Principles of downstream techniques in Bioprocess – a short course
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Lecture-14
Drying/ Lyophilisation /Stabilization

This lecture we are going to talk about certain downstream operations, which are carried out at the very end operations like drying, lyophilisation and stabilization. So here if you want to remove whatever moisture that is present in the product, it is assumed to be solid. So either drying which may be involved slightly higher temperature, lyophilisation involves low temperature and then finally we add stabilizers to prevent degrading. It could be anti oxidants or anti ozeoners or some other enzymes which prevents hydrolysis or deduction and so on actually and that is what we are going to talk about today.

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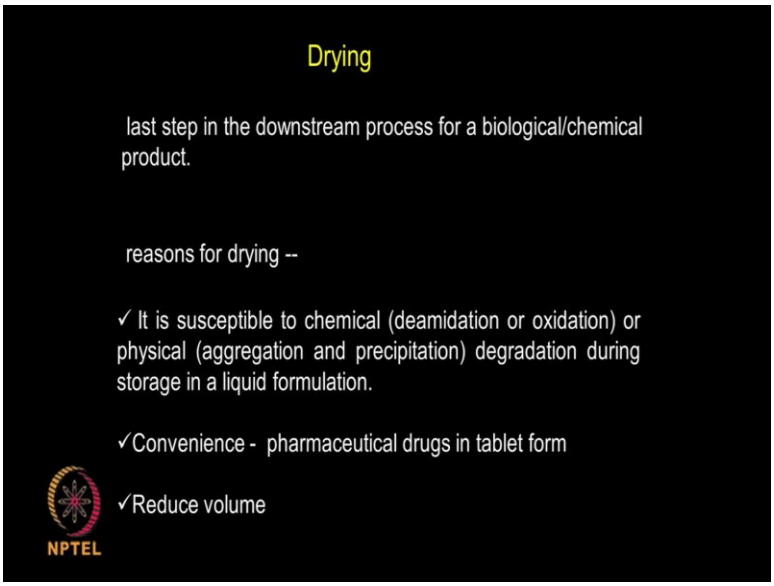

Drying, what is drying? This is generally the last step and we are using a higher temperature sometimes with vacuum and without vacuum to remove the moisture that is present. So we need to do that because you want to remove whatever liquid that is present okay, because if your product is in the liquid form, it may degrade because of oxidation or deamidation. We do not want that to happen.

It is convenient because pharmaceutical products drugs may be in tablet form, it reduces the volume because its storage you are using less volume or less space if it is in solid form rather than in the liquid form.

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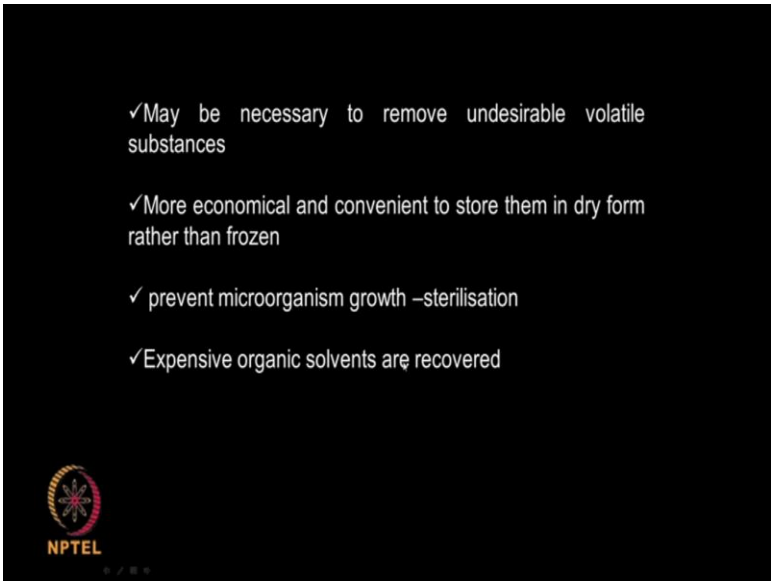

May be necessary to remove undesirable volatile substances, its more economical because it may like to store them in dry rather than frozen condition. We can prevent microorganism growth because there is no moisture, so the bacteria might not grow. If there are expensive organic solvents then by doing a drying may be removing those organic solvents or recovering those organic solvents.

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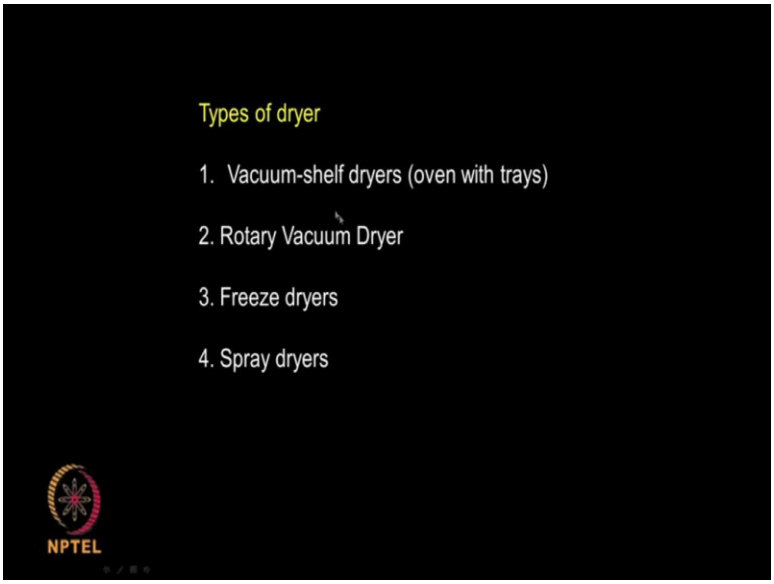

The types of dryers you have vacuum shelf dryers, that is oven with trays. So this is generally batch type of operation, rotary vacuum dryer this could be a continuous operation, freeze dryers this is almost like lyophilisation and then spray dryers, so it uses atomizations and vacuum and so on actually. So we look at each one of them little bit in more detail later.

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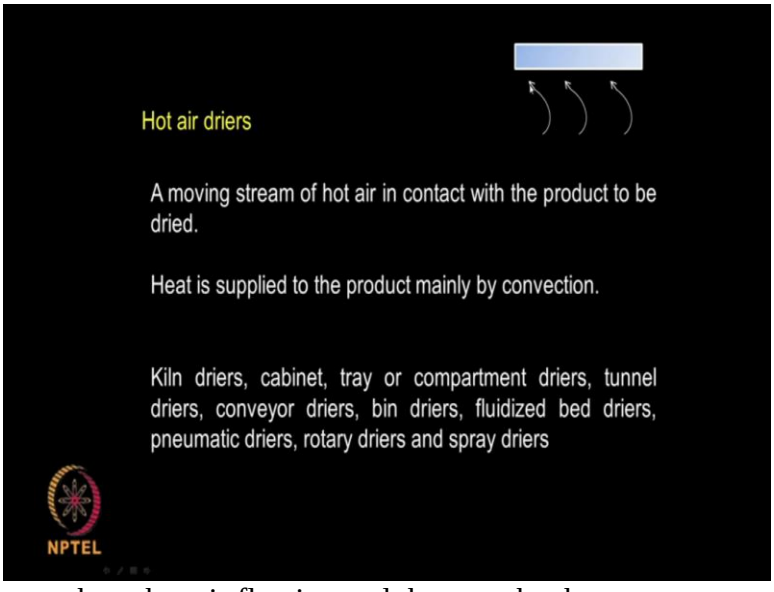

So hot air driers, so you have hot air flowing and then maybe there are trays on which the solid is kept, which gets dried, so moving stream of hot air in contact with the product. It is generally by convection like kiln driers, cabinet driers, tray driers, compartment driers, tunnel driers, conveyor driers, bin driers, fluidized bed driers, pneumatic driers, rotary driers and spray driers

and so on actually. So lot of driers, different types of driers and all of them are based on hot air flowing.

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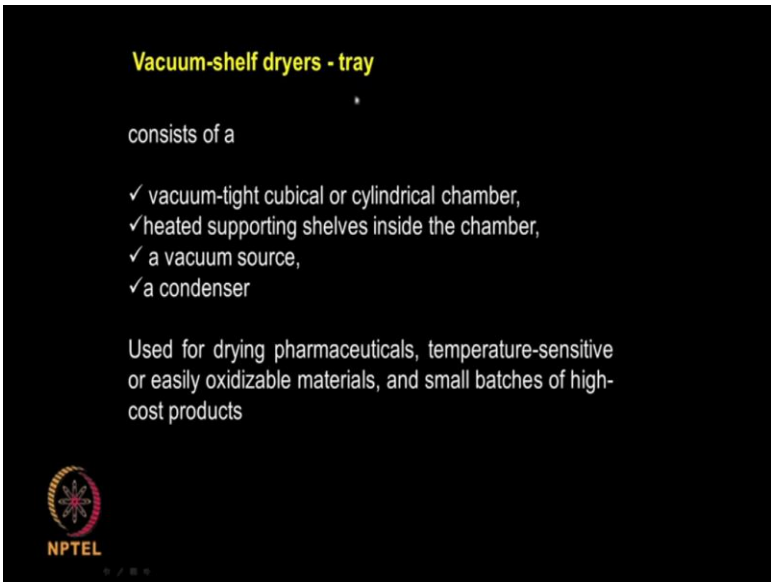

Vacuum-shelf dryer they are also called tray dryers. It consist of a cubical chamber heated support shelves inside the chamber, there is a vacuum source there is a condenser for collecting the solvent or water. It is widely used in pharmaceutical temperature sensitive or easily oxidazable material and we can do in small batches for high-cost products.

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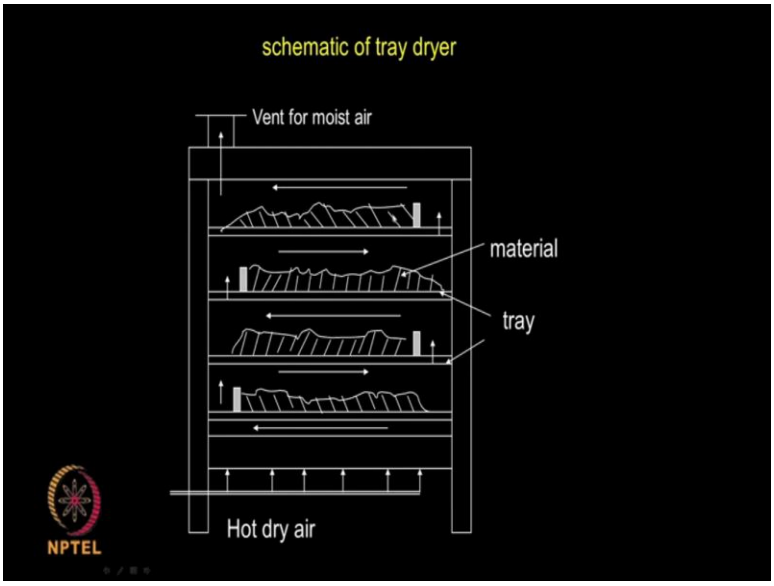

So this is the tray dryer, so you have lots of trays, you have your product which is collected initially, the wet product and then hot air flows upwards. So the air flows through these beds and

thereby it is driving the material. So it is by connection. So once it is done, you stop the drying process, cool it down, take the solids down again, charge with the again wet solids and so on. So generally it is carried out in batch mode.

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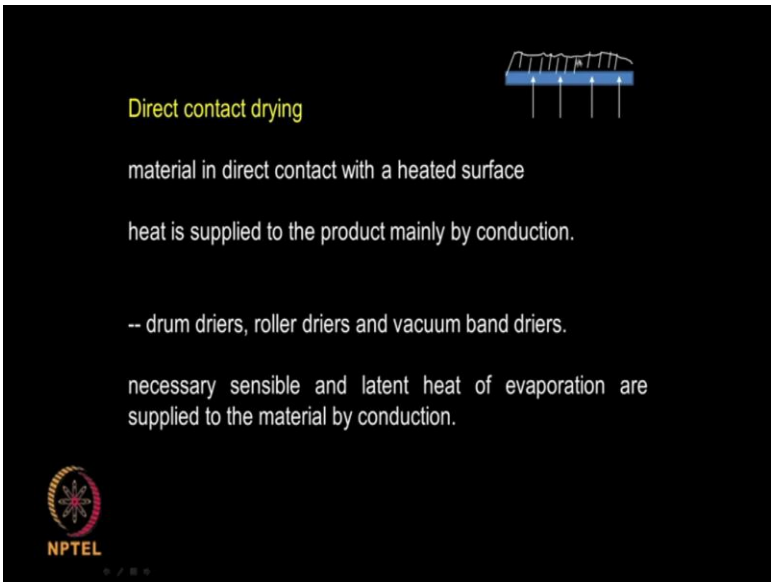

So direct contact drying you may the material which is hot, so your solid which needs to be dried is dumped on this and more of a conduction based heating. So it gets dried because of this conduction actually. Like drum driers, roller driers, vacuum band driers, you may even have belt driers. So the belt itself may be very hot, the drum itself may be very hot and the solids wet solids when it comes in contact, so the moisture is removed. So that is based on conduction approach.

