Course on Industrial Biotechnology
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Lecture 26
Module 5
Downstream processing: liquid-liquid extraction, distillation, chromatography

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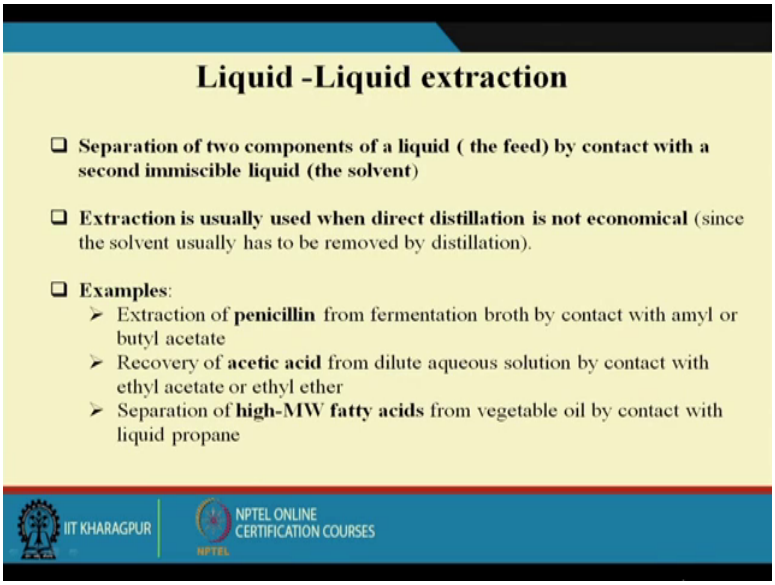

Let me welcome you back in the Industrial Biotechnology course, now I shall continue with downstream processing and in the in this downstream processing we will take care two different aspects one is liquid-liquid separation process and what you call distillation process is largely used by the industry. Now liquid-liquid separation process usually use where we have partition coefficient of the tube liquid is different as for example with respect to a particular solute will be different as for example I can tell you in case of penicillin penicillin separation process that we use butyl acetate as a solvent.

Now butyl acetate when you decrease the pH of the liquid to penicillin solution to 2, the solubility of the penicillin in butyl acetate is more as compared to the water, so when you mix these two layers then we will find that penicillin will go in more in the solvent layer then then since butyl acetate is insoluble in water so you can separate because if separate the layer we can separate the solve that that butyl acetate from the aqua space.

Now if we take water and we increase the pH to 6, 7 then we find that solubility of penicillin in aqueous space is more as compared to the solvent so the penicillin will go to the solvent

phase aqueous phase. So like this we can purify this penicillin and after that as you know penicillin usually marketed in two different forms, one is called in the form of penicillin capsule another form is penicillin liquid that fluid that you know injection fluid. Now when injection fluid we use one we should imagine that you know this that directly injected to the blood steam.

So you should be 100 per cent free from any kind of contaminants is called pyrogen free material but when we use this that you know in the form of capsules even you have little contaminants is there because since I was (())(03:1) is acidic pH of a 2 it will take care. So any how that now I am going to discuss about the liquid-liquid extraction process which largely used by the industries.

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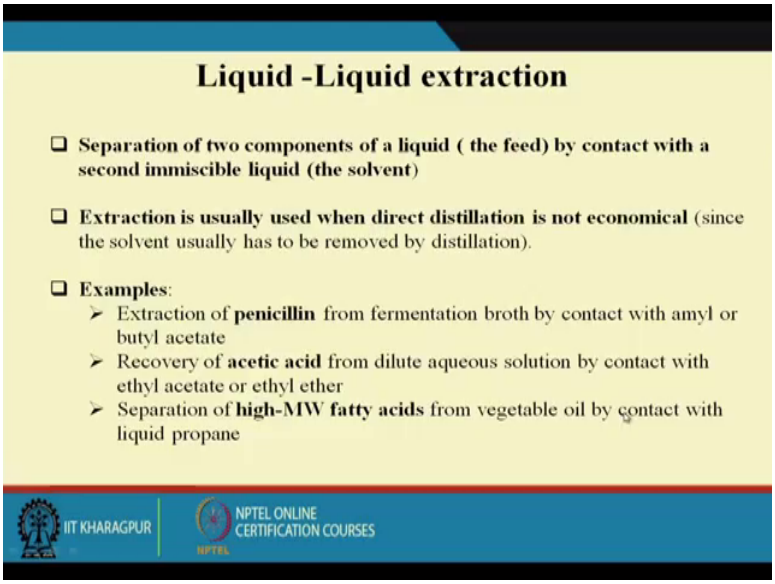

Now the separation of the basically it is it says that separation of the two components of a liquid the feed by contacting with other second immiscible liquid the solvent. This is the solubility of this solute in one solvent will be more as compared this is on the basis of partition coefficient, so the extraction is usually used when direct distillation is not economical.

The examples are penicillin I just extraction of penicillin from fermentation broth by contacting amyl or butyl acetate, then recovery of acetic acid from dilute aqueous solution by contact with ethyl acetate or ethyl ether then separation of high molecular weight fatty acid from vegetable oil by contacting with liquid propane.

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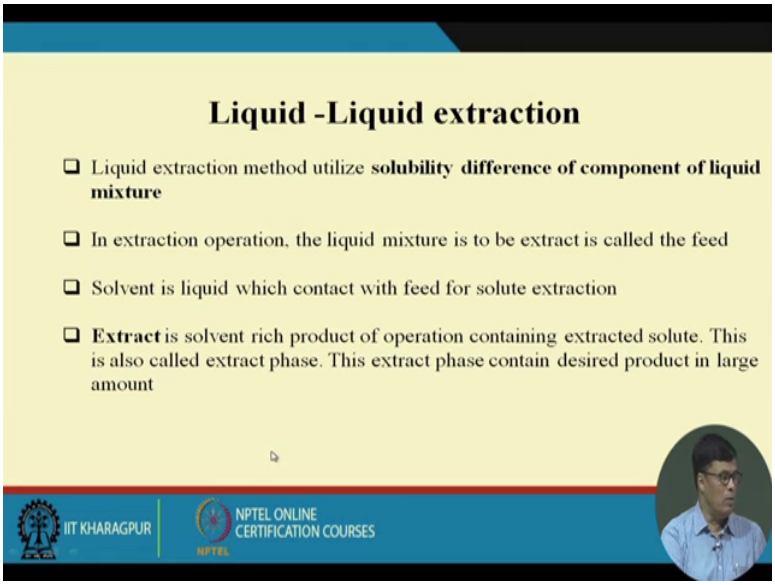

Now liquid-liquid extraction method utilized the solubility difference of the component in liquid mixture this is very important until and unless we have the solubility difference this method is not cannot be used.

