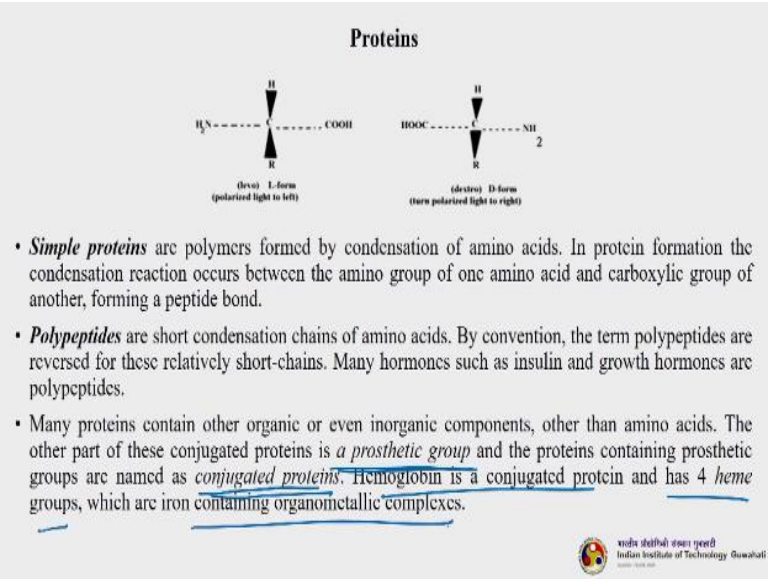
Structural proteins; which are glycoproteins, collagen, keratin. And then we have catalytic proteins which are enzymes. Transport proteins; haemoglobin, serum albumin. We have regulated proteins like hormones, and then we have protective proteins such as antibodies and thrombin.

(Refer Slide Time: 32:18)



So let us see the structure; how it looks like? So proteins this is a alpha helical coil, so these are fibrous proteins so globular proteins looks like this, this is a tertiary structure of a single chain globular protein the bind together along its own chain actually and forms a structure something like this, this is called globular proteins.

(Refer Slide Time: 32:41)

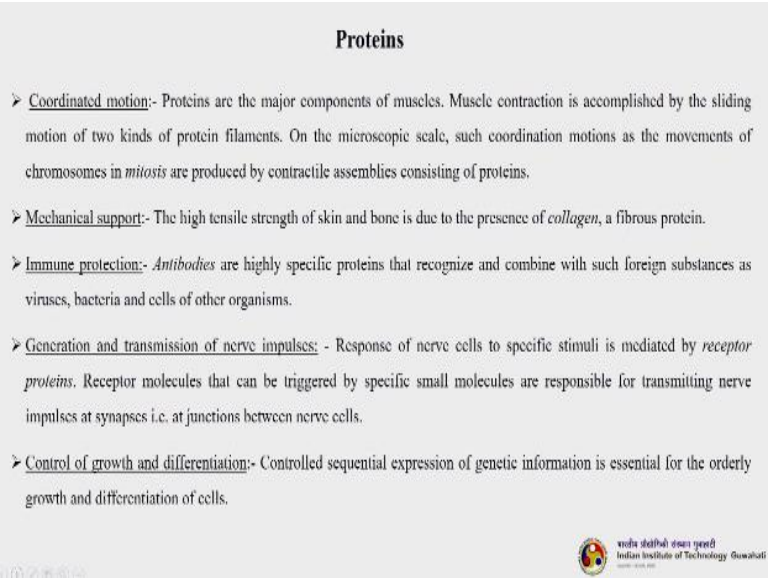


Now simple proteins are polymers found by condensation of amino acids. In protein formation the condensation reaction occurs between the amino group of one acid and carboxylic group of another forming a peptide bond. So polypeptides are short condensation chains of amino acids. By convention, the term polypeptides are reversed for these relatively short chains. Many hormones such as insulin and growth hormones are polypeptides.

So many proteins contain other organic and even inorganic components other than amino acids, so, the other part of this conjugated proteins is called a prosthetic group and this proteins contain a prosthetic groups are known as conjugated protein. The proteins which contains a prosthetic all protein is do not contain prosthetic groups, so those can contain a prosthetic groups are called conjugated proteins.

So hemoglobin is a conjugated protein and has 4 heme groups, which are iron containing organometallic complexes, you know hemoglobin is responsible for the transfer of oxygen in the cells.