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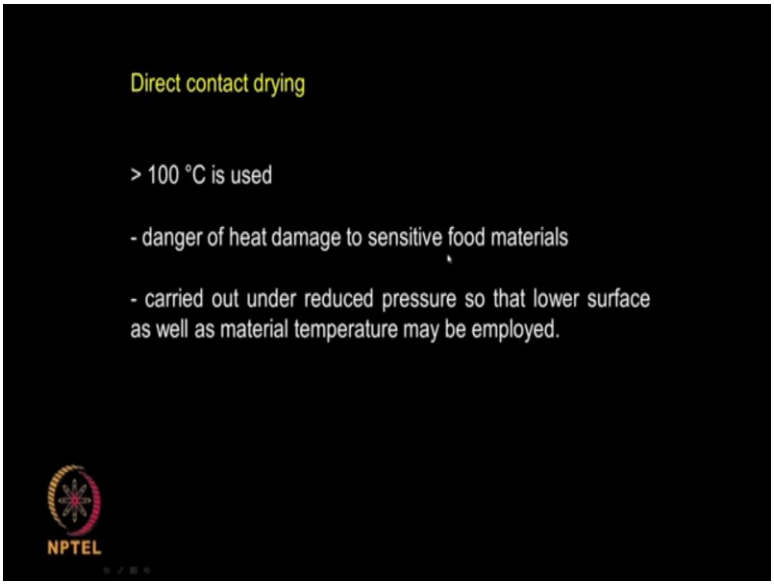

So 100 degrees is used, one problem is the heat sensitive material like food material may get damaged. So we can get carried out under reduced pressure and lower surface temperature, so that if it is temperature sensitive material, it does not get damaged.

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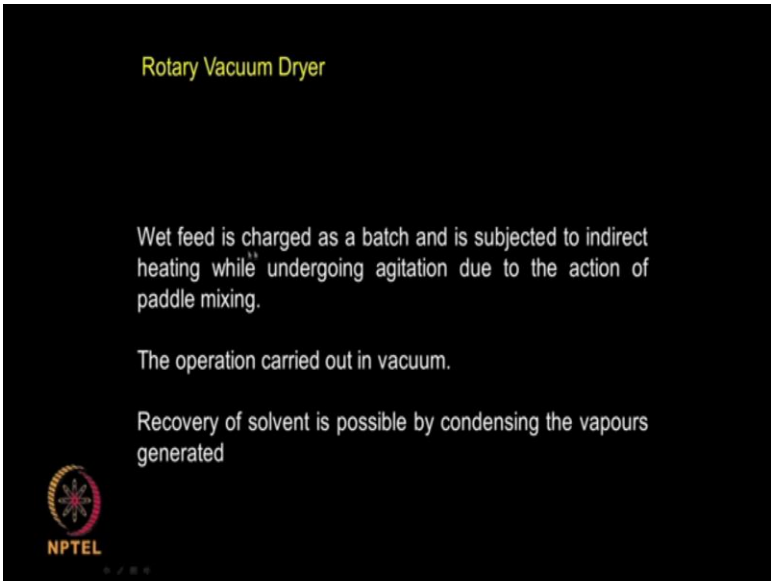

Rotary vacuum dryers, so wet feed is charged as a batch and it is subjected to indirect heating, there could be agitation also. This could be carried out in vacuum okay. So that is called rotary vacuum dryers okay. So we can even recover the solvents especially in this type of dryers whereas in other types it may be more difficult to recover the solvents.

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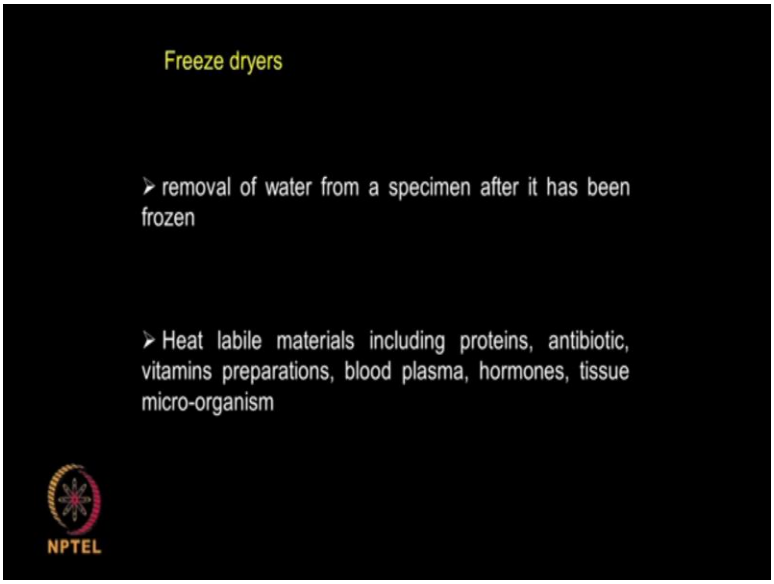

Freeze dryers this is done at low temperature this is almost like a lyophilisation we will talk about it more detail. So removal of water from a specimen after it has been frozen. So it is very good for heat labile materials including proteins, antibiotics, vitamins, blood plasma hormones, tissue, micro organisms and so on actually.

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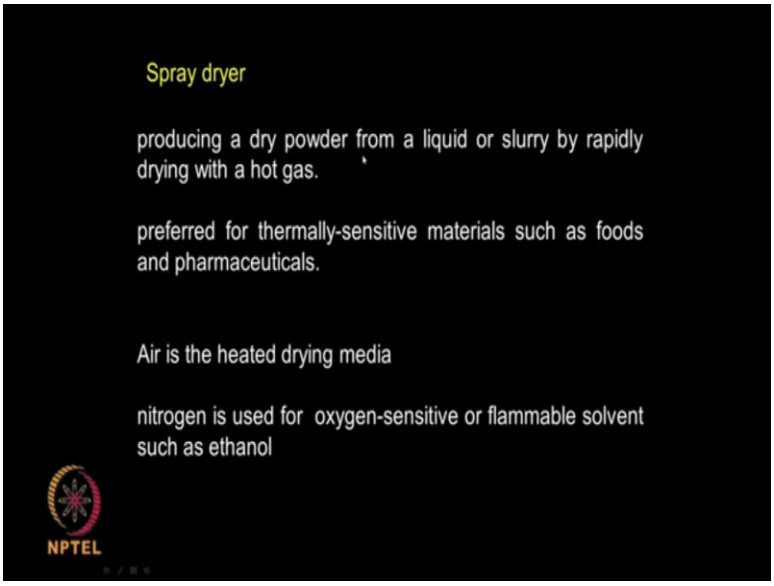

Then you have the spray dryers like your coffee powder or instant milk powder and so on. So you can get a dry powder from a liquid or a slurry, it is very good for thermally sensitive material. So you do not have to heat much but you apply very high vacuum and air is heated and

it flows and carries away the moisture. Sometimes you can even use nitrogen, hot nitrogen if the material is oxygen-sensitive or it is flammable solvent is there.

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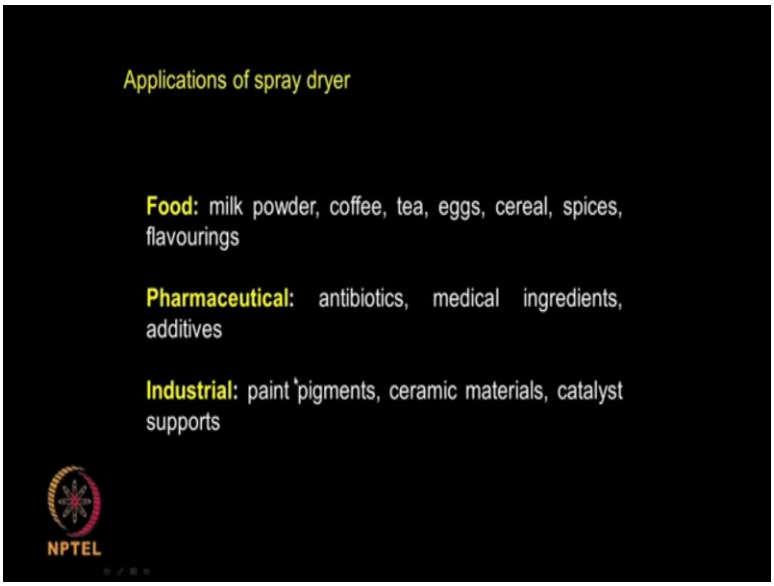

So where do you use them? You use in food industry, pharmaceutical industry, even industry like paint, pigments, ceramic, so lot of applications nowadays this spray dryer has come into quite a lot.

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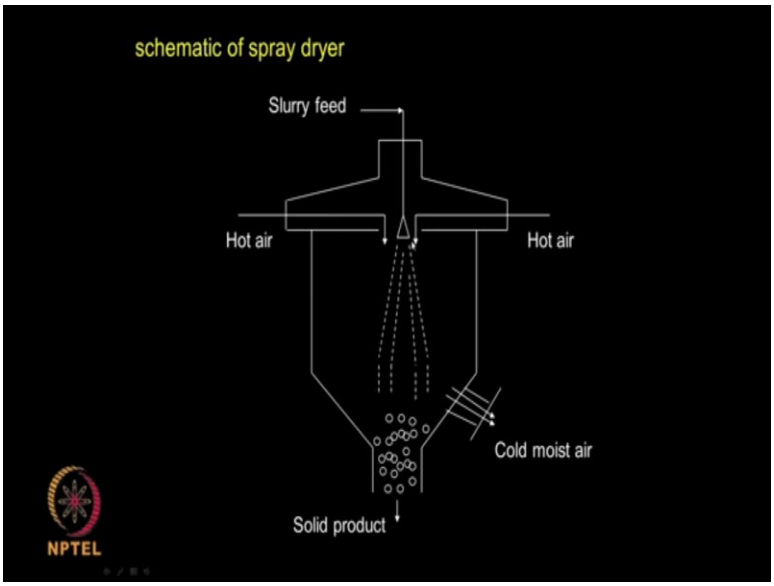

Typically it looks like this there is a long tall chamber, so you have the slurry feed and it is atomized and it comes out as fine droplets, there is hot air coming in. So it carries the moisture

okay as it comes down because it is fine particles you end up with solid particles which are corrected. So it could the solid particles we are talking in terms of microns. So we could get very fine micron particle using this type of technique.

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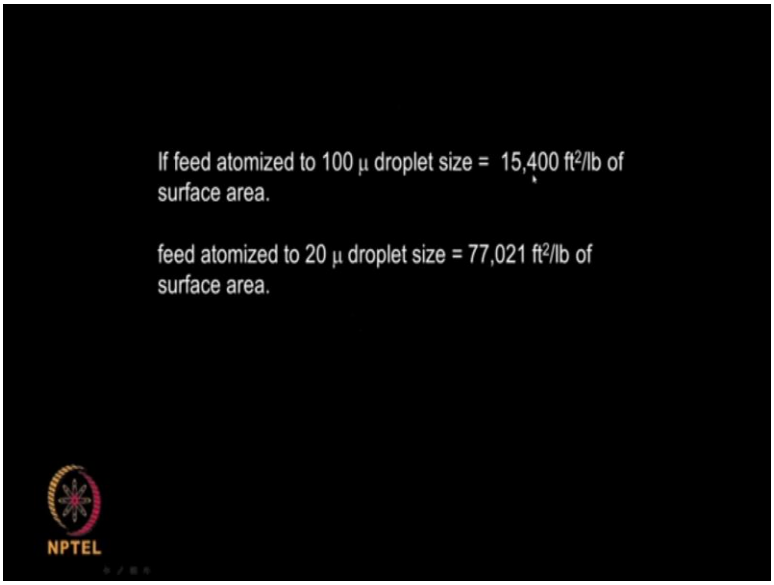

So the feed is atomized to 100 micron then the droplet then you have 15400 feet square per pound of surface area. If the feed is atomized to 20 microns then we are talking about 77000 feet square per pound of surface area okay. So the surface area is almost like d square okay, so final surf surface area where it then finer the surface area that means will become smaller and smaller. So the surface are becomes larger and larger actually that is the advantage of this type of dryer.