In extraction operation liquid mixture is to be extracted is called feed and the solvent is liquid which contact with the feed for solute extraction. The extract is solvent rich product of operation containing the extracted solute, this is called the extract face this extract face contains the desired product in large amount.

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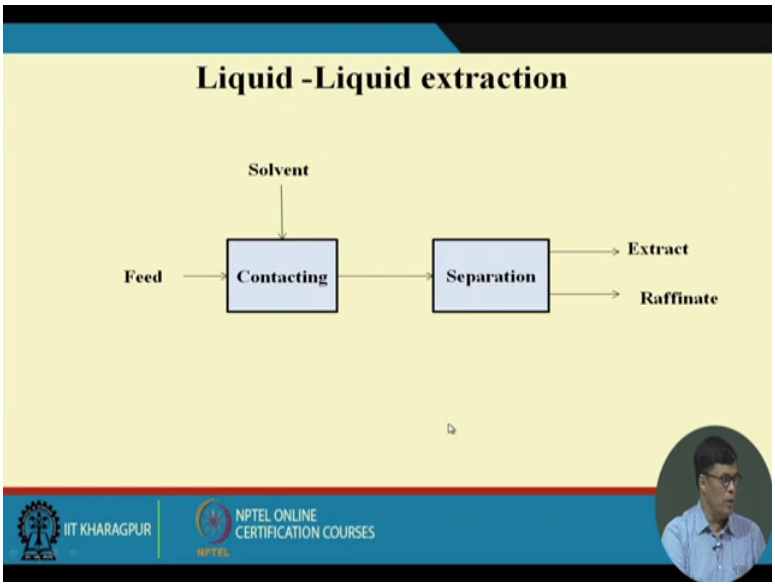

I can give a schematic diagram on that this is like this the block flow diagram. This is feed is coming this way and this is solvent is coming then they are mixing together the and then the separation take place we have extraction we have raffinate. Raffinate means extraction means here we have mostly the solute here we do not have much of solute that we present that.

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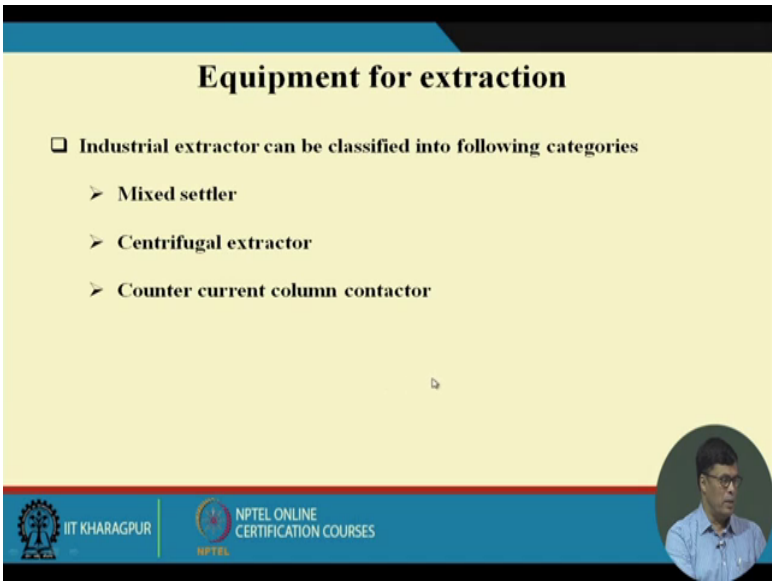

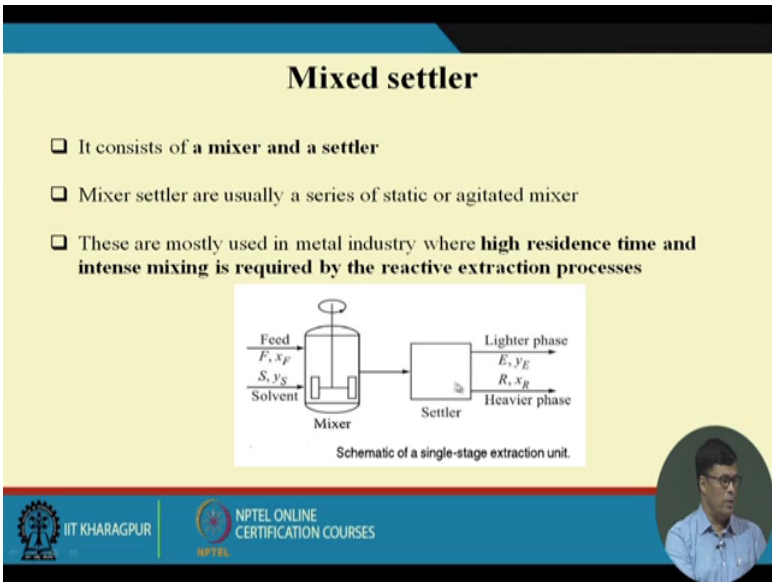

The equipment for extraction industrial extractor can be classified in the following category one is mixed settler, centrifugal extractor and counter current contractor, this are the different techniques that we have that I shall explain and now mixed settler is consists of mixer and settler. So you have feed and solvent both the things we have we mix it with the help of starrer then we settle that and then the one phase will be separate to other lighter phase we take it out heavy phase we will take it out from the bottom.

So these are mostly used for metal industry where high residence time and intense mixing is required for reactive extraction process this has little application in the biochemical industries but I told you in the penicillin industry also that your solvent is lighter than aqueous layer that is go to the top and the aqueous will be bottom that also but this is mostly used for the metal sepra metal industry.

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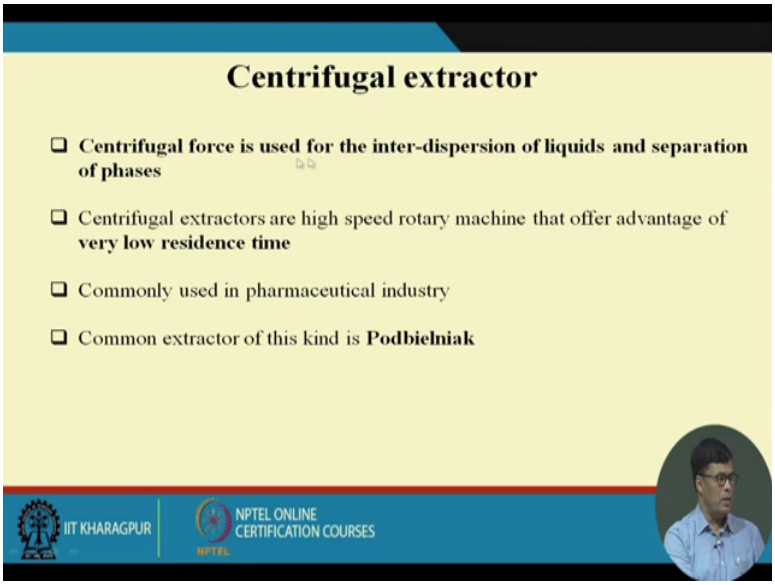