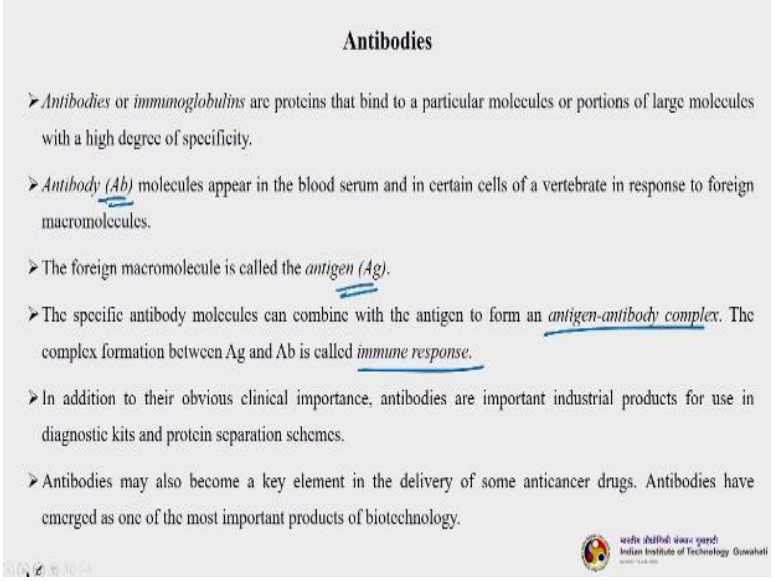
(Refer Slide Time: 33:43)



So protein play crucial role in virtually all biological processes. The significance and remarkable scope of their functions are; the proteins play an important role in enzyme catalysis, transport and storage and then coordinated motion, mechanical support, immune protection, generation and transmission of nerve impulses, control of growth and differentiation.

So you can later on just read these slides, there are little description for your understanding. And this skipping them and let us move ahead.

(Refer Slide Time: 34:13)

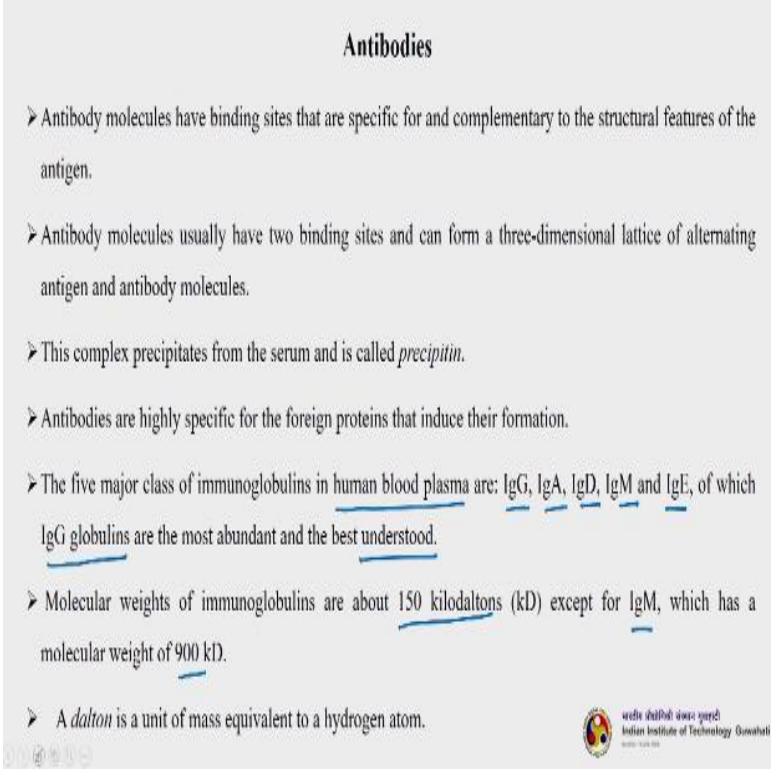


So then what are antibodies? So antibodies or immunoglobulins are proteins that bind to a particular molecules or portion of large molecules with a high degree of specificity. Now antibody usually written as Ab. like this. So molecules are the molecules appear in blood serum and in certain cells of a vertebrate in response to foreign macromolecules. So when some virus is attacking us some antigen.

So, as a consequence of that, the cell will secrete or generate antibodies. Now this antibody will bind to the antigen by forming a group, antibody-antigen complex and then it will somehow go away from the cell, so there are different mechanisms that are, anyway we will not discuss in those things. So as I told the foreign macromolecule is called the antigen, so antigen, antibody. when they form so that is called antigen-antibody complex, so the complex formation is between antigen antibody is called the immune response of the cell.

Now in addition to their obvious clinical importance antibodies are important in industrial products for using diagnostic kits as well as protein separation schemes. So antibodies may also become a key element in the delivery of some anticancer drugs. Antibodies have emerged as one of the most important products of biotechnology.

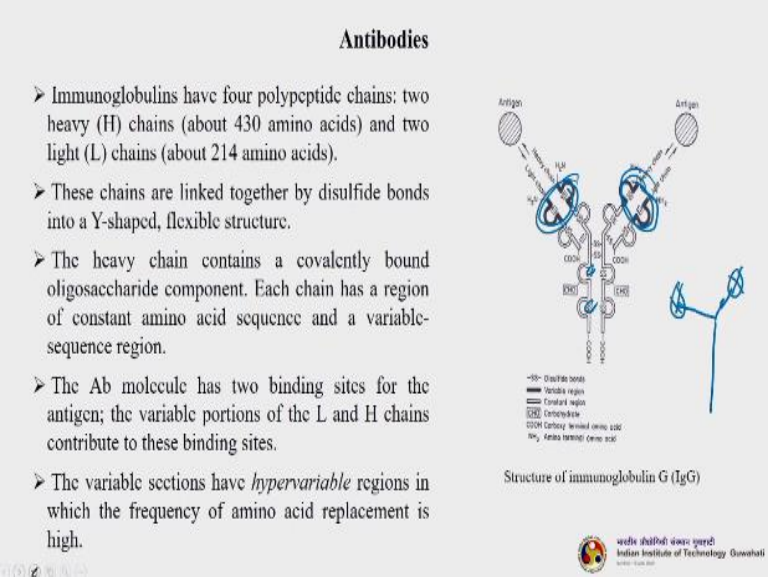
(Refer Slide Time: 35:37)