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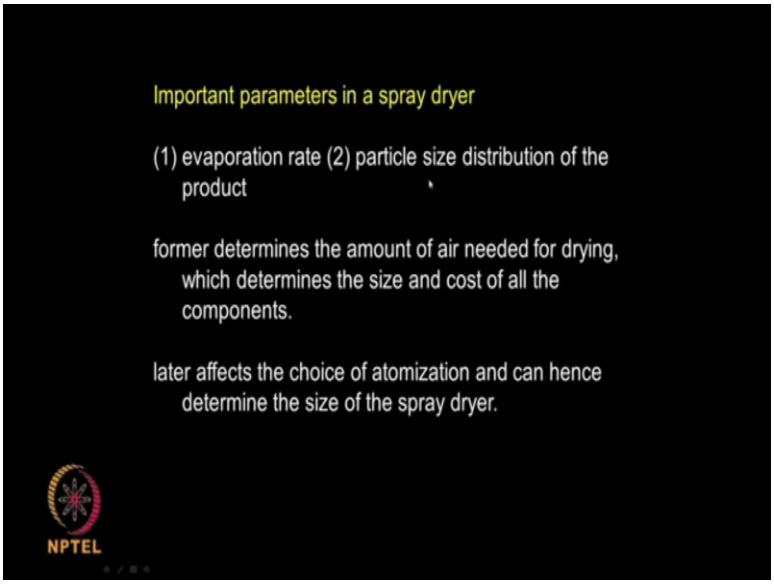

So what are the important parameter in a spray dryer? Evaporation rate, particle size, distribution of the product, so evaporation rate determine the amount of air needed if I want to evaporate at certain that means, I am evaporating the solvent or the moisture. So it tells you how much air I will require for drying. So that will determine the cost of the air handling system in a compressor or a blower.

The particle size distribution that affect the choice of how to do the atomization. So that will tell you what will be the size of the spray drying unit. So the former will tell you the size of your air handling system the later will tell you the size of your spray drying chamber.

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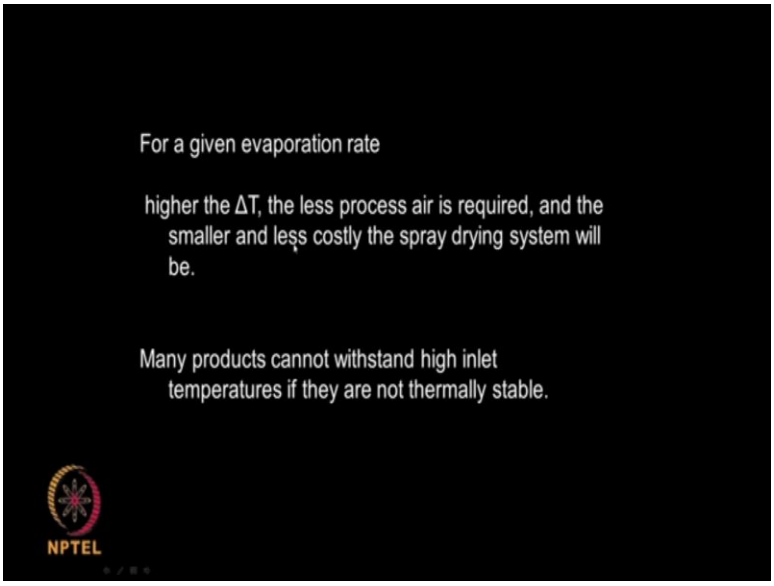

For a given evaporation rate, higher the delta t you will require less process air. So if you require less process air you will have a smaller and less costly spray drying system. Of course if we are going talking about high delta t, you have to be very careful your material does not degrade okay. So, but if it cannot withstand very high temperature then your delta t has to come down. So if it can if it in the material can withstand then, we can have a higher delta t the system will be quite small.

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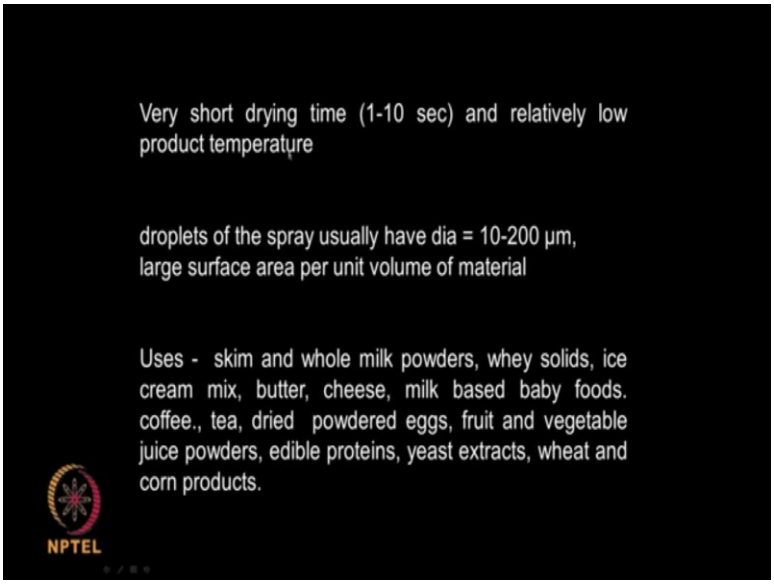

Very short drying time that will give you a very low product temperature. So your product will not degraded, droplet's of the spray usually have diameter in this rate into 200 micron. So it

gives you very large surface area okay. So it is used to as I said quite a lot in food industry and milk processing milk product and so on pharmaceutical products and so on actually.

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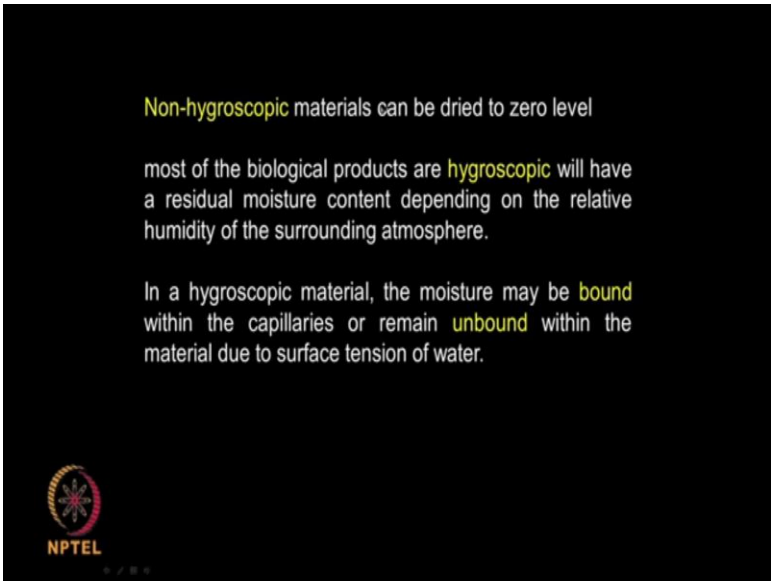

Non-hygroscopic materials can be dried to almost 0 level because most of the biological products are hygroscopic. So it will have some residual moisture content depending upon the relative humidity. Hygroscopic materials you will have bound moisture and unbound moisture. So bound moisture is within the capillaries whereas the unbound moisture is generally present in material as surface tension okay.

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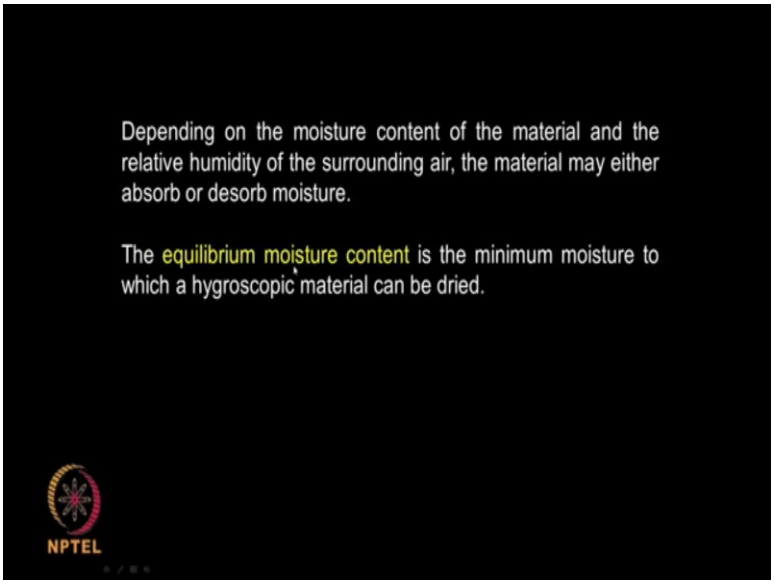

So then you have the equilibrium moisture content that is the moisture content which the material will hold at a given relative humidity. So at a given relative humidity we cannot bring the amount of moisture below that equilibrium moisture content.

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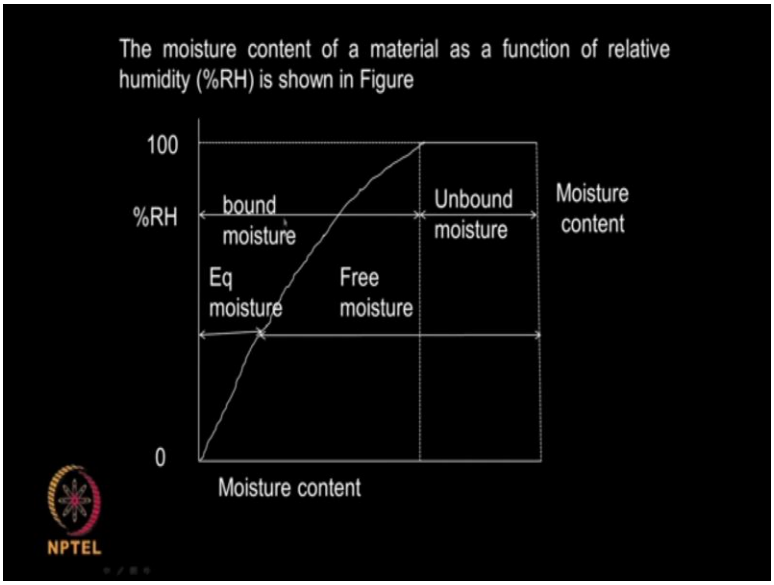

Okay so if you look at the graph between moisture content on the x-axis and the relative humidity on the y-axis okay, so as the moisture content increase the relative humidity increases okay. This is called the equilibrium moisture, so we have the bound moisture here and we have the unbound moisture here. Okay. We have the bound moisture, unbound moisture here and this is the equilibrium moisture whereas this is the free moisture. So we can remove all the free

moisture but as soon as you reach the equilibrium moisture content at a given relative humidity, you cannot go below that moisture content.

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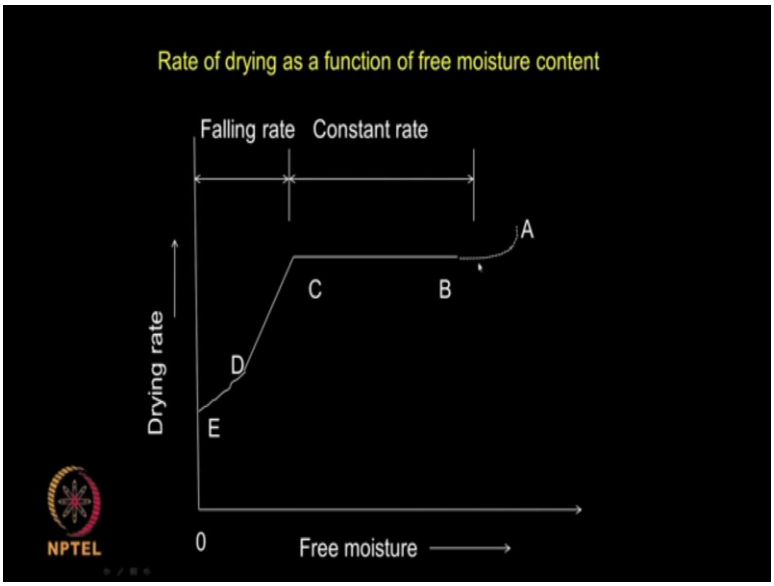

Okay so when you are drying material okay initially the moisture is so much, that at a constant rate the moisture is removed, that is called the constant rate drying. Once the moisture amount goes, moisture is present only inside the capillaries. So the water has to come up come up to the surface and then it has to get evaporated. So the drying rate goes down.

So here the controlling factor is how the water diffuses out from the capillary to the outside. Initially you have plenty of water on the surface, so water is evaporating fast that is why you have constant drying rate but once that has depleted water that is present inside that the solid, inside the capillaries has to come out or diffuse out to the surface and then dry and that is called the falling rate okay. And finally you reach the equilibrium moisture content, okay beyond that you cannot dry the material further okay this is how it looks like.