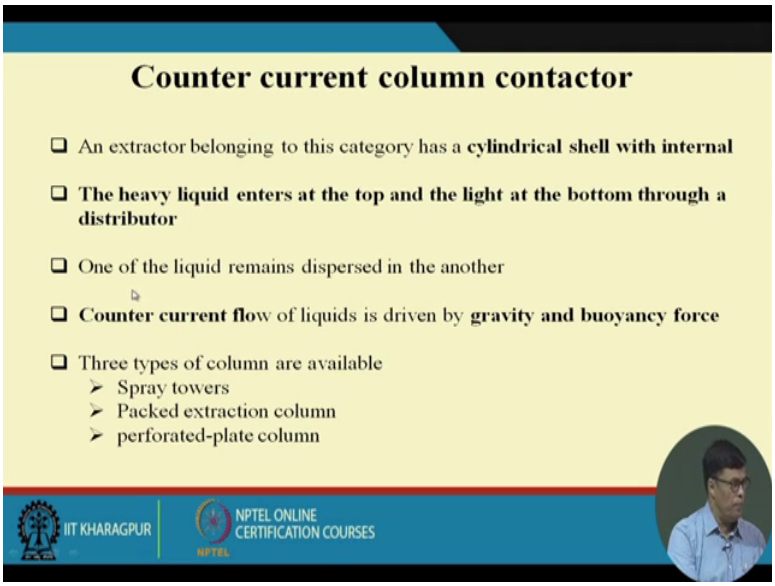

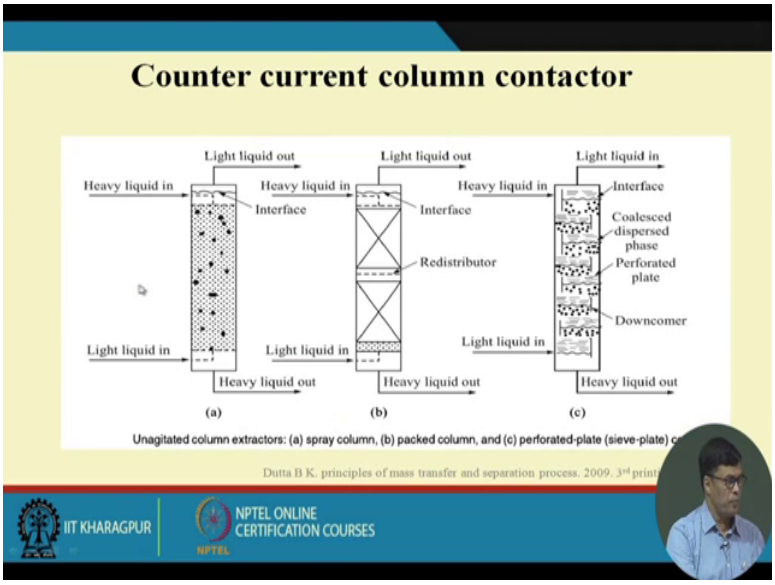

Now centrifugal extractor we have centrifugal is used for inter dispersion of liquid and separation phase.

The centrifugal extractor are high speed rotary machine that offer the advantage of very low residence time and commonly used pharmaceutical industry, common extraction of this kind is Podbielniak. Now counter current column extractor the extractor belongs to this category has the cylindrical shell with the internals the heavy liquid enters from the top and light liquid from the bottom through a distributor I can show you I think this will be very clear to you that how it is so we have this is the spray column we spray the heavier liquid here and this is lighter liquid here and when it goes like this then they mix together.

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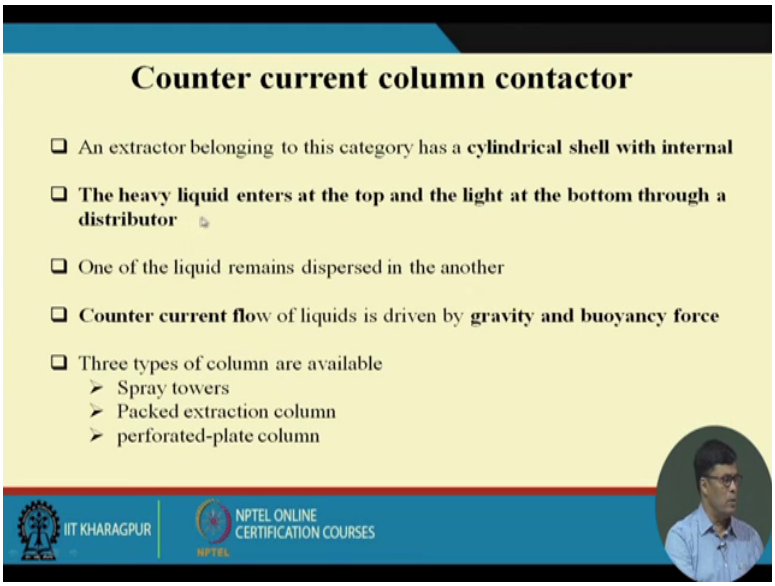

And this is called this is heavier liquid from the top and lighter (())(07:42) through the this is the counter current column contractor that we have.

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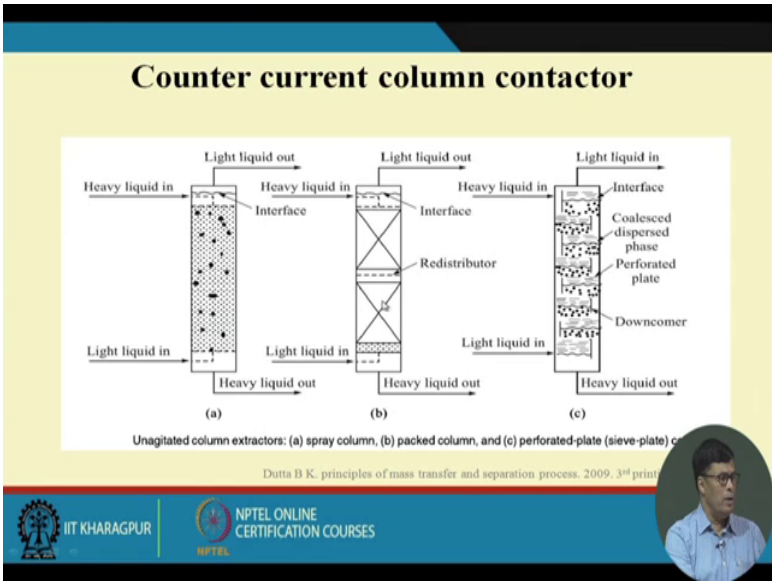

Now another things we have that is packed column. Packed column means here we pack the some kind of solid materials and then heavier liquid is coming here a perforated material that (())(08:02) some porosity then lighter material when they cross each other then they mix they have the intimate mixing and we can separate that light liquid will goes out from the top heavy liquid goes out from the bottom.