So, antibody molecules having binding sites that are specific for and complimentary to the structural features. That is why affinity chromatography can play a big role in antibodies separation. Antibody molecules usually have two binding sites and conform to 3 dimensional lattice of alternating antigen activity molecules. So this complex precipitates from the serum and is called precipitin.

Antibodies are highly specific to the foreign proteins that induce their permission the five major class of immunoglobulins in human blood plasma are IgG, IgA, IgD, IgM and IgE of which IgG globulins are the most abundant and the best understood. It is called immunoglobulin g. So molecular weights of immunoglobulins are about 150 kilodaltons except for IgM, which is little high 900 kilodaltons. So Dalton is a unit of mass equivalent to a hydrogen atom. This we know we have discussed earlier also.

(Refer Slide Time: 36:33)

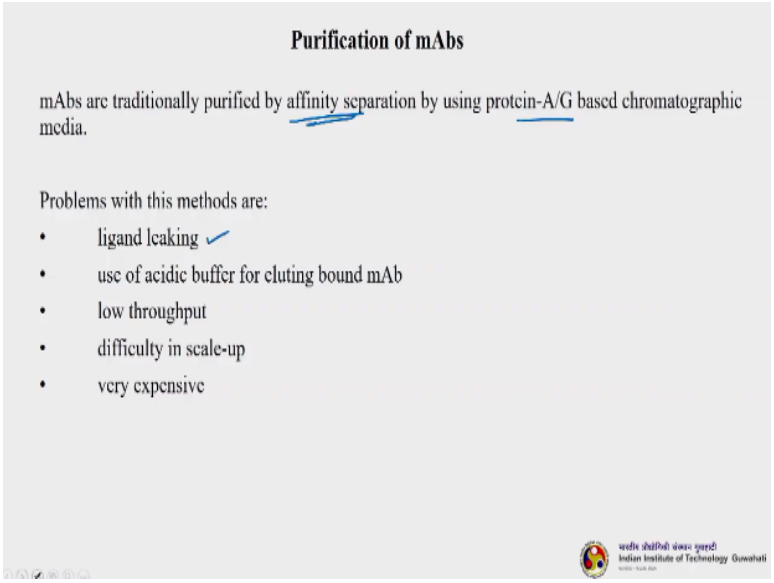


So this is how antibodies looks like, you can see; so Y separate structure; is just a Y separate structure like this. You can see so much of disulfide bonds here, these are the disulfide bonds then this black areas are the variable regions and whatever the white area that is actually constant region. So, immunoglobulin have 4 polypeptide chain; two heavy chains and two light chains so they contain about 430 amino acids and 214 amino acids, respectively.

So these chains are link together by a disulfide bonds into Y-shaped, flexible structure. Now the heavy chain contains a covalently bound oligosaccharide component and each chain a region of constant amino acid sequence and a variable. so this is how it helps and so this is see the antigen is coming here and it will bound to this heavy chain to this particular region here. So there are two regions with in which antibodies can be antigens can be bound.

Now the Ab molecule has two binding sites for antigen and the variable portions of the L and H chains, so the variable sections of the hyper variable regions in which the frequency of amino acid replacement is high. So once the bound then it can be separated.

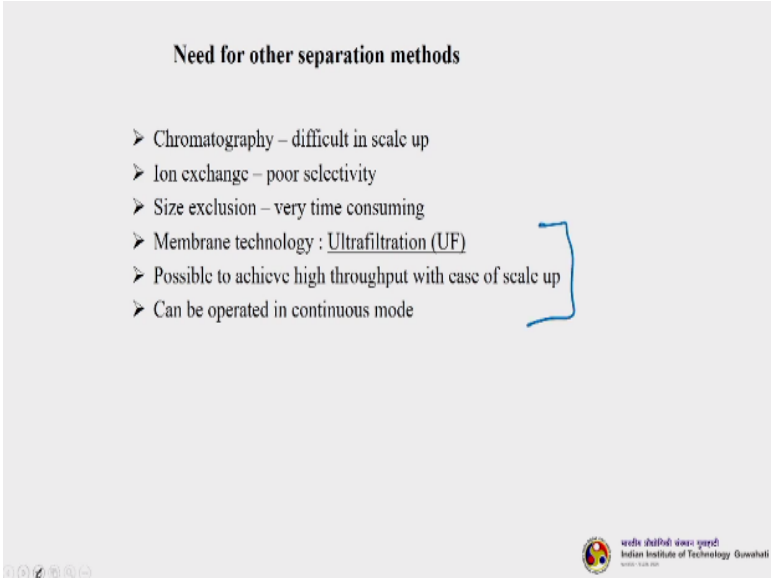
(Refer Slide Time: 37:51)



Now let us understand how it purify the monoclonal antibodies. So that traditionally purified by affinity separation by using a protein A/G based chromatographic media. So problem with this media is the ligand leaking. So this is a ligand; its a common column chromatography, the ligand starts leaking it is a big problem that will come into the product. Then when you are using acidic buffer to elute the bound mAb.