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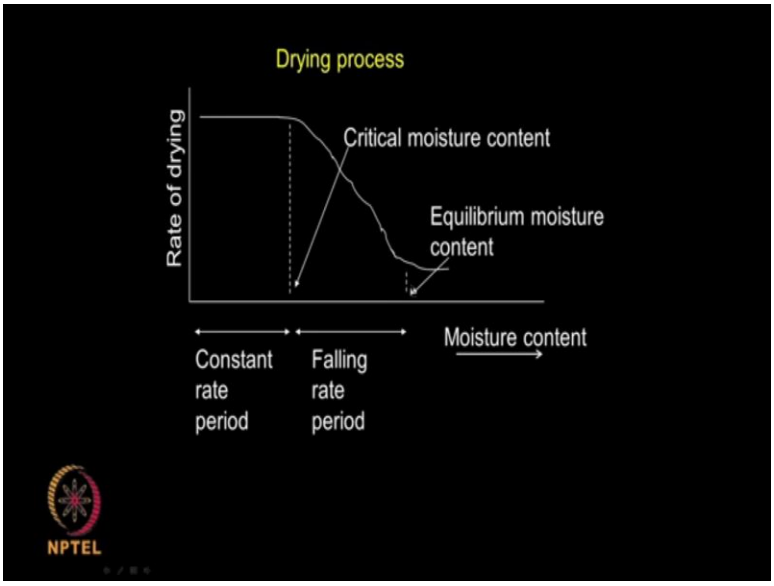

Whereas if you look at the rate of drying, you have the constant drying period and then the falling drying period and then this is the equilibrium moisture content. Okay. This is the constant drying where the rate of drying is constant. So the concentration where there is a shift form constant to falling rate that is called critical moisture content. So equilibrium moisture content is the final value which you can reach for the final relative humidity whereas critical moisture content is the connecting between the constant rate period and the falling rate period. So.

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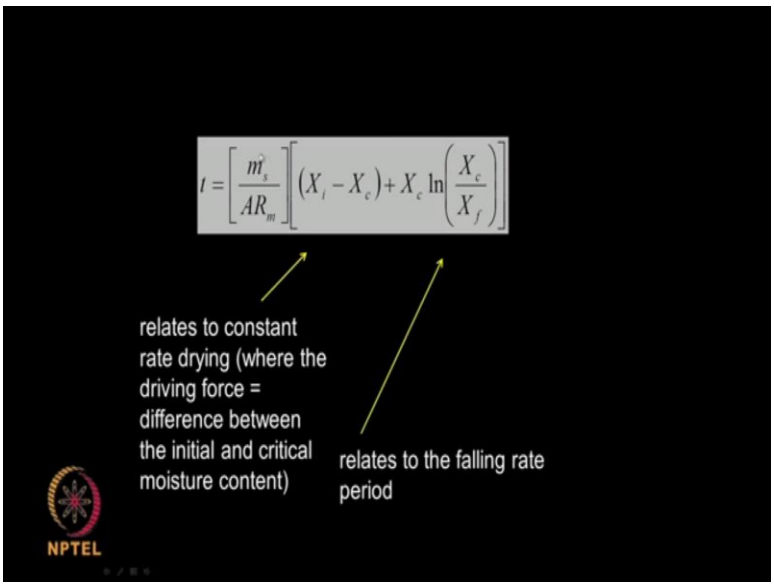

Generally there is an equation like this okay, so you have the amount of rate of moisture removal as function of A and R is the constant rate drying, okay so it and then you have 2 terms here, one

relates to the constant rate and one relates to the falling rate. So the one, this one is falling rate, this one is constant rate and then we come to a term called the critical moisture content, okay. So until critical moisture content it will be the constant rate and after that it will be falling rate period until it reaches the equilibrium moisture content. Okay. This is the typical equation simple equation which relates all these parameters.

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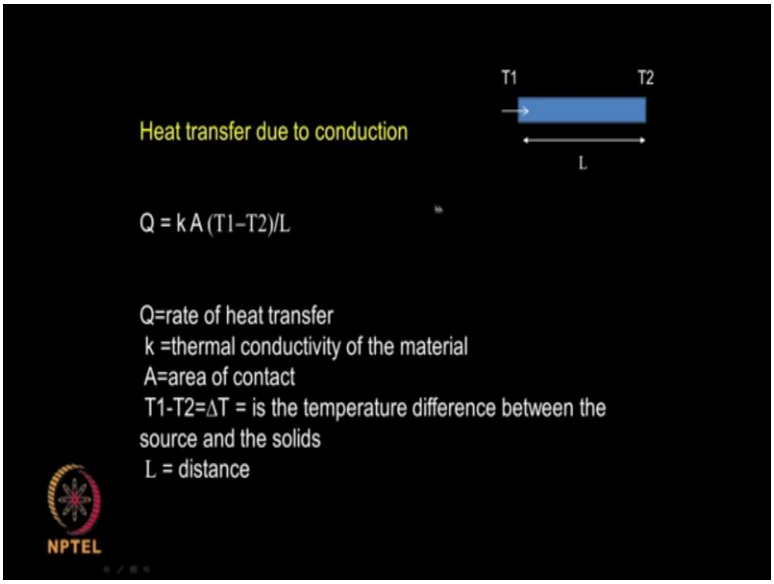

So heat transfer can happen because of conduction and you all know this equation, must have studied long time back rate of heat transfer = k thermal conductivity area a t1 - t2, okay is the driving force / the l l is the length. So t1 - t2 is the driving force that is in the conduction.

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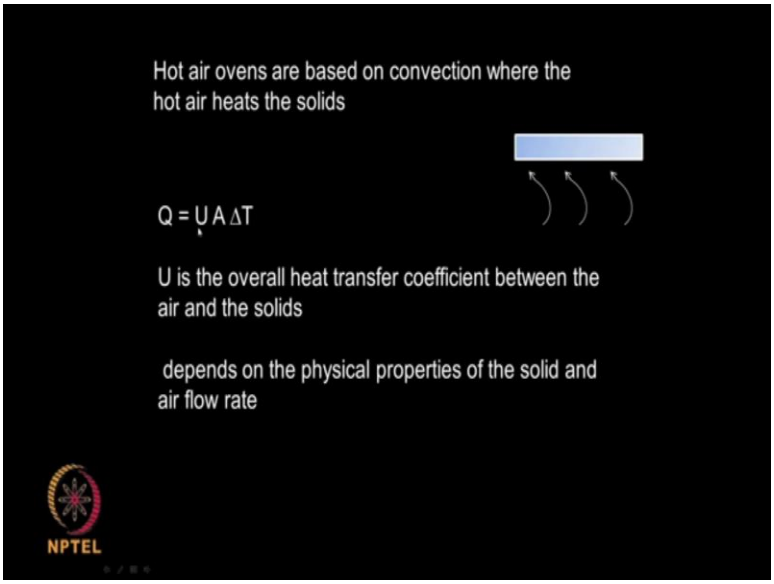

If you take conduction heat read heat, q amount of heat, you need to supply u a delta t a is the area, u is the heat transfer coefficient overall heat transfer coefficient, delta t is the driving force okay. U depends on the physical properties of the solid and the air flow rate and So on actually okay.

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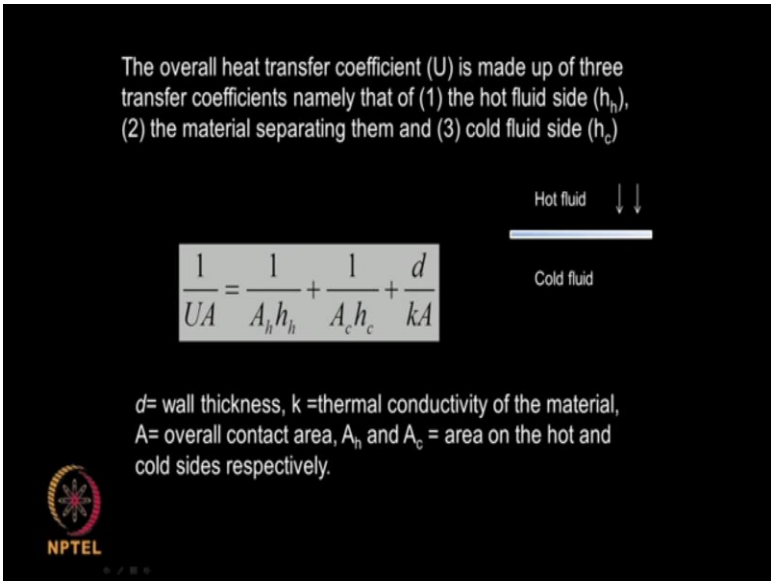

So u is related to individual heat transfer coefficients, okay heat transfer coefficients related to the hot fluid side, heat transfer related to the cold fluid side and the thermal conductivity of the material. Again you might have read this equation long time back. So this relates the overall heat transfer coefficient with the individual heat transfer coefficient.

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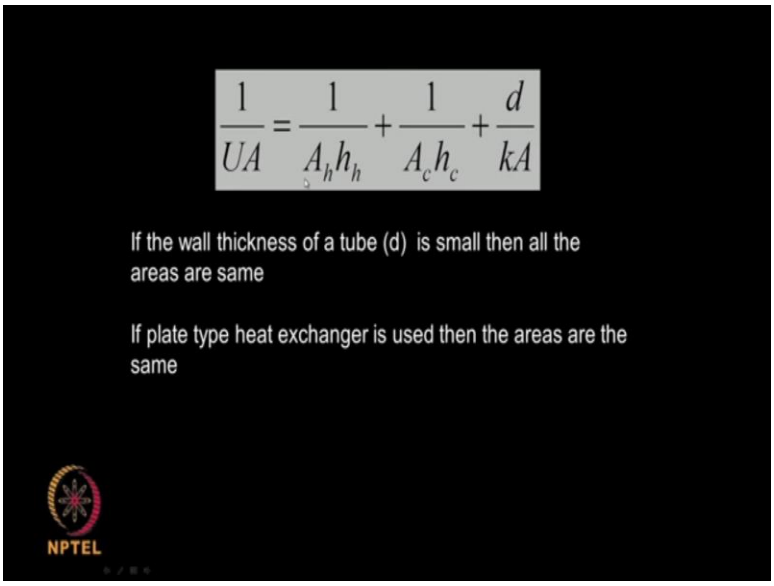

So if the wall thickness of the tube is small then all the areas will be equal okay. So we have equation of this form actually simple equation of this one.

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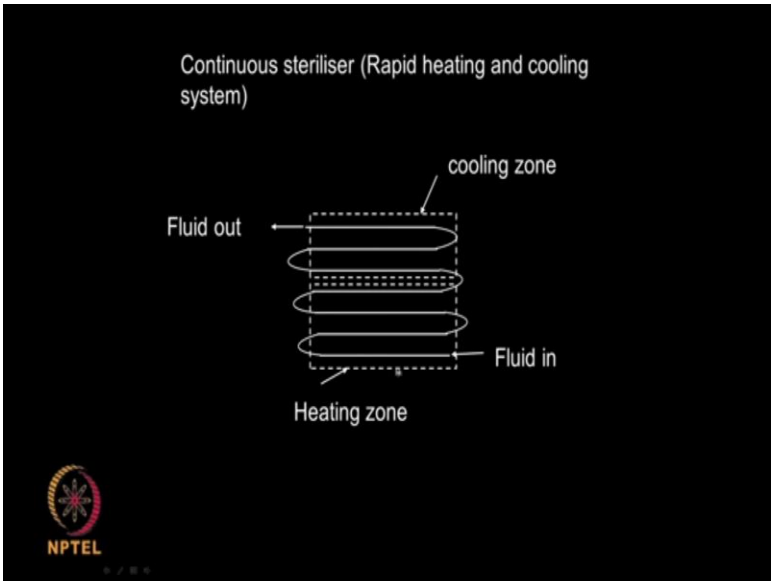

We have continuous sterilizers where you have rapid heating and cooling taking place okay you quickly heat and then you quickly cool generally it is used for pasteurizing milk. As you know milk you do not want to quick heat and keep it for a very long time because it may get denatured.

So this type of rapid cooling and sorry rapid heating and rapid cooling is done to kill the bacteria at the same time, not to lose the more the flavor of the milk.

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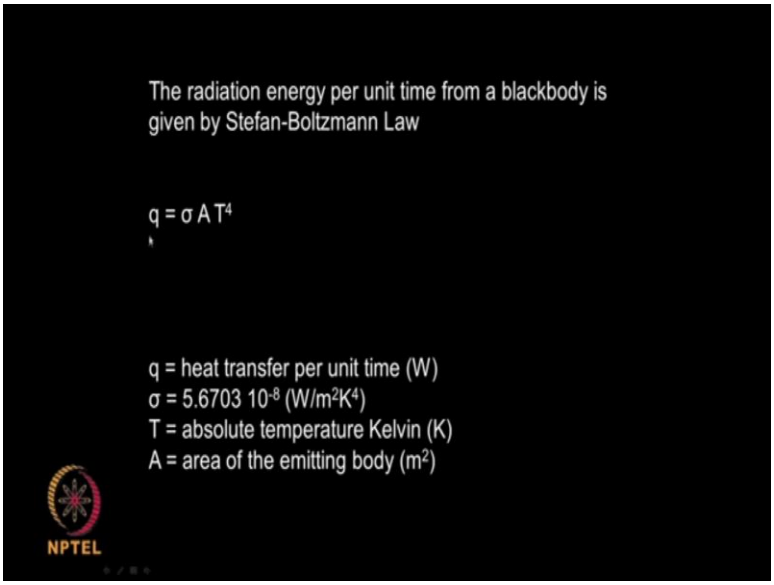

So if you go to the heating because of radiation, then you must be knowing this heat transfer = sigma at power 4, a is the area and t is the temperature, absolute temperature okay and sigma is a constant, so we looked at drying. Now let us look at lyophilisation. This is very common in biological systems because most of the biological products, biomolecules are temperature sensitive. We do not want to heat up and make lose it is activity.