Now another things we have what you call perforated plate the sieve plate column now you can see that some kind of the sieving plates are there the liquid is going like this it cross like this and heavy liquid is come this is also we can separate the light liquid and the heavier liquid like this. This is called counter current column contractor.

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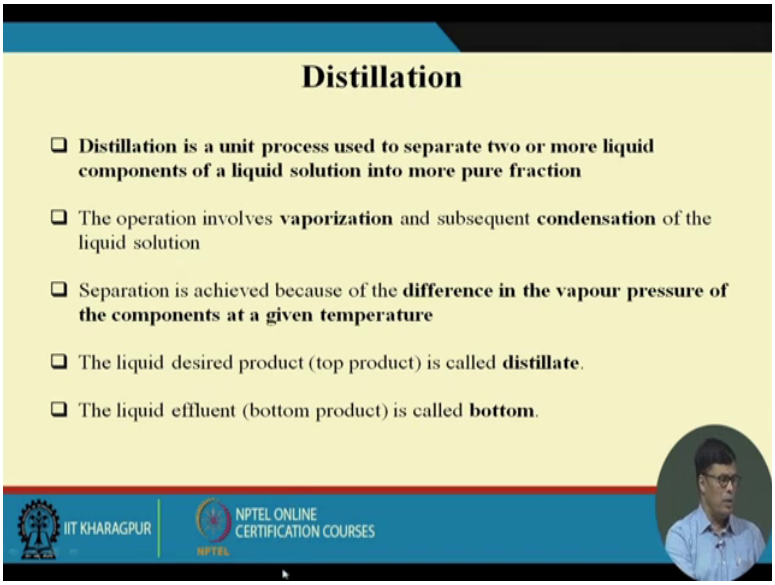

Now distillation is largely used by the industry as for example that alcohol industry we largely use this is on the basis of mostly the temperature of vaporization of the particular liquid that your particular solvent you are going to separate.

The distillation in the unit operation separation of two or more liquid components of a liquid solution into more pure fraction I can give a simple example then the alcohol fermentation process we during the fermentation process not only the ethanol production take place but besides ethanol there are fusel oil also formation take place. Fusel oil is nothing but higher alcohol so that you can separate on the in the fractional distillation column. The operation involves the vaporization subsequent condensation of the liquids, separation is achieved because of the difference of vapour pressure of the components at given temperature.

Liquid desired product is called distillate and the bottom product is called bottom or it called raffinate.

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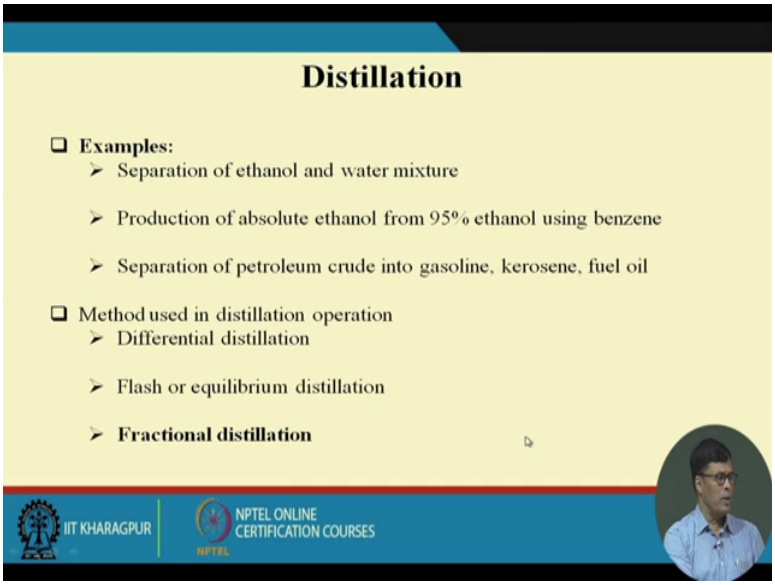

Now distillation examples we have the ethanol and water mixture that is largely used by the industry because ethanol has the boiling point 78 degree centigrade and water has the boiling point 100 degree centigrade so when you heat this solution and we collect the fraction as about 78 degree centigrade we get mostly the ethanol but with the separation process we can get maximum 95 per cent of ethanol we cannot have more because 95 per cent ethanol plus 5 per cent water they form the azeotropic mixture.

It is very difficult it is not possible to separate, if you want to separate produce the absolute alcohol which contains 100 per cent alcohol so we use the benzene as a solvent then and only then we can produce the absolute alcohol. Separation of petroleum crude from gasoline, kerosene and fuel oil also can be done through the distillation process, method used for the distillation process is differential distillation flash or equilibrium distillation and fractional distillation. Fractional distillation is largely used by the industry so that we can collect the different fractions after the distillation.

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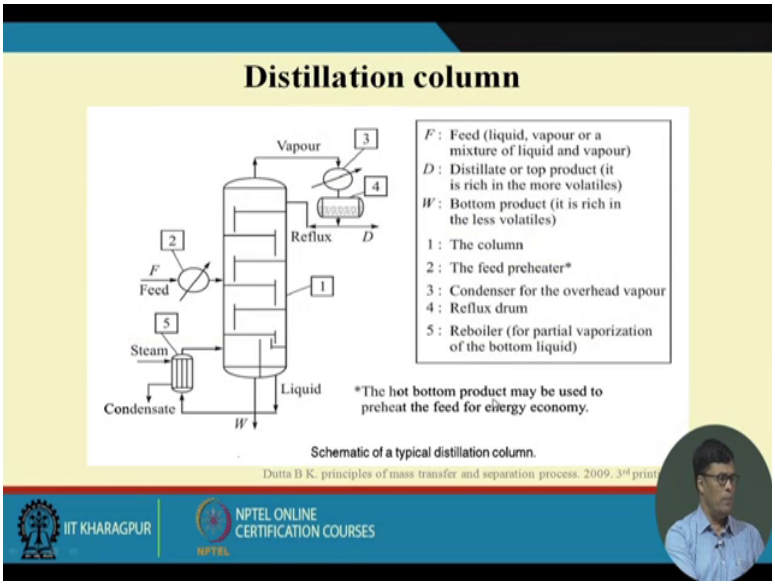

So this is the schematically how this process can be explained that we can heat this and then we pass through this column and you can see that we can collect it the different fractions that that we have.