That is another product because the acid may also contaminate the final monoclonal antibodies. Throughput is very less difficulty in scale up and very, very expensive process.

(Refer Slide Time: 38:29)

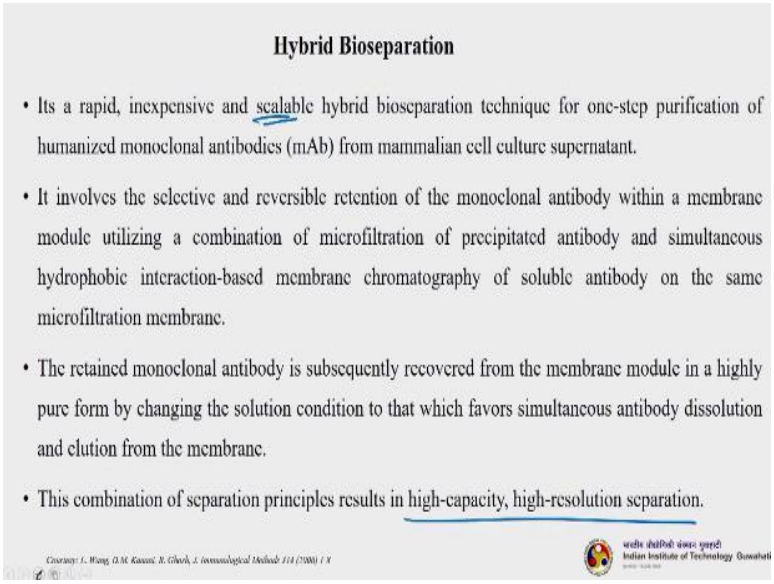


So there are need for other separation methods, so we have chromatography is difficult in scale up, ion exchange is poor selectivity, size exclusion is very time consuming. So whereas membrane technology ultra; filtration is providing is one of the best solution. So it is possible to achieve high throughput with each scalar and can be operated with continuous mode, so let us see, how it is happening?

(Refer Slide Time: 38:53)

So try to see quickly the latest developments in bioseparation using membranes.

(Refer Slide Time: 38:58)

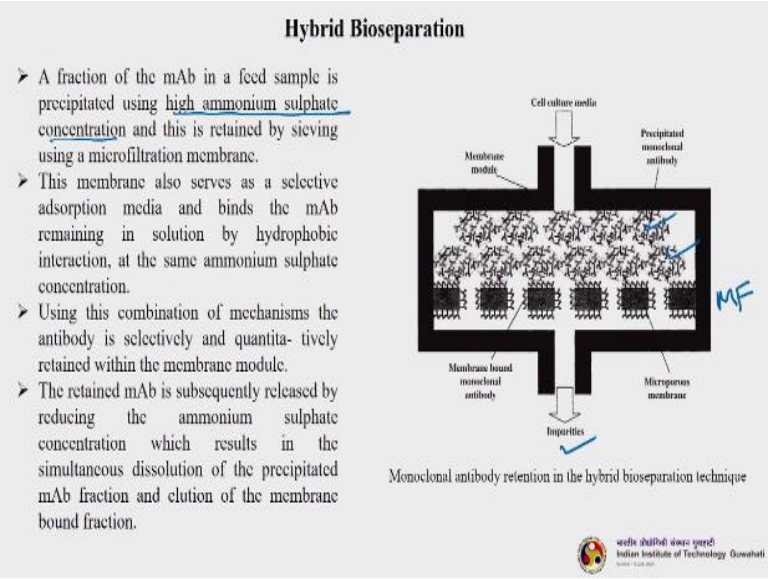


So there is a new hybrid bioseparation, which is proposed by Wang et. al. So it is a rapid, inexpensive and scalable very, very important. Hybrid bioseparation technique for one step

purification of humanized monoclonal antibodies from mammalian cell culture supernatant. So it involves the selective and reversible retention of the antibody with the membrane module, utilizing a combination of microfiltration of precipitator antibody.

And simultaneous hydrophobic interaction based membrane chromatography of soluble antibody on the same microfiltration membrane, so the retained monoclonal antibodies subsequently recovered from the membrane model in a highly pure form by changing the solution condition to that which flavours whichever simultaneous antibody dissolution and illusion so this combination of principle suppression in high-capacity high-resolution.

(Refer Slide Time: 39:45)



Let us see how it happens actually, so this is how this is actually a microporous membrane, so this is a MF membrane. So here the monoclonal membrane is bound with the monoclonal antibodies. And when you are passing the cell culture supernatant, so you have precipitated monoclonal antibody is getting retained on the surface of the membrane and all other impurities are permeating through.