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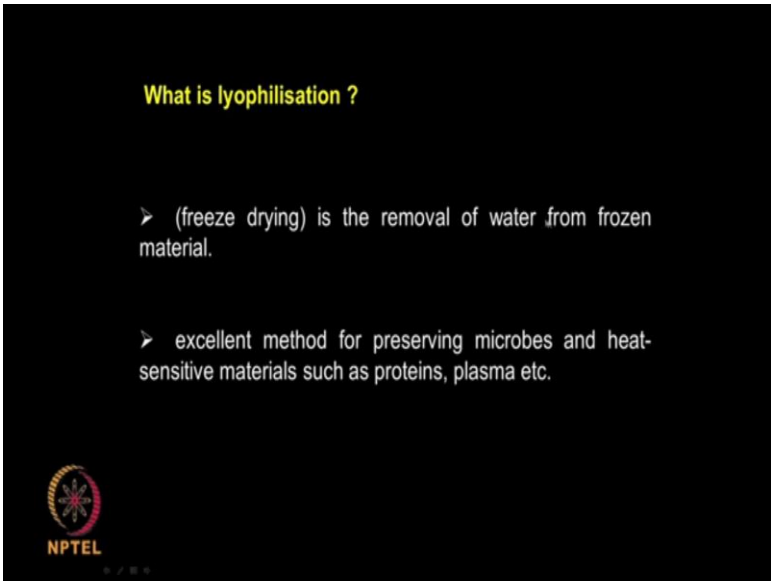

So generally lyophilisation is resorted to so that you handle it at low temperature. So it is freeze drying removal of water from material, it is very good for preserving microbes and heat sensitive materials such as proteins plasma and so on.

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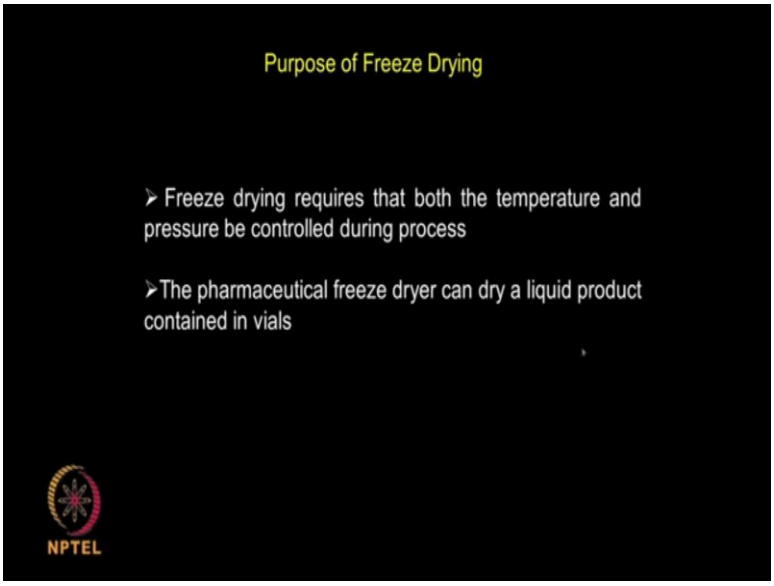

So this requires both the temperature and pressure to be controlled during the process okay it can dry a liquid product contained in vials also, it can dry products into solids and so on actually.

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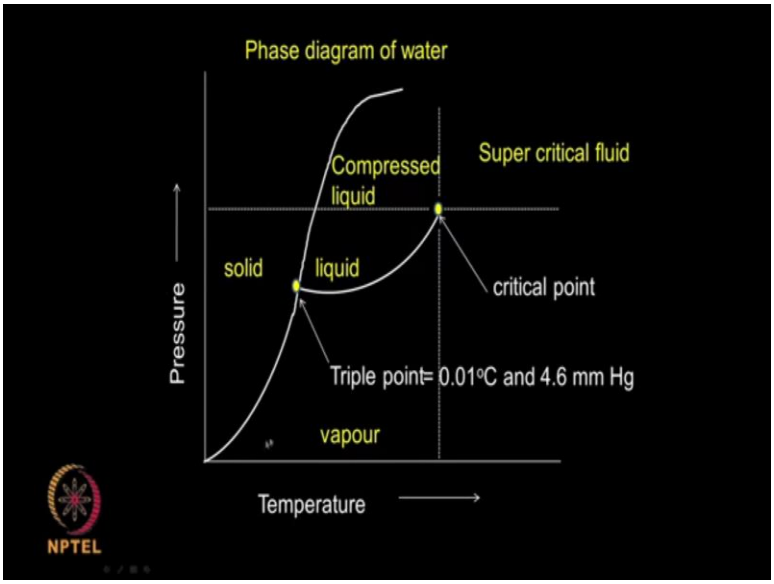

So if you look at the phase diagram for water, what is the phase diagram? You have the temperature, you have the pressure, you have the solid liquid vapor okay, solid at lower temperature, okay vapor at higher temperature and lower pressure okay liquid at higher pressure and higher temperature okay. At one point here this is called a critical point, we have the super critical fluid here, then we have the compressed liquid.

There is another point which is called triple point, where the solid, liquid and the vapor are in equilibrium okay. This is called critic triple point, solid, liquid and vapor are in equilibrium. This is where you operate. For example for water if you look at it is - sorry 0.01 centigrade and 4.6 mm Hg, so I can move from liquid to vapor and then I can move to solid and then I can move to liquid or I can move from solid to vapor without going into liquid at this triple point.

Otherwise normally if you are here for example you have to go from solid to liquid, then you come to vapor okay if you are heating ice, you go to water then you will go to vapor but if you are here you can directly from solid you can go into vapor okay. That is a beauty of it, so that is what the lyophilizer makes use of. That is the principle of it so this is the triple point 0.01c centigrade and 4.66 mm okay. So we can directly move from solid to vapor okay.

So if I am having I liquid water, I can move from liquid to solid okay make into ice and then I do not want to go again to liquid, I want to go into vapor. So what I will do? I will reduce the

pressure and then i will increase the temperature, so it will go from solid to vapor. So I am here. So from here I go like this, like this and like this. So liquid is converted into vapor at all at low temperature. Remember that. That's the beauty of lyophilizer okay.

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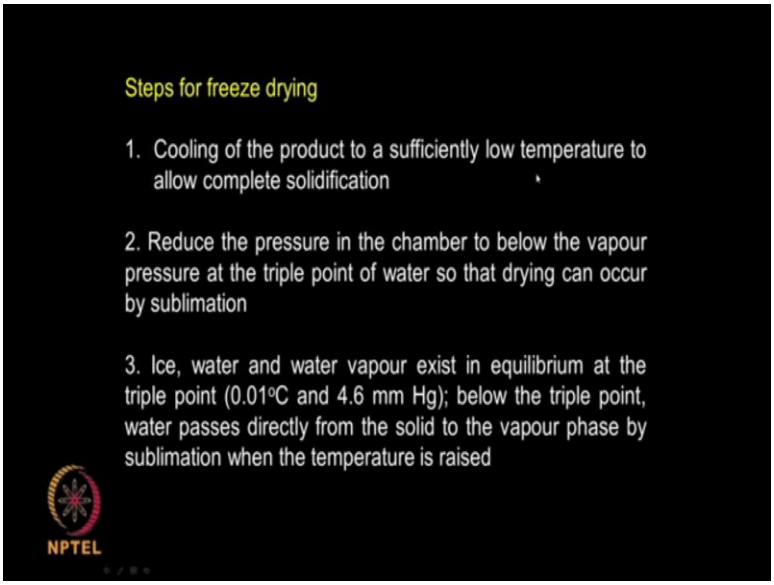

So cooling of the product to a sufficiently low temperature to solidify, reduce the pressure in the chamber to below the vapor pressure at the triple point, okay and then increase the temperature So that it passes directly from solid to vapor. That's what it is. Right?

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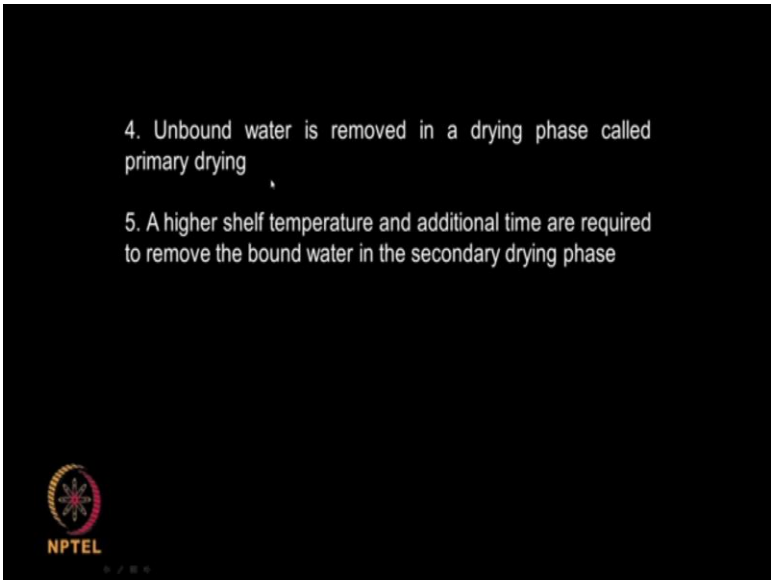

So unbound water is removed in drying phase called primary drying and then higher shelf temperature and addition time required to remove the bound water in the secondary drying. So you have a primary drying unbound water, secondary drying where the bound water is removed okay.

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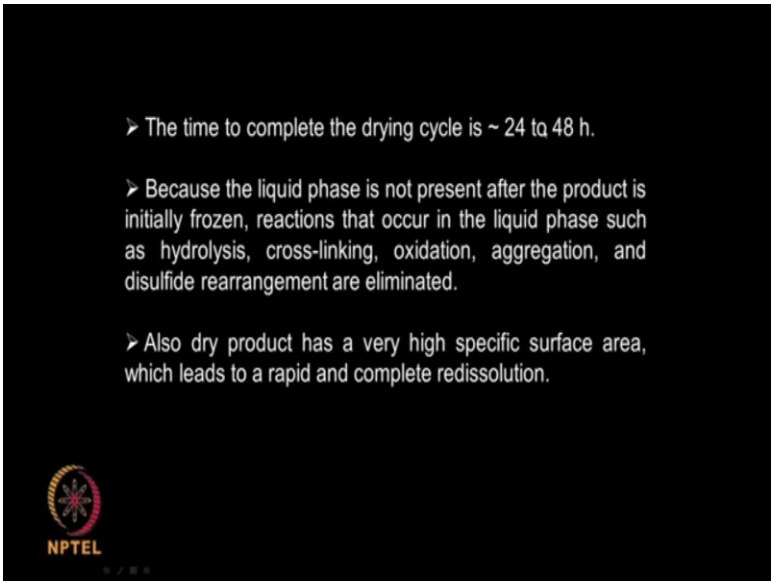

Generally it takes long time almost 48 hours for performing a lyophilisation. So we can do lyophilisation of lot of products using this type of technique actually.

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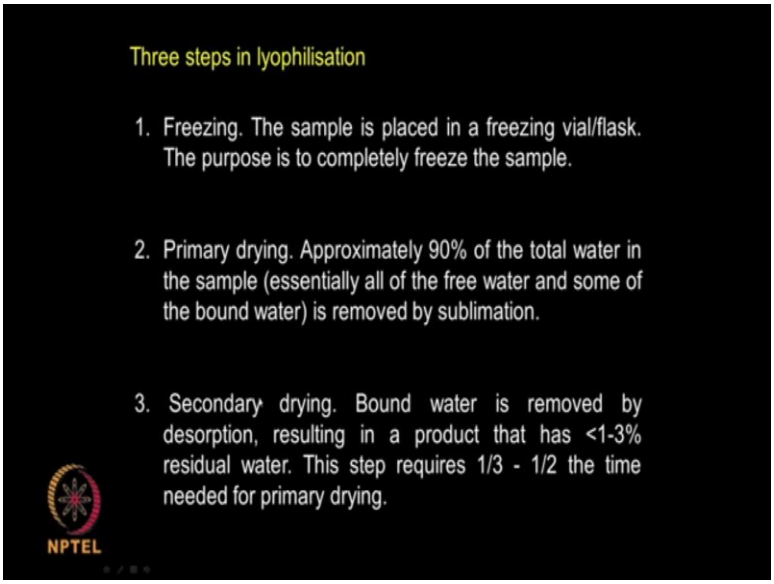

So freezing primary drying secondary drying so approximately 90% of the water is removed in the primary drying, that is by sublimation and then secondary drying where we have the bound water, you can bring down the moisture content to less than 1 % here okay using this secondary drying. So bound water is removed by desorption resulting in product that has a very very low amount of moisture.