We have this is this is one is the column two is the feed preheater this is the preheater feed is coming like this and then condenser for the overhead vapour this is the condenser overhead vapour, this is the four is the reflux drum you want to reflux is you can do that and reboiler partial vaporization of this. So this is why this is how you can separate so here basically at the bottom that you get what you call raffinate that is actually the waste material that is un-volatile material that comes out from the bottle and volatile material we collect it from the top. This is kind of distillation technique that we use.

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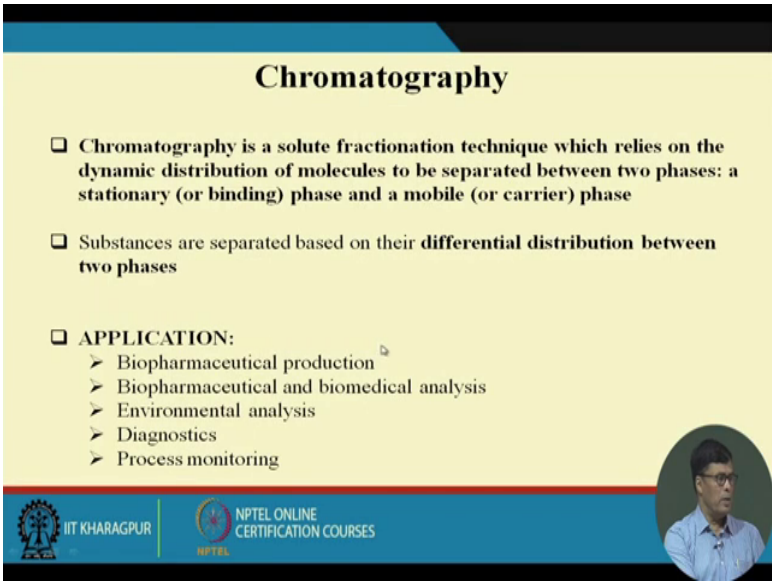

Now another technique that is largely used by the industry is the chromatography technique. Chromatography is a solute fraction technique that relies on the dynamic distribution of the molecules to be separated between the two phases is stationary or binding phase and a mobile or a carrier phase. So you know that this is the different type of you know chromatographic techniques we have. Particularly I can give you the example of streptomycin fermentation process where we use this chromatography technique for the separation of streptomycin.

Substance different substances are separated based on the differential distribution between the two phases application is the biopharmaceutical production, biopharmaceutical and biomedical analysis, environmental analysis, diagnostics and the process monitoring.

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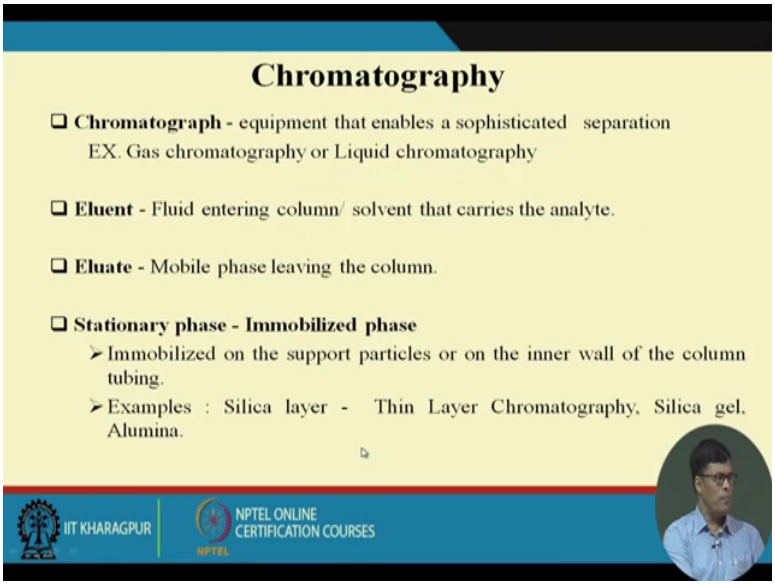

Now chromatography we have the chromatography is equipment that enable a sophisticated separation as for example we have gas chromatography, we have liquid chromatography. The basic difference between the gas chromatography and liquid chromatography is that mobile phase in case of gas chromatography is the gas. So we use gas as a as a carrier because when we inject the sample and then it vaporizes.

If it is gas, that gas will remain in already in the gas phase so another but with that gas you have to have a carrier gas, you have sample, sample is the gas but you have to have carrier gas that carrier gas will take the sample through the your column where the separation of different component present in the gas mixture that will take place then it goes to the detector where we can detect the temperature.

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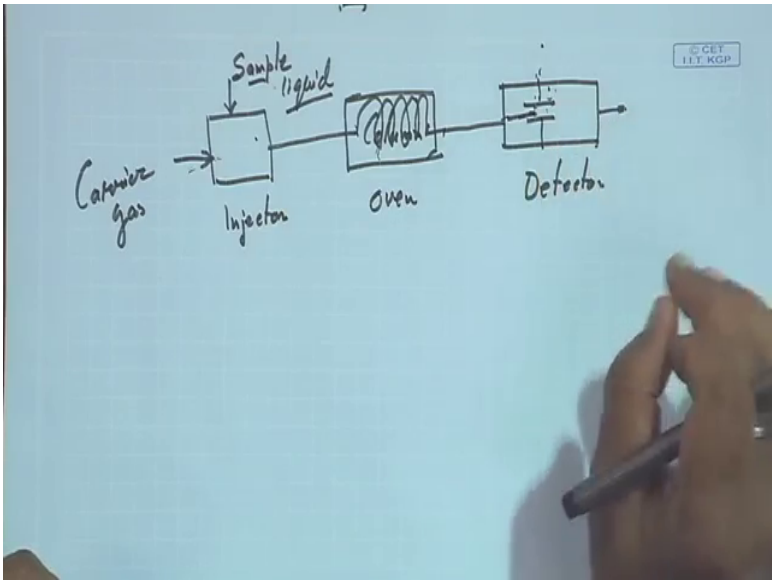

So in case of gas chromatography we can we have three different section one we call injector this is called injector and another we call oven where we have column and another we have detector. So what you do here we put the sample measured amount of sample with the help of syringe then here we put the carrier gas, now here everywhere we can maintain the temperature we have different temperature control here we maintain temperature here everywhere you have heater you can maintain the temperature.

So we here we maintain the temperature suppose the sample is liquid as for example, now in case of in case of gas chromatograph we can only analyse the volatile liquid we cannot analyse the non-volatile liquid. The volatile can be (())(15:41) out. So you get the liquid suppose I want to use the acetic acid the acetic acid has a boiling point is about 121 degree

centigrade now the temperature of the injector should be more than 121 degree centigrade because as soon as we inject this acetic acid in the injector this should be vaporizes out

And once it vaporizes out then what will happen this carrier gas will bring the material here so here here have column, column is like this it may be capillary it may be packed column, so when the column comprises of different packing material when we pass through the column the different component present in the samples that will be separate from each other and then it comes to the detector here also we maintain the temperature everywhere we maintain as per the required temperature and temperature should be higher than the boiling point of the samples that you are going to analyse because if it is lower than boiling point there is the possibility of sedimentation condensation of the particular liquid in the column.