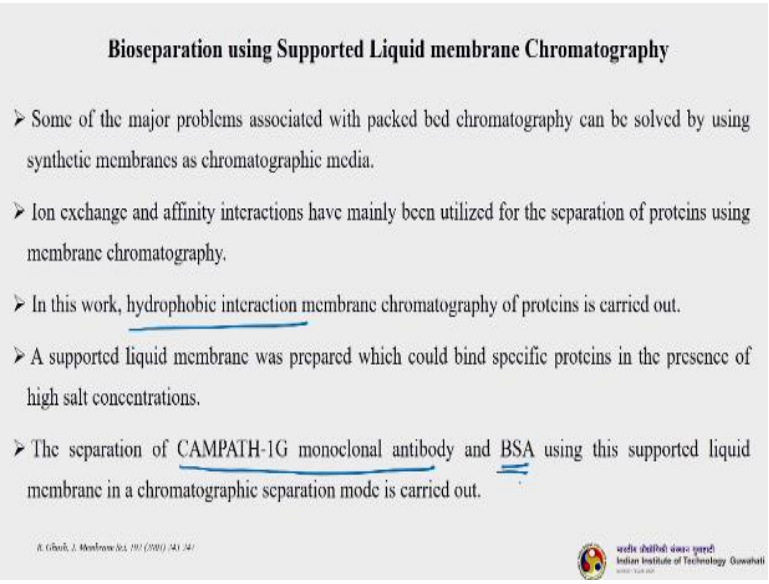
So, fraction of the mAb in a feed sample is precipitated using high ammonium sulphate concentration and this is written by sieving through a microfiltration membrane. So what you are doing is you are using ammonium sulphate. High concentration ammonium sulphate that will

precipitate monoclonal antibody, then you pass this precipitation through the membrane. So the microfiltration membranes.

Since now they have become aggregates, it will be using their these are become aggregates, something similar to your Micellar-enhanced stuff, so this aggregates due to their size big size will be retained on the surface of the membrane and other all impurities will be passed through. So using this combination of mechanism the antibodies selectively and quantitatively retained within the membrane module.

Now the retained, this mAb is subsequently released by reducing the ammonium sulfate concentration which results in the simultaneous dissolution. So what you are doing? So you are slowly reducing the concentration of the ammonium sulphate. So that the aggregate becomes the aggregate there will dissolve so that you will get free monocular antibiotic and you can take it up.

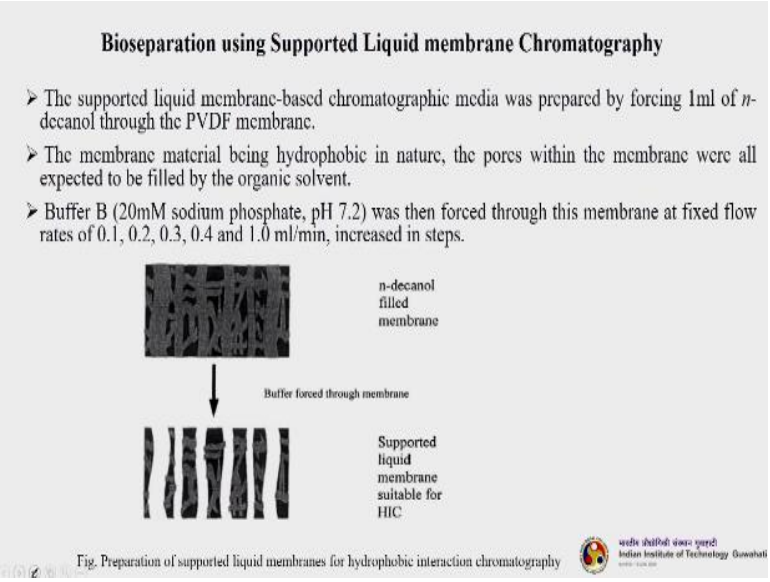
(Refer Slide Time: 41:14)



There is another technology which was proposed by Professor R. Ghosh. So this bioseparation using supported liquid membrane chromatographs, you have not studied liquid membrane but just let us try to understand we will of course in detail study liquid membrane in our subsequent lectures. So some of the major problems associated with packed bed chromatography can be solved by using synthetic membranes as chromatographic media.