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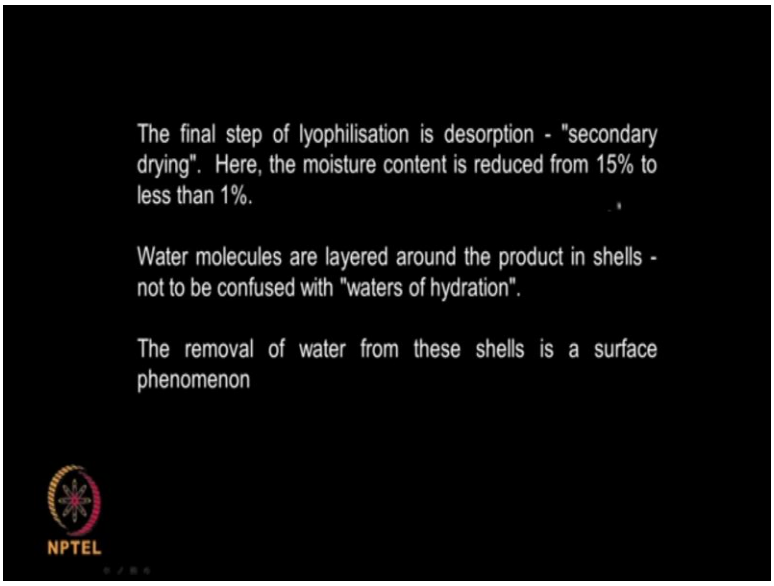

So in the secondary drying the moisture content is reduced very less to almost 1 % then removal of water these shells is a surface phenomenon actually.

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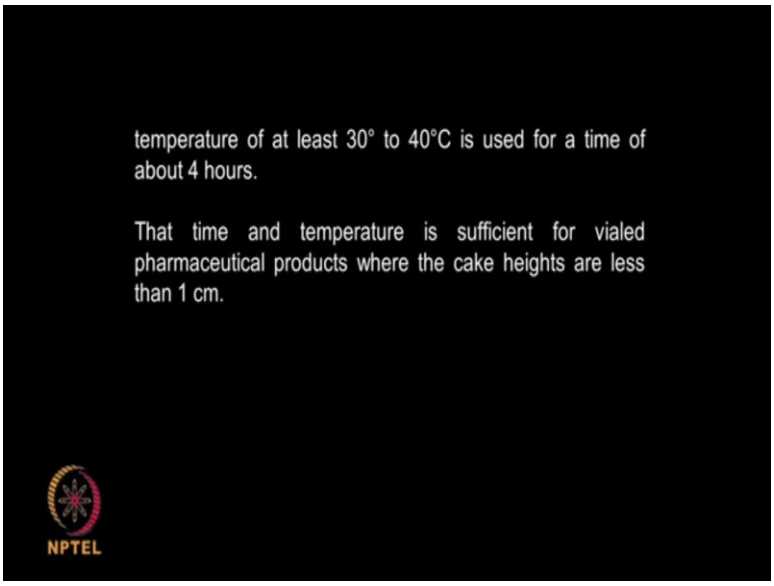

So you also use 30 to 40degrees for 4 hours to remove all the whatever moisture is there from the product okay.

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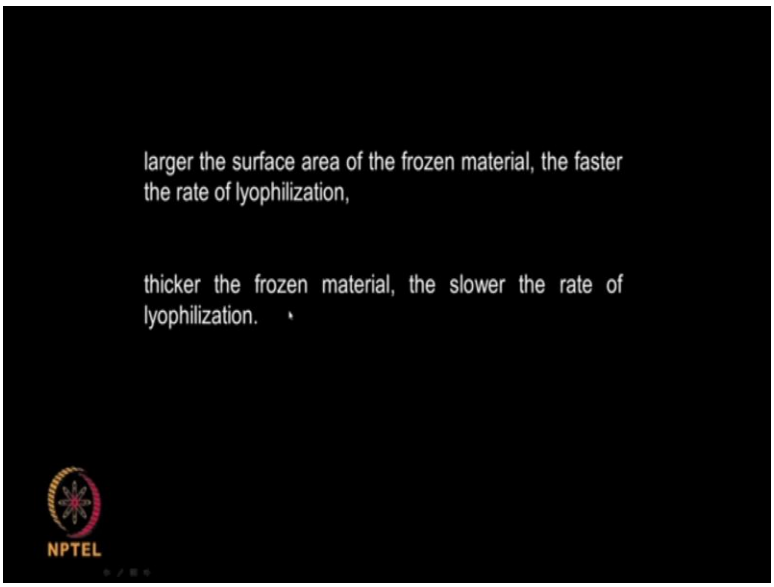

Larger the surface area of the frozen material, faster will be the rate of lyophilisation, thicker the frozen material, slower the lyophilisation, these are very logic. Right? So you want to spread out the material to a very large surface area so that lyophilisation is very good okay.

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So what are the advantages and disadvantages advantages of lyophilisation? A preservation of chemical and biological potency the final product protection from solution effects and chemical degradation speed and completeness of rehydration compared with dry powder okay. It can be quickly again rehydrated by following lyophilisation.

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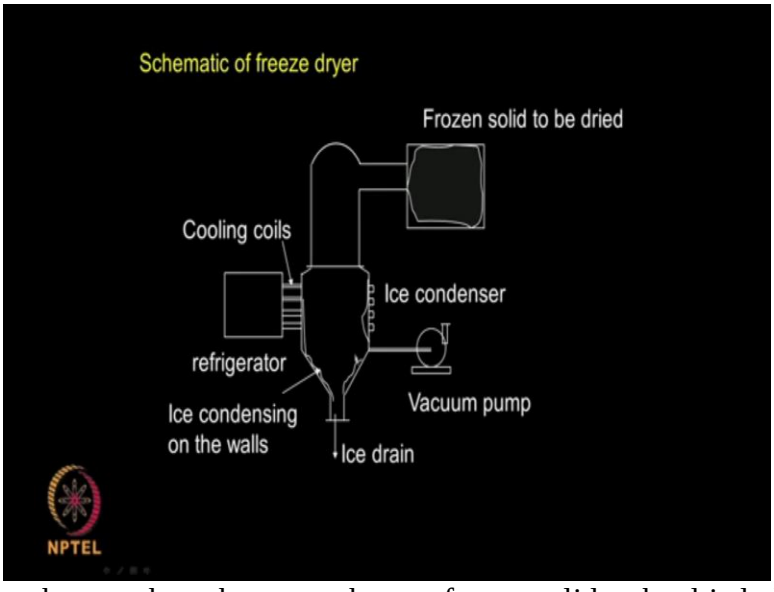

So typical setup so what you have here, you have a frozen solid to be dried okay then the triple below the triple point becomes vapor and then it is condensed as ice here and then if it ice drained so you are applying a vacuum here. So it is generally a batch process.

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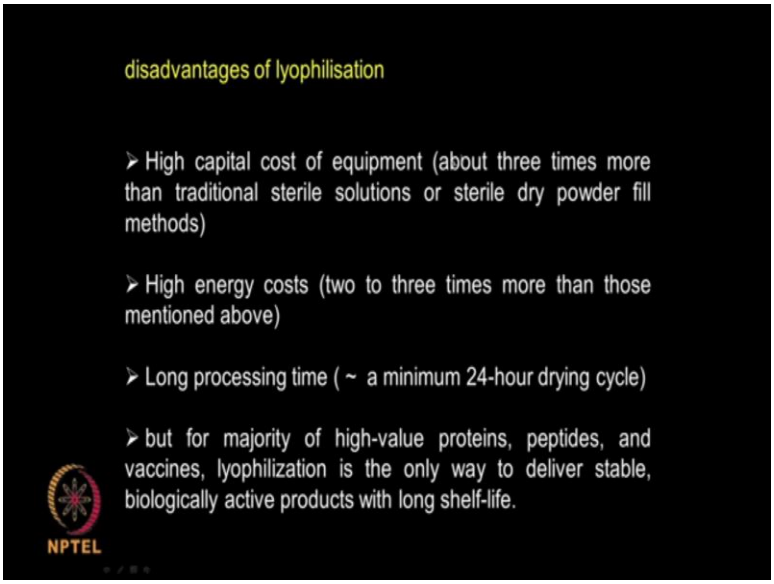

What are the disadvantages? High capital cost of the equipment, it is three times more than a dryer that's much cheaper, dryer high energy cost because you use lot of cooling vacuum long processing time we are talking almost 48 hours but for majority of high-value proteins peptides there is no other way except resorting to lyophilisation. Let us look at stabilization you want to stabilize your product, if you have say enzyme or a protein so that you can increase the shelf life, you do not want it to degrade because of say hydrolysis or oxidation or amidation.

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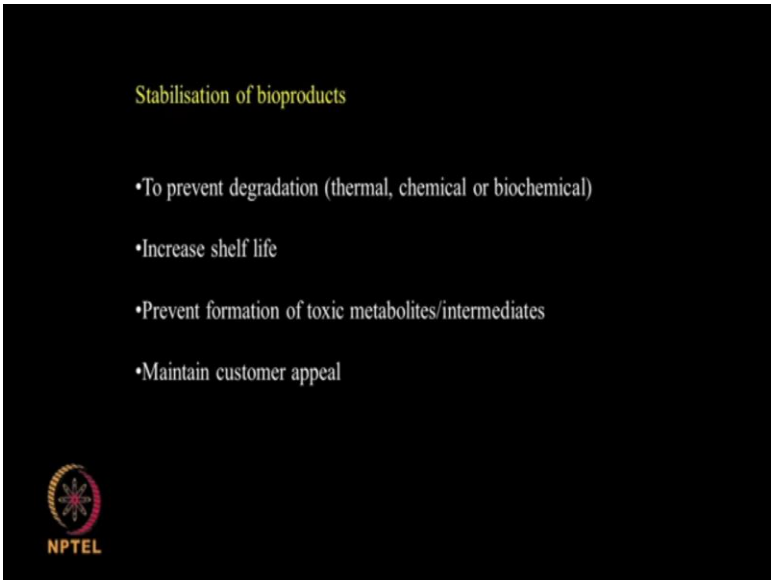

So to prevent degradation could be thermal, chemical, biochemical, increase shelf life prevent formation of toxic metabolites maintain customer appeal. That's why you do this type of stabilization.

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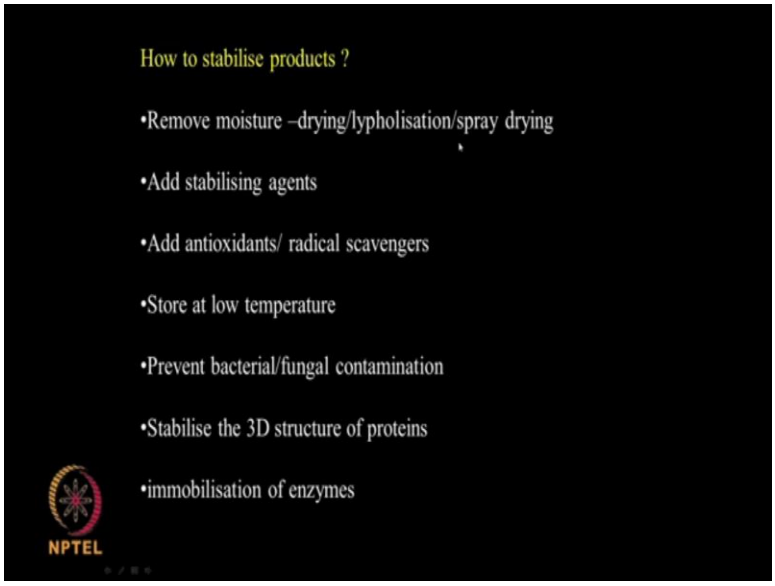

So what do you do? How to stabilize? we can remove moisture by drying lyophilisation, that is a very good stabilization, even spray drying add a stabilizing agent, add antioxidants which are radical scavengers. It will not allow oxidation to takes places okay if there are any radicals or if there are any oxidant formed it scavenges. Store at low temperatures, prevent bacterial fungal

contamination, stabilize 3d structure of protein, immobilization of enzymes that also improves the stability of the enzymes.