So you know you have to we shall have to keep the temperature higher than the boiling point of the that liquid. Then detector that detect that particular component A, B, C, D whatever is that one after another it goes out like this so here you have the kind of electrodes and through the electrodes pass through the connected with kind of wheat stone bridge arrangement where there is a change of voltage or there is a change of current on the basis of that they detect that what are the different components present in the in the mixture.

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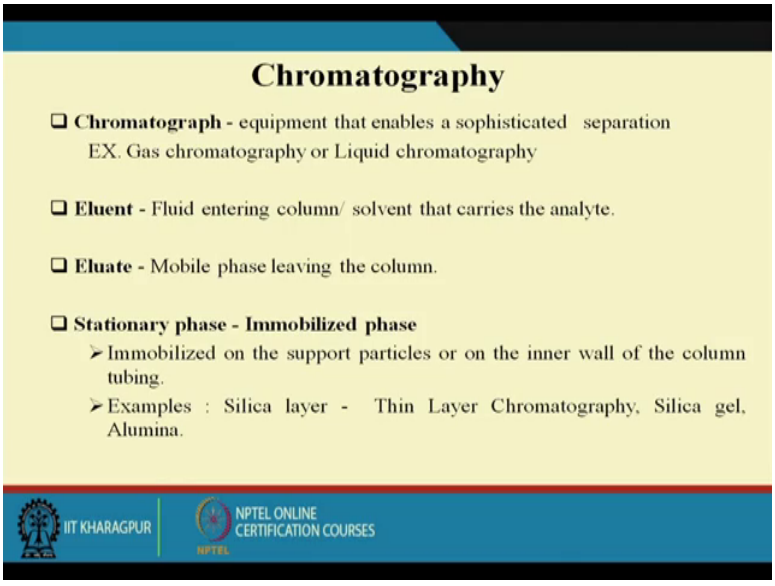

So this is largely used this is called gas chromatograph. In the liquid chromatograph this mobile phase will be liquid so here the only the advantage of this of this liquid chromatograph that we can analyse both the volatile and non-volatile component present in

the liquid though whatever suppose we I can give the example of glucose, glucose is a non-volatile organic material.

So this cannot be separated in the in the gas chromatograph. Now we if you want to find out that what is the glucose concentration present in the sample we can pass through the liquid chromatograph and then there we can find out. I can give the different example of citric acid also, citric acid is a non-volatile acid so that also you can find out, different types of proteins also we can get analyse in the liquid chromatograph.

Now eluent the fluid, eluent is the fluid entering the column or solving that carries the analytes and eluent is the mobile phase leaving the column that you know which is leaving the column that is eluent. The stationary phase is the immobilized phase immobilized of the support particles on or on the inner inner wall of the column tubing as per example we have silica layer, thin layer, chromatography then we have we have silica gel, alumina like this we have different type of packing material we use in the column.

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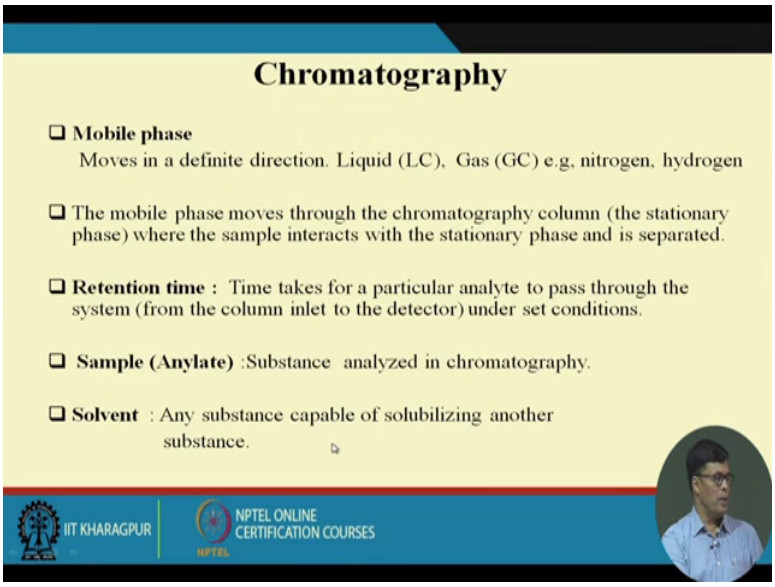

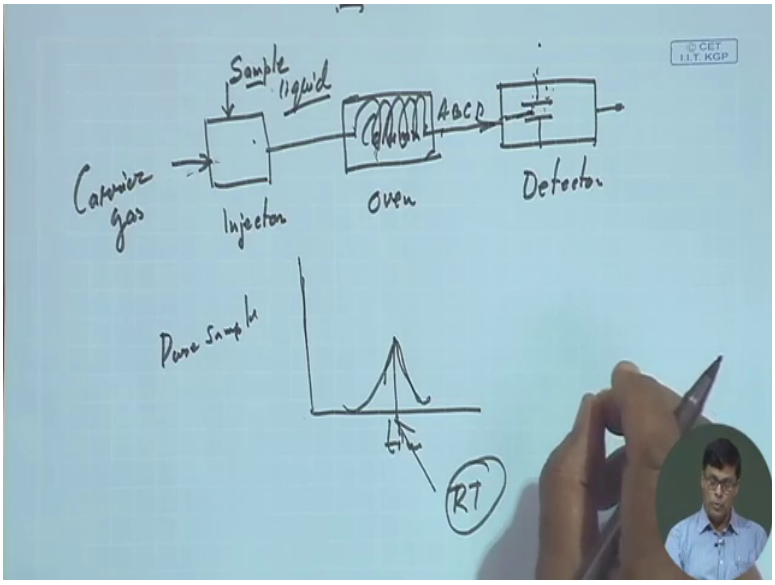

Now we have mobile phase as I told you mobile phase we have liquid we have gas, gas we use nitrogen hydrogen we use helium. The mobile phase moves through the chromatography column the stationary phase where the samples interact with the stationary phase and is separated. The retention time is very important as for gas chromatography is concerned that time required to separate because when the sample this coming here then the suppose sample contain A, B, C, D different components first A suppose is coming then B will come now initially how initially what you do we first we shall have to find out what is the probable component present in the that sample, so we can use the pure sample pure sample we inject and then we find out that at what time this is time and what time the peak is coming, this peak we can find out this peak is coming at what time this this is called retention time.

This is the called what you call retention time, retention time of a particular component that varies from each other it depends on the characteristics of the column also. Sometime it may so happen that depending on the characteristics of the column is such that both A, B has same retention time.