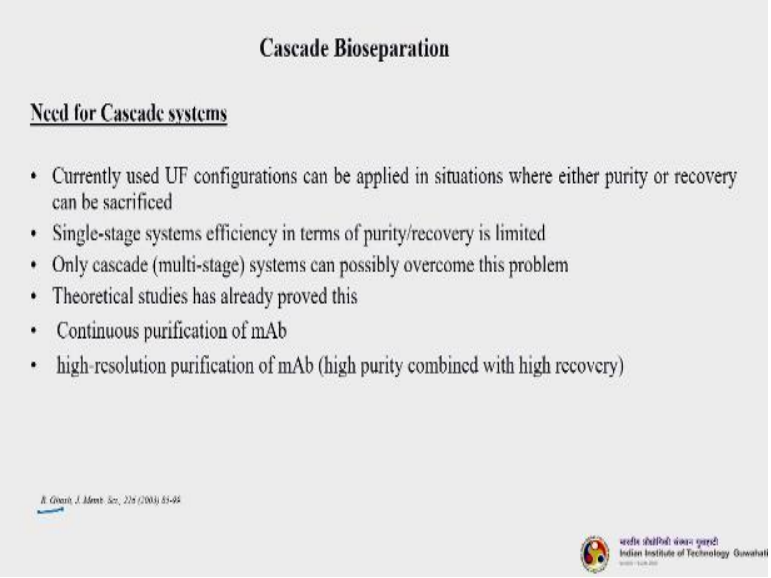
So ion exchange and affinity interactions have mainly been utilized for the separation of proteins using membrane chromatography. In this work hydrophobic interaction membrane chromatography of proteins is carried out. So supported liquid membrane was prepared which could bind specific proteins in the presence of high salt concentration. So in this particular study, they have separated CAMPATH-1G monoclonal antibodies and BSA using supported liquid membrane technology.

(Refer Slide Time: 42:02)



This is how it has happened. So this is a micro filtration membrane through which, PVDF membrane through which n-decanol is filled. So n-decanol is prefilled inside the membrane pores. So the membrane material being hydrophobic in nature the pores within the membrane were all expected to be filled with the organic solvent. Now buffer which is 20 millimolar sodium phosphate, at pH 7.2 was then forced to this membrane at fixed flow rates, and in increase in steps. So that will help in retaining the CAMPATH-1G.

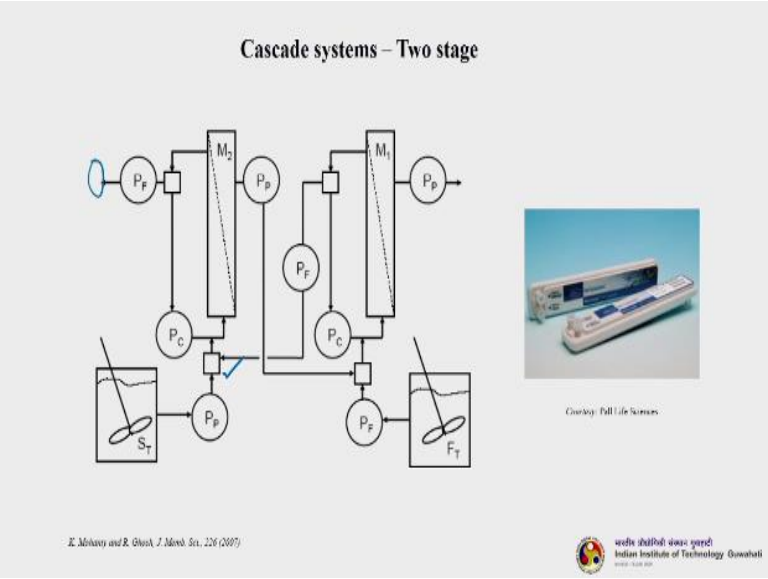
(Refer Slide Time: 42:39)



So then there is another latest development which has come which is called cascade bioseparation. So you know what is the need for cascade system because you know, the current ultrafiltration configurations can be applied in situations where either purity or recovery can be sacrificed in a single stage system. So in a single stage system, the efficiency is usually limited, only cascade or multitask systems can possibly overcome this problem.

So theoretical studies by Professor Ghosh has already proved that. So continuous purification of monoclonal antibody has been reported high resolution purification of antibody that is high purity combined with high recovery is already predicted.

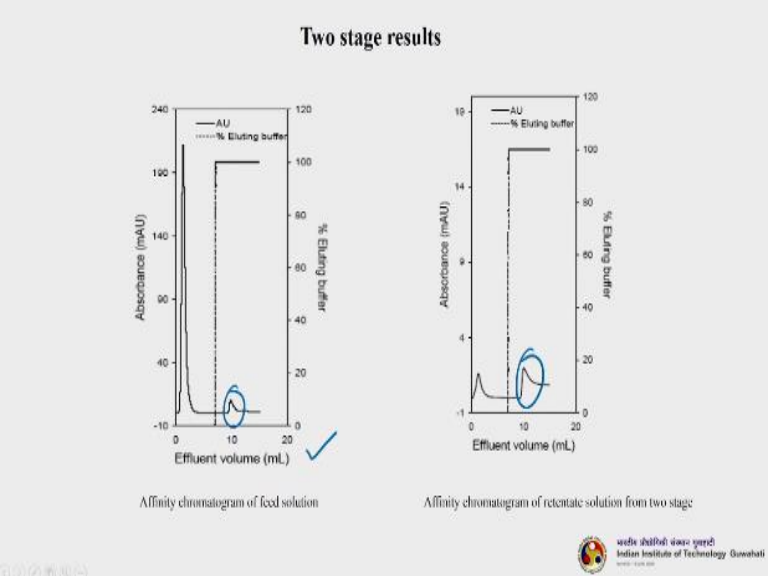
(Refer Slide Time: 43:21)



So, I will show you one of the works which actually, I carried out in professor Ghosh labs in McMaster University Canada. So what we have done to initially we have used it two stage cascade systems. So you can see this; there are two membranes so this is membrane 1 and membrane 2. So your feed is being fed to the membrane 1 you can see here, and the retente is being partly recycle and the partly is being feed to the membrane 2.