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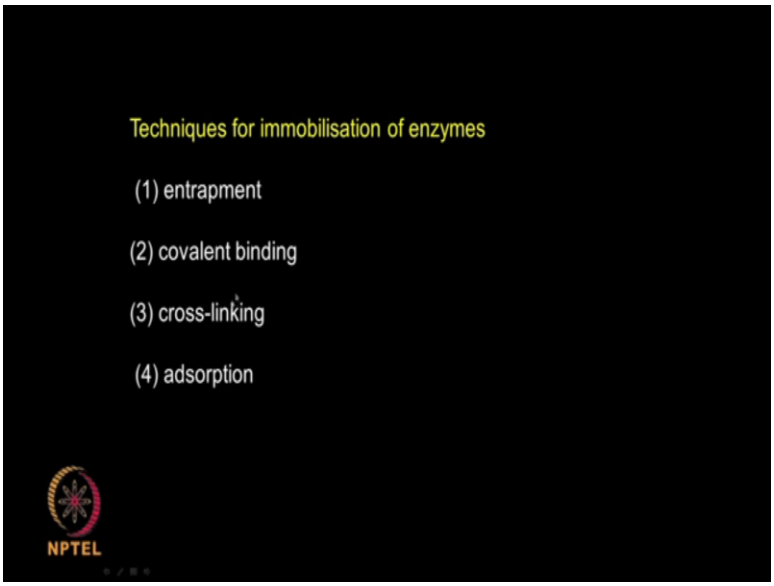

So what are the different techniques for immobilizing enzymes? We can do entrapment, covalent binding, cross-linking adsorption, let me show it in the next slide.

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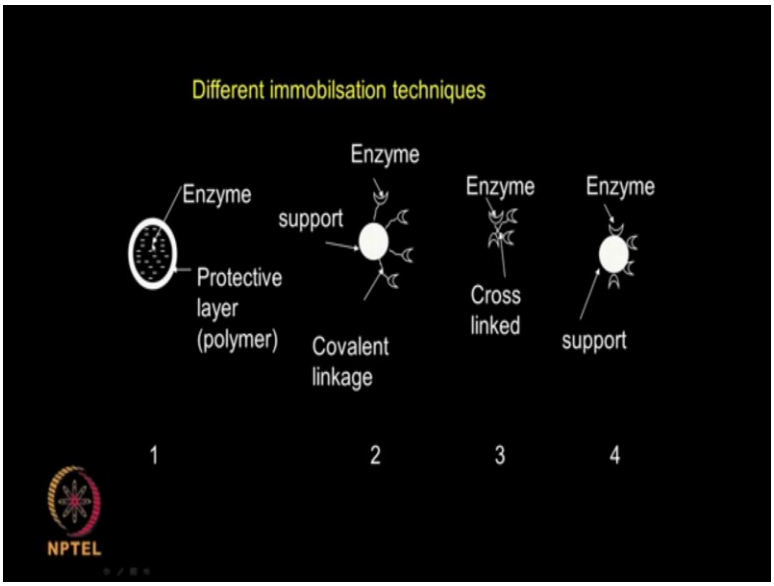

So we can entrap our enzyme on through a protective layer of polymer, so the enzyme is inside. So there is a layer of polymer so the enzyme does not denature. Another approach is covalent

binding so the enzyme and the support are covalently connected here. So the enzyme is covalently bound to the support so the enzymes retains it is activity. Cross-linked, the enzymes can be cross-linked okay to each other so that they do not lose their activity.

Another approach is using enzyme with the support okay that is the enzyme is adsorbed on the support. So mostly it is non-bonded interaction unlike the covalent immobilization where there is a bonded interaction between the support and the enzyme and here it is more non-bonded type of interaction. All these will prevent the stability and the activity of the enzyme of course the enzyme elute lose little activity but generally they are kept for a very long time, especially immobilized enzymes are very active much longer than the native enzyme and if you are doing any reactions. We can do it for many cycles without any problem.

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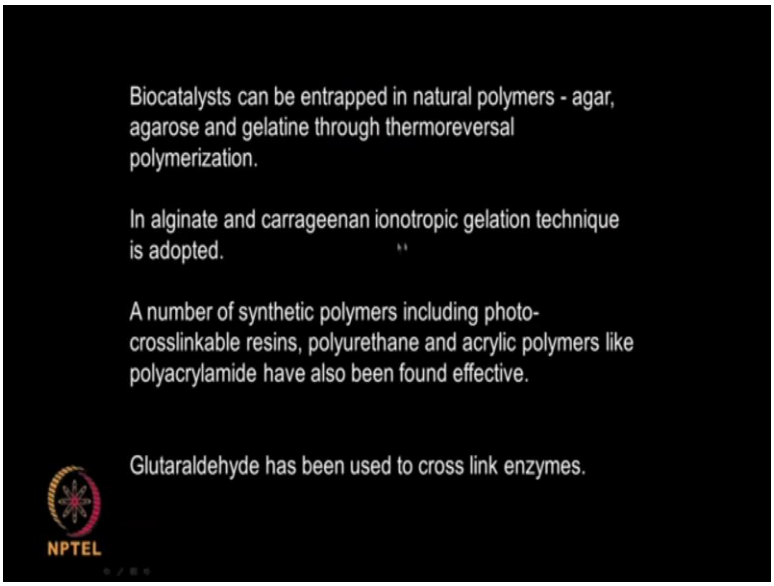

Biocatalyst can be entrapped in natural polymers like agar, agarose, gelatin, alginate, carageenan. So we can use all these type of biopolymers okay number of synthetic polymers also we can use like polyurethane, acrylic polymers, acrylamide, okay glutaraldehyde, also been used for cross-linking enzymes to in support, so different approaches can be adopted for immobilizing these on to support.

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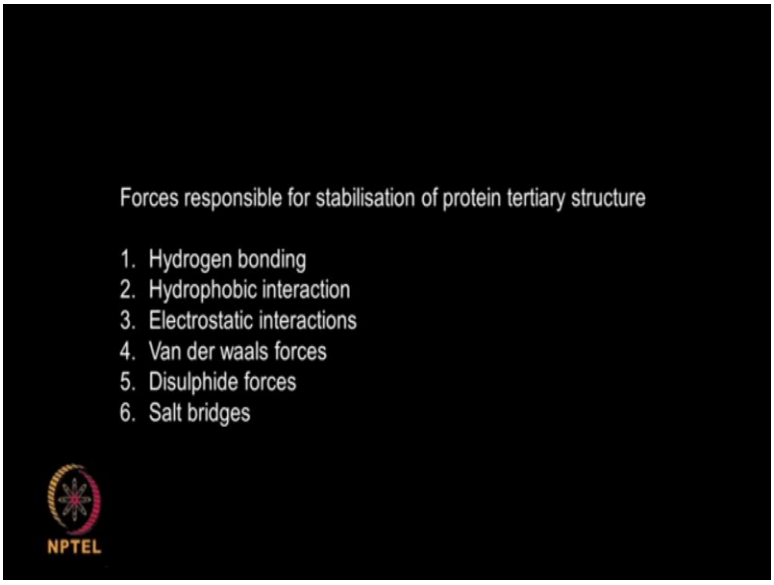

So what are the forces? Hydrogen bond, hydrophobic interaction, electrostatic, Vander Waal's forces, disulphide forces, salt bridges. So all these are various forces which stabilization of the protein can be achieved.

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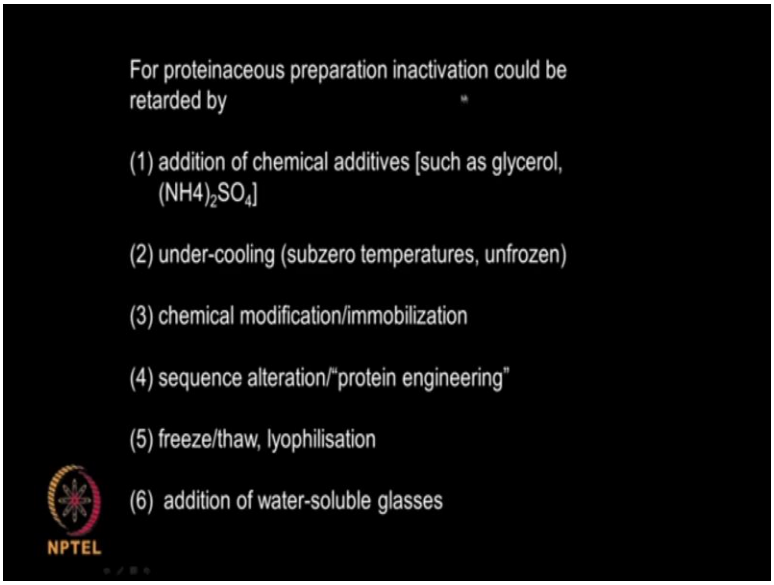

So proteinaceous preparation inactivation. Why do they get inactivated it could be addition. So we can retard it, retard these inactivation by adding chemical additives like glycerol cooling low temperature, chemical modification like immobilization, sequence, alteration that is protein engineering, freeze thaw, lyophilisation, addition of water-soluble glasses.

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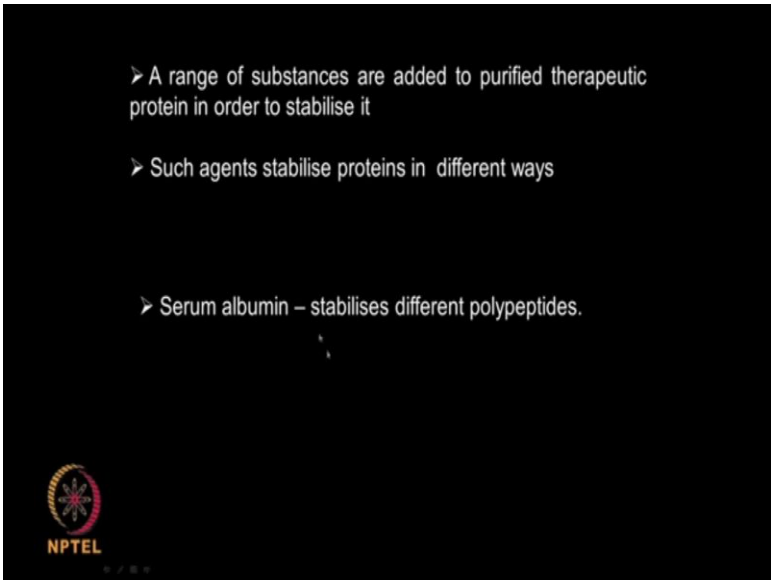

So all these are different techniques by which we can slow down. A range of substances can add if I purify therapeutic protein in order to stabilize such as stabilizing protein in different ways serum albumin, it can stabilize polypeptides. So there are different other approaches by which we can stabilize that.

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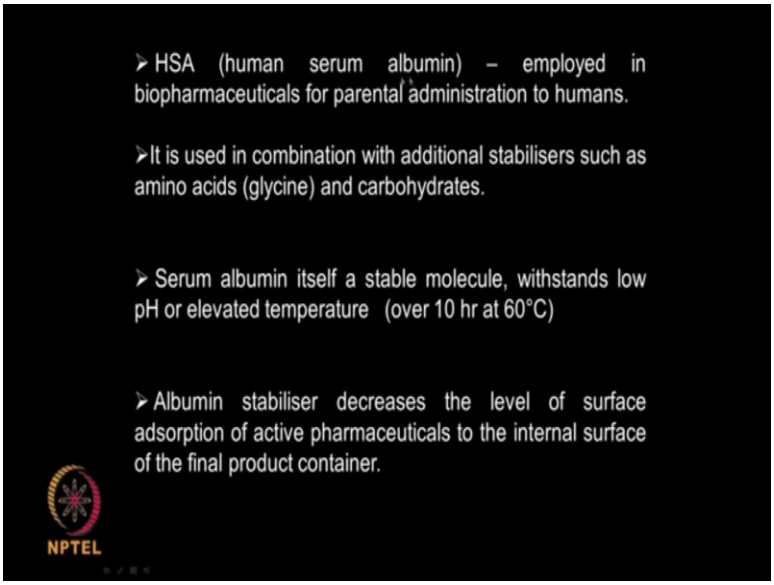

For example HSA, human serum albumin is used to stabilize some biopharmaceutical parental administration to humans, it is used in combination with additional stabilizers such as amino acids and carbohydrates. Serum albumin itself is a stable molecule, it can withstand low pH or even elevated temperature. So when we use this it decreases the level of surface adsorption of

active pharmaceuticals of the internal surface of the final product container, okay. So that is the advantage of using HSA human serum albumin okay. So that is a good stabilizer because it can take up very low pH as well slightly higher temperature.