They are very close to each other so they cannot be separate by using in that case you have to use the different other column which can separate A and B from each other then and only then you can get the different peak, until and unless you get the different peak you cannot correct you cannot say correctly how much amount of A present in the in the sample how much amount of peak present in the sample. So this retention time is very important time takes for a particular analyte to pass through the system from the column inlet to the detector under the set conditions.

The sample the substrate analysed in chromatography that is the that is the sample we use solvent any substance capable of solubilizing another substance that is we use as a solvent.

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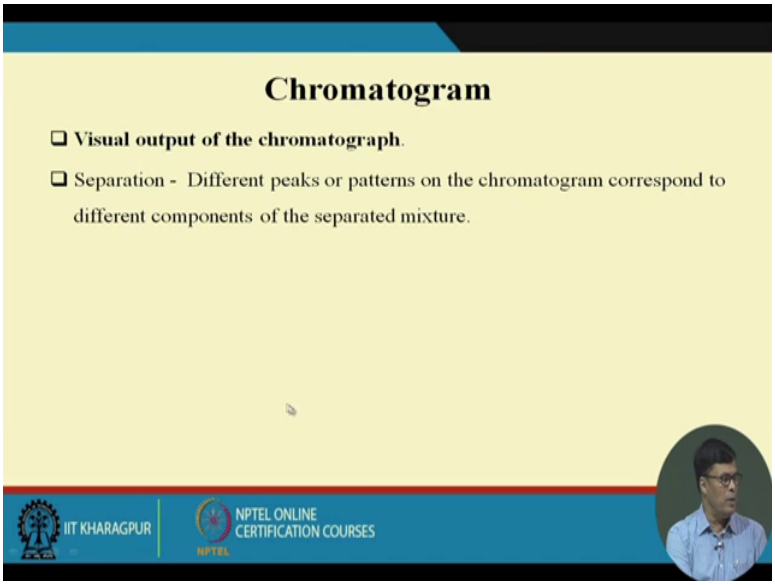

Now the visual output is the chromatograph because as I told you that this chromatograph may be this is on the basis of two type of detection we have they are either potentiometric or amperometric mostly we have the potentiometric and through the potentiometric we can note the change of voltage this voltage and this we correlate the concentration of the different component present in the reaction mixture.

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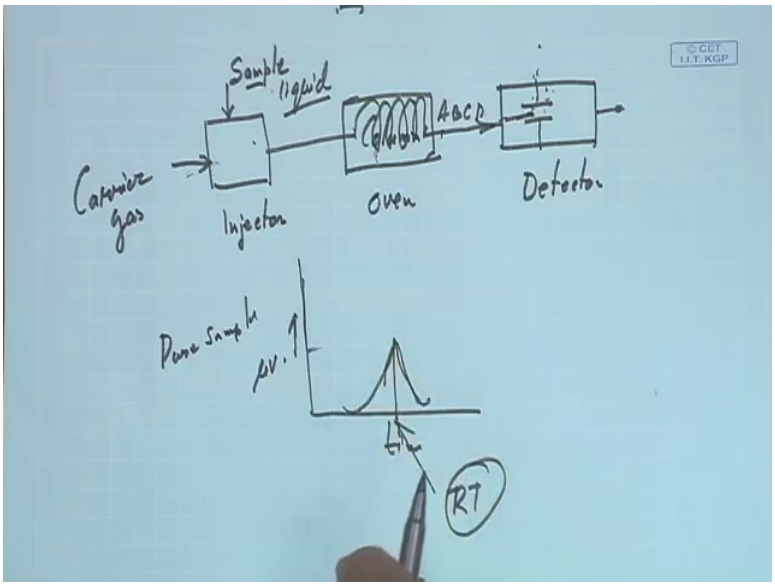

So maybe we have some micro volt here that change of microvolt, this we correlate with the kind of concentration of the component present in the sample. This is the visual output that we have the separation different peaks and patterns of the chromatogram corresponds to the different components of the of the separated mixture.

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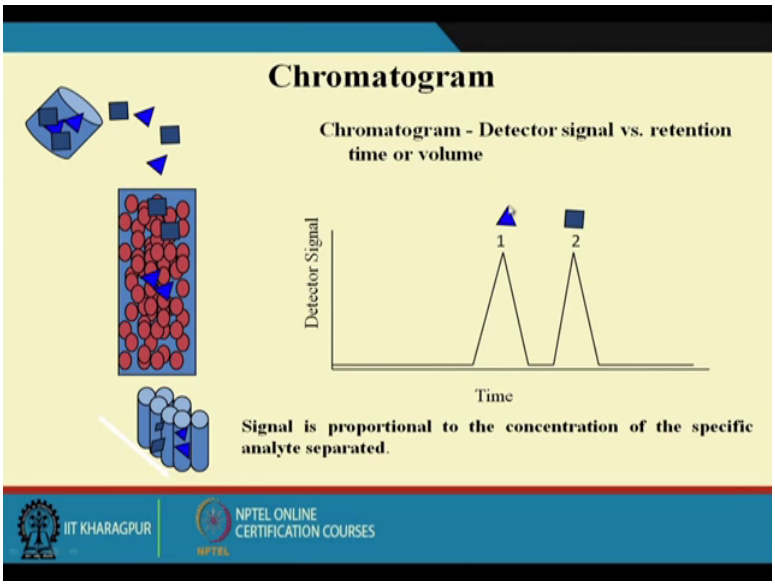

Now as I told you that here the picture is very clear suppose this this is a mixture of two particles and this you can see that you know that configuration of the two particles are different one is triangular another is square. So when it pass through the column one is separated from others and so you will you will get the different peaks because first might be triangle is coming and second is the square is coming so you have two retention time.

So two components has two retention time so if you know that that when you inject the standard you find out that what is the retention time what is this components stands for and when what is the components stands for, so with the respect of the standard you have to find out that what is that. Then you have to initially you have to first you have to identify what are the different components present then you have to you have to inject the desired amount because this chromatograph is used for dual purpose for both for qualitative analysis and quantitative analysis.

Qualitative analysis we find out that what are the different possible components present in the sample and quantitative analysis means how much of the different components present in the sample. When you do the any kind of want to do the quantitative analysis then we inject the

standard sample with desired concentration with respect to that we find out how much concentration of that particular component in the sample.