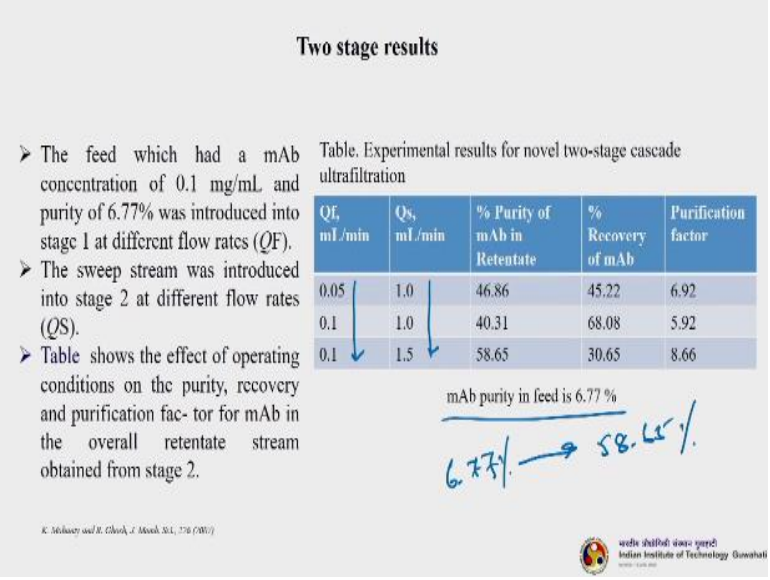
And then here this is when you get your, this one product of interest. So basically two stage system so the retente from one stage is feed to the another stage that we have used this tangential flow filtration systems, which were given by the pole light sensors.

(Refer Slide Time: 44:09)



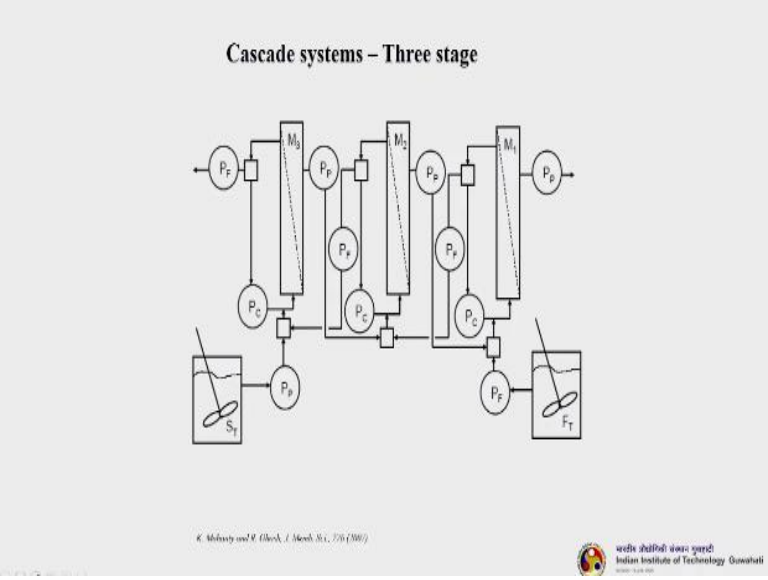
We can see the initial value is actually 6.67 and the purity you can see this is a single stage system, this is single stage system. And with the two stage system we can see the purity has increased. This is affinity chromatograms.

(Refer Slide Time: 44:26)



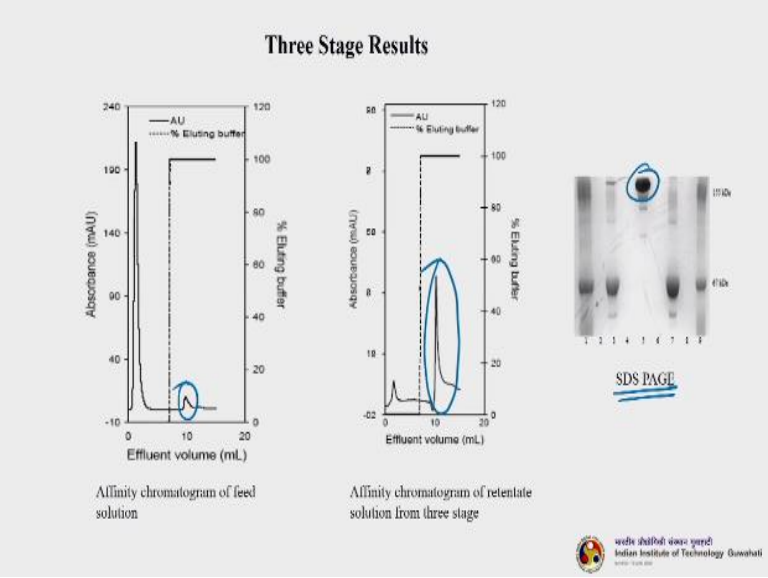
So let us see the result actually in quantification terms. So I have varied the feed flow rate point, we have increased 0.05 to 0.1. Then the Qs of the sweep flow rate 1 to 1.5, there is the percentage of purity of mAb in retentate is 46, 40, 58.6 you can see that the initiality 6.77%, you need two stage systems, only 2 stage systems, it has increased from 6.77 to 58.65. This is in percentage, so you can understand that, how does 2 stages doing the up concentration? Then the same system we have added another one. So you have added one more membrane to the two stage system.