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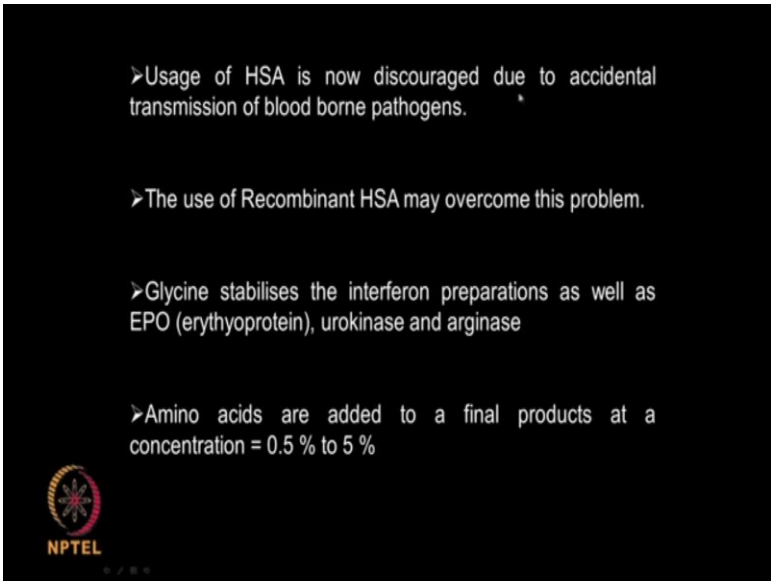

HSA is now discouraged due to accidental transmission of blood borne pathogens. So there are recombinant HSA's human serum albumins are coming into glycine also stabilizes the interferon preparations as well as epo ethylprotein okay. Amino acids are also added to a final products at a concentration that also can act as a stabilizer okay.

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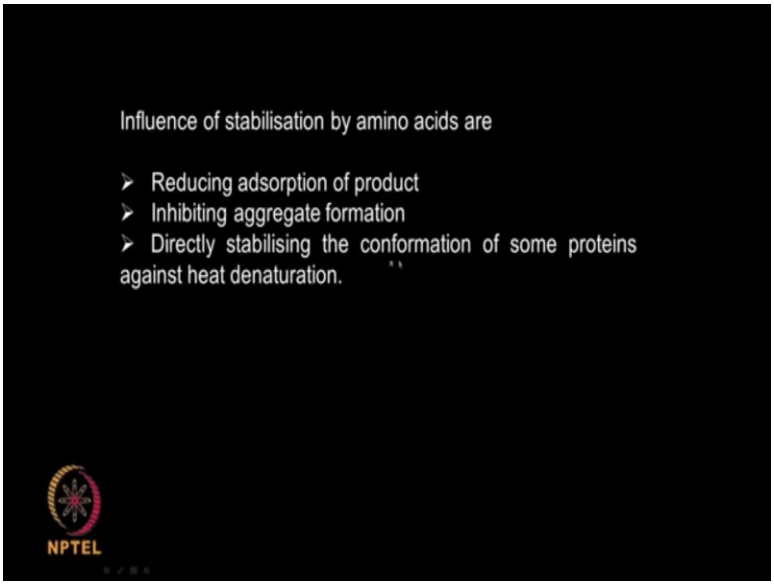

So these amino acids can reduce adsorption of the product inhibition, inhibits aggregate formation by directly stabilizes the confirmation of some proteins against heat denaturation okay.

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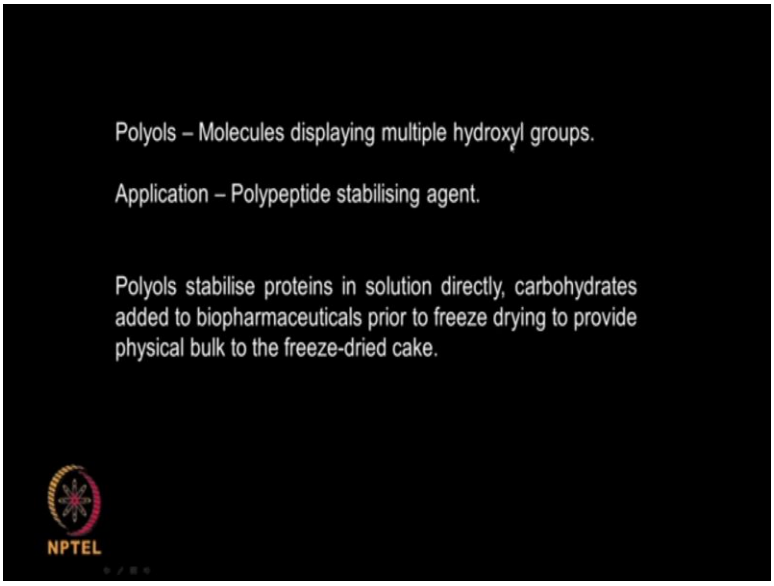

Polyols these are molecules displaying multiple hydroxyl groups that's why there are called polyols. So they are very good for polypeptide stabilizing agent. So the polyols can stabilize the proteins in the solution directly carbohydrates added to biopharmaceutical prior to freeze drying prio to provide physical bulk to the freeze-dried cake. So polyols can also act as a very good stabilizing agent.

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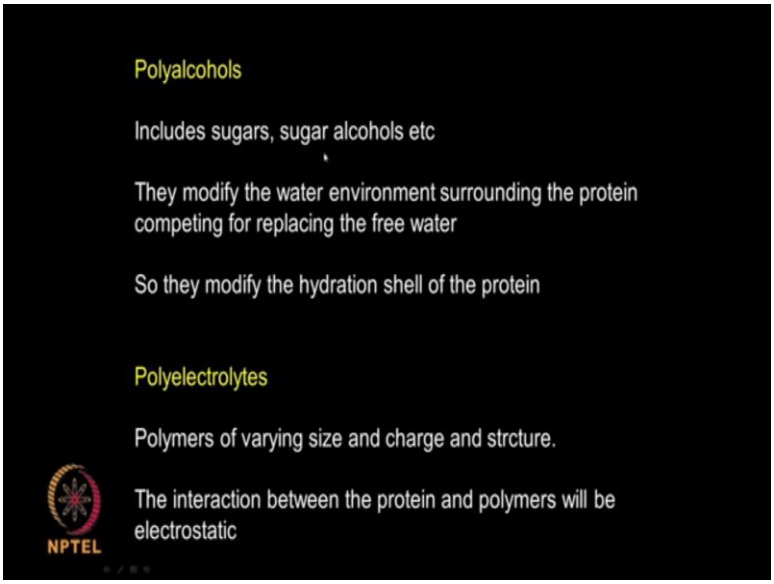

Polyalcohols includes sugars, sugar alcohols, etcetera. They modify the water environment surrounding the protein competing for replacing the free water, so they modify the hydration shell of the protein. Basically they also compete with the water that is present in the protein. So the modi the that way they stabilize the protein. You can even stabilize it with polyelectrolyte that is polymers of varying sizes and charge okay they can also act as a stabilizing agent okay. So they interact with the proteins through electrostatic forces so they stabilize the protein.

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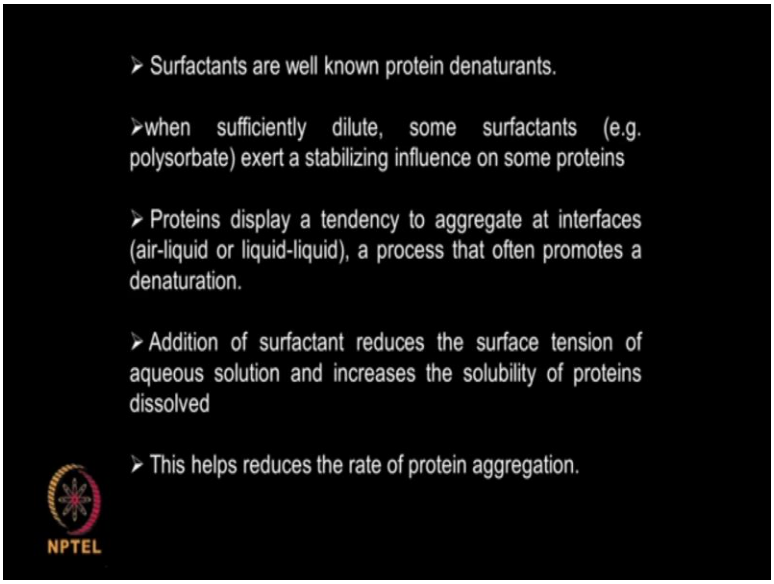

There are surfactants which are well known for protein denaturants when they are sufficiently dilutes, you know when they are very dilute, they also exert a stabilizing influence like polysorbate. Proteins display a tendency to aggregate interfaces especially air-liquid or liquid-liquid this is also promoting denaturation. But if I add surfactant what happens is the surfactant reduces the surface tension of the solution and increases the solubility of these proteins.

So these proteins will not come to the air-water interface or air liquid interface okay or liquid-liquid interface. Thereby protein remains in the solution and so it does not get denatured at all or it does not allow the protein to aggregate.

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So there are many excipients added to proteins especially in biopharmaceutical industries which stabilize like serum albumin, various amino acids, various carbohydrates, alcohols, polyols, surfactants. So all these help especially when they are added as a finish during the finished product to stabilize a biopharmaceutical products.

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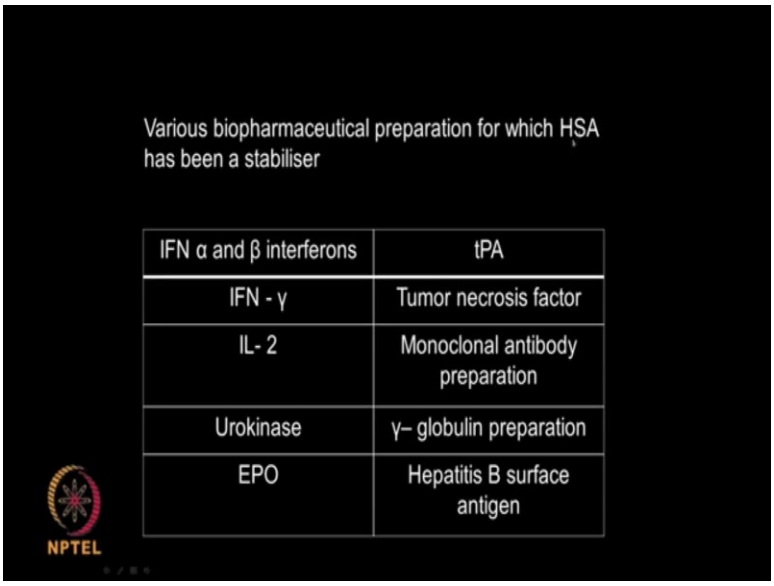

Human serum albumin is also added as a stabilizer as you can see for a large number of interferon gamma interferon alpha and beta and so on HSA acts as a very good stabilizer okay.

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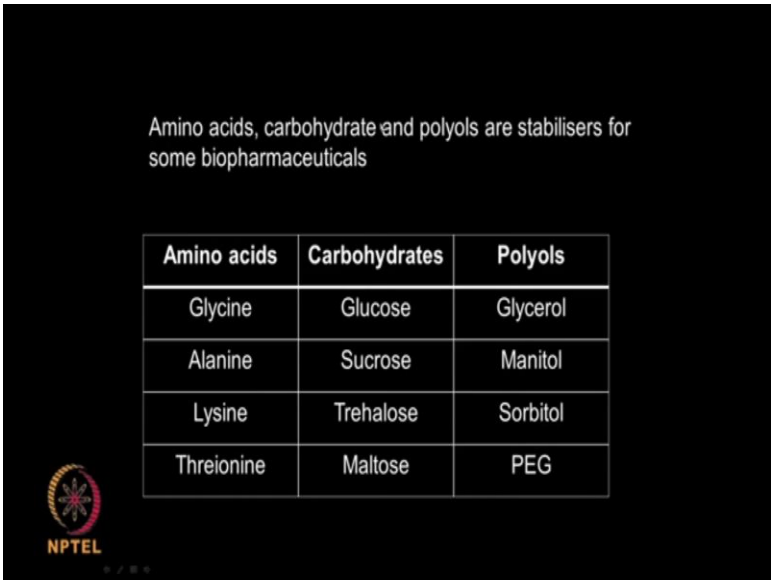

Amino acids, carbohydrates and polyols, amino acids like glycine, alanine, lysine, threionine, carbohydrates like glucose, sucrose, trehalose, maltose, polyols like glycerol, manitol, sorbitol, peg, all these are very good stabilizers, that are used in biopharmaceutical industries and they are also biocompatible. So they enhance the shelf life of the biopharmaceutical product and also prevent the protein aggregation okay. So today we talked about a mixture of downstream activities.

Generally these downstream activities are resorted to at the end of the operation that means at the end of the product purified product, either I want to make the product into a solid and then dry it using temperature I want to either lyophilize it at low temperature, so that I end up with solid product. So prior to that I may like to add a stabilizer because I do not want my product lose it is activity a protein in or a or an enzyme. So I add different types of stabilizers.

So I talked about different types of stabilizers if you have enzymes, you can immobilize the enzymes thereby increasing the shelf life of your enzyme. Thank you very much.