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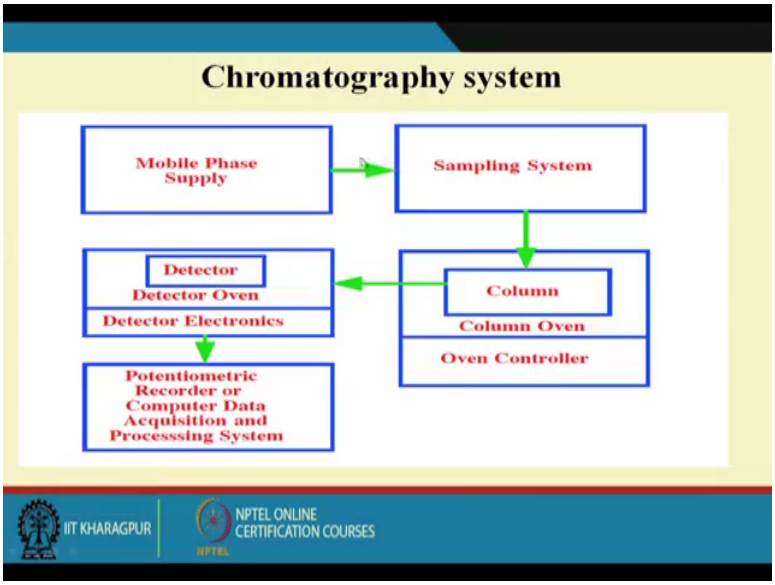

Now this is schematically it can be explained with the flow diagram is like this mobile phase supply the sample system then here suppose you have gas or liquid whatever you have here you put your sample then it goes to the column and that present in the column oven that is I told you that everywhere we have we maintain the temperature because we maintain the temperate in such a way that the sample should not be condensed in the any different part of this particular chromatographic system.

So we pass though the column where the different components will be separated out then we detector and after detector we have potentiometric recorder and computer data acquisition and processing system that is how we can monitor the different concentration of the material present in the sample.

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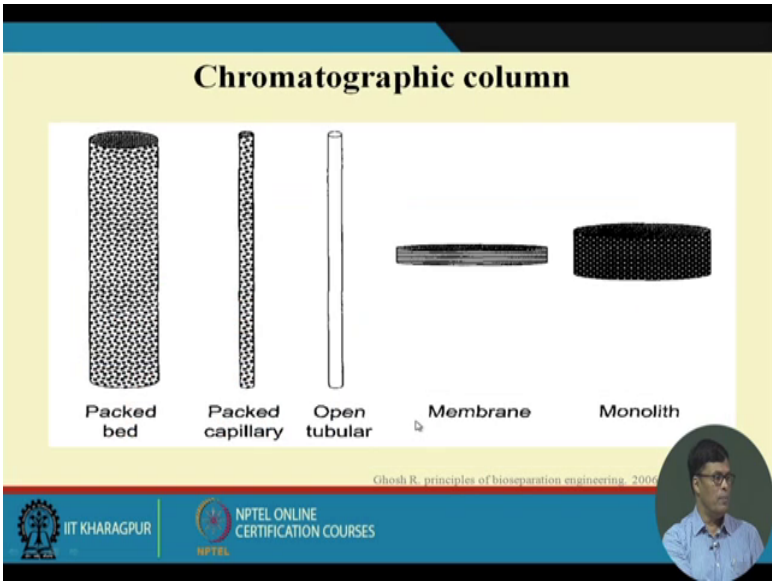

Now the characteristics of the material is basically they are different there might be two type of column we mostly use. One is called packed column another is capillary column so the capillary column is very very we have we can see the capillary column is quite long so on the basis of when it passes through this capillary column the different components present there there will be separate from each other and this is the packed column this is usually made of stainless steel and this is made of polymeric material and this is made of stainless steel.

This is this is not this is this is competitively this bit shorter and this is very longer, this is we have this.

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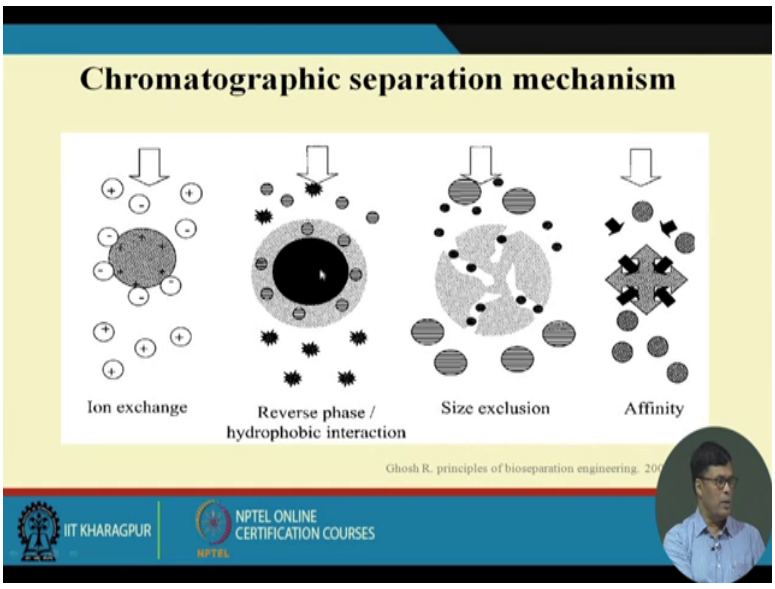

And then that you know how the particle the separate from each other that ion exchange reverse phase hydrophobic interaction sizes exclusion and affinity. The different type of techniques that we have through which the particles can separate from each other affinity that this suppose sample contains two type of particles but you know your packing materials the affinity for this only. So other particle will separated out and then you use some kind of eluent so that you know it deters this this from the solid surface you get the pure this component in your liquid.

The here you see this is size exclusion that you have bigger particles and smaller particles when it passes through this smaller particles would be separated out will be adjust will be will be other here and bigger particles will go out so and then you can you can separate from bigger particle from smaller particles. Now here the reverse phase and hydrophobic interaction through which also we can we can easily separate out the particles from one after another and ion exchange also we can analyse.

So what I what I want to tell that in this particular lecture I tried to concentrate that you know that two different techniques largely used by the biochemical industries or in the downstream processing particularly liquid-liquid separation techniques. I told you that penicillin industry is largely used for because we use the butyl acetate or amyl acetate to separate the penicillin from the fermentation broth to the solvent phase and then I told you at the same time that streptomycin is used by using some kind of chromatographic technique because just to separate.

We use the column through which the streptomycin is basically absorb and we use sulphuric acid as a eluent we do we take the streptomycin sulphate as the eluent. So then we have I told you the distillation technique where that ethanol is separated on the basis of the particular boiling point of the particular solvent present in the in the mixture. So we heat it and then we passes though the distillation column at different temperature we can separate out the different fractions and we can have different solvent in the system.

So this is all about the downstream processing and from that we have a clear cut idea that the different downstream processing has different conception and different way of separation that we have so that is how different biochemical industry they differ from each other because all the biochemical industry they have different downstream processing and they do not have the same type of downstream processing depending on the nature of the product they have to adopt the downstream processing accordingly. Thank you very much.