(Refer Slide Time: 45:11)



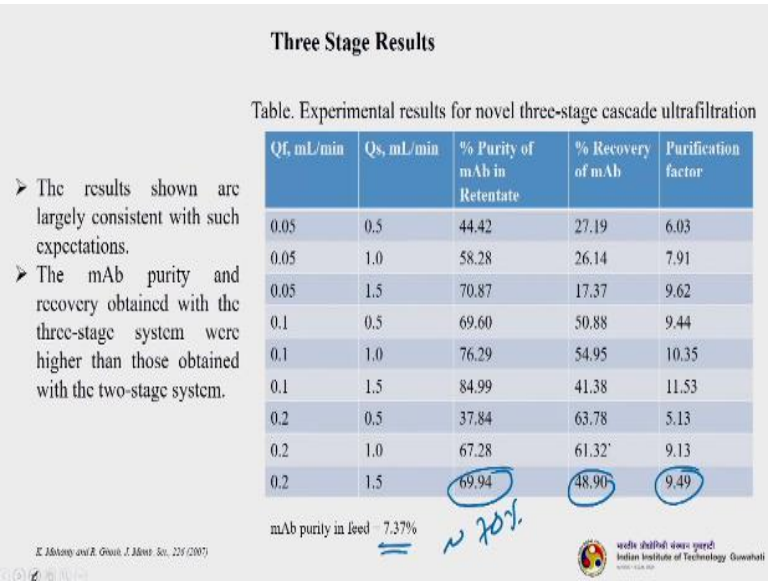
Now, this has become 3rd stage system.

(Refer Slide Time: 45:13)



So in the third stage system what we are getting you can see this is the initial feed and we can see how the, maybe purification has been increased and this is SDS PAGE, you can see this is the concentrated monocular antibody, its completely separated there is almost no impurity present here, in this layer.

(Refer Slide Time: 45:36)



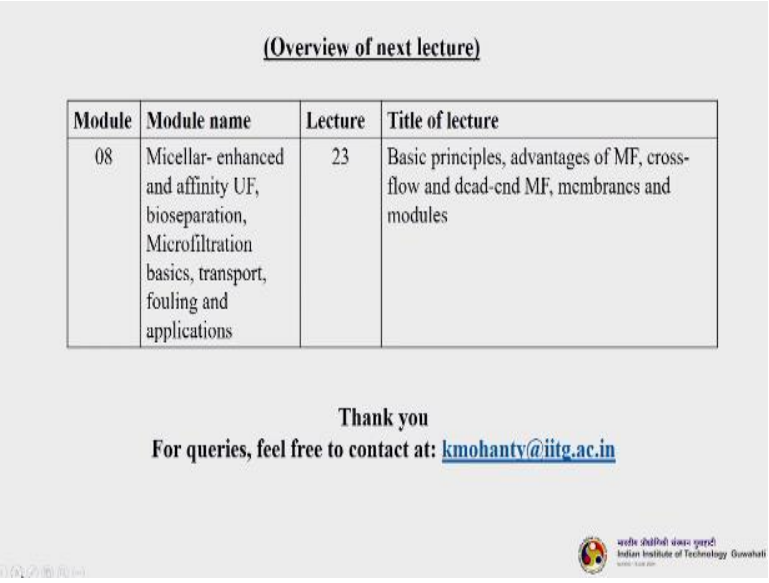
Let us see the 3 stage system here, the initial purity is 7.37, from 7.37, we have gone almost to 69.94%, so it is close to 70%. So you can see that from 6 to 7% of the initial purity in a two stage, we got almost 60% in 3 stage we got almost 70%. So, the mAb purity and recovery obtained with the three state system are higher than this obtained with the two stage system

recovery is also very important because these are very costly products, the purification factor also 9.49, which is very good.

(Refer Slide Time: 46:15)

So today's lectures are mostly taken by various books. So you can refer little of, K. Nath and Baker as well as Mulder. So in case you have any query please feel free to write to me at kmohanty@iitg.ac.in.

(Refer Slide Time: 46:35)



So in the next class, we will discuss about, we will start microfiltration. So we will understand the basic principles and advantages of microfiltration system cross-flow and dead-end

microfiltration system, different types of membranes and modules which are utilized in microfiltration systems. So, thank you very much.