Novel Separation Processes
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Lecture No. # 39
Electrophoretic Separation Methods

Good morning everyone. So, you are we are looking into the ion exchange separation processes in the last class we have looked into the operating principles of ion exchange separation processes. Now, into this class we just do the calculations relevant to the ion exchange processes and then you will move to the next separation process that is the electrophoresis separation processes it is.

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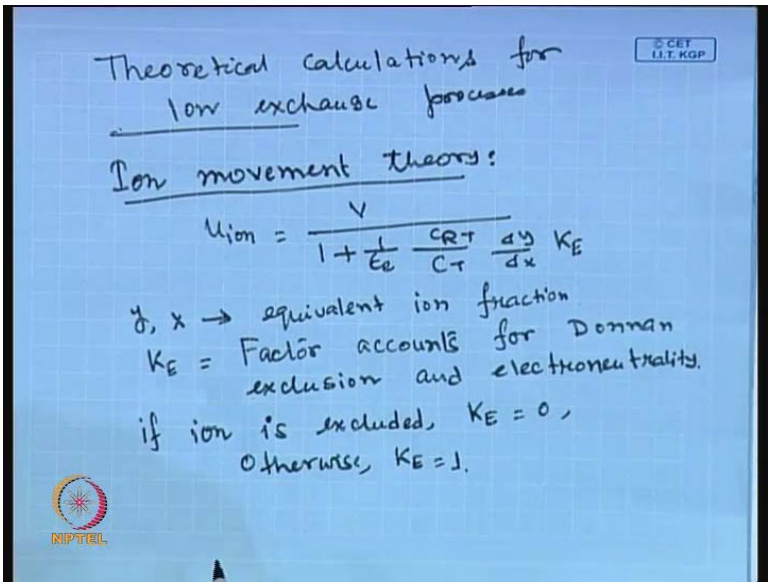

So, looking to the theoretical calculations those are relevant for ion exchange separation processes again here they would have a column here and they have eventual in this almost similar like solute movement theory in the chromatographic column. The ion movement will be almost similar to the chromatography by the velocity found that we have calculated for the chromatographic column. And the ion movement velocity will be related as v divided by 1 plus 1 over epsilon e C R T divide by C T delta y by delta x times K E. Where y and x are equivalent ion fraction in resin face or in the solution face, K E is a factor that includes the donnan exclusion and electro nutritive factor accounts for donnan exclusion and electro neutrality.

If ion is excluded then K E will be equal to 0 excluded K E equal to 0 otherwise K E will be equal to 1.

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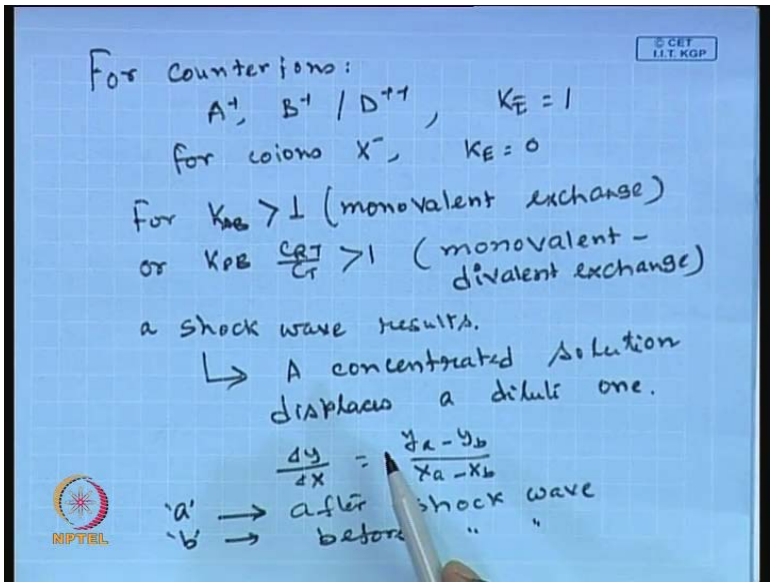

Now, for counter ions like A plus B plus or D plus plus or divalent or monovalent counter ions K E will be equal to 1. And for co ions x minus K E will be equal to 0 for K A B it will be a constant bigger than 1 this is for the monovalent exchange. Means you are exchanging a monovalent ion by a monovalent ion or K D B multiplied by C R T over C T is greater than 1 that is for the monovalent divalent exchange. it results a shock wave, a shock wave that means for these values greater than 1 shock wave results. And as you are shock waving in the alert less that if a concentrate solution displaces a dilute 1 than it say shock wave.

An as you are talked about (( )) if that delta y, in case of shock wave delta y by delta x will be written by this will be y a minus y b and x a minus x b, a refer to after and b refer to the condition before the shock wave. So, a is after the shock wave and b refer to conditions before shock waves. And in case of defused wave that means, when you are displacing a concentrate solution by a dilute solution that means, in the case of regeneration of any column. In the case of regeneration of any column you will be displacing the concentrate solution by the dilute solution. In that case there will be gradual change of concentration and the mole fraction in the solute case and in the dilute case so therefore, delta y by delta x will be replace by d y d x.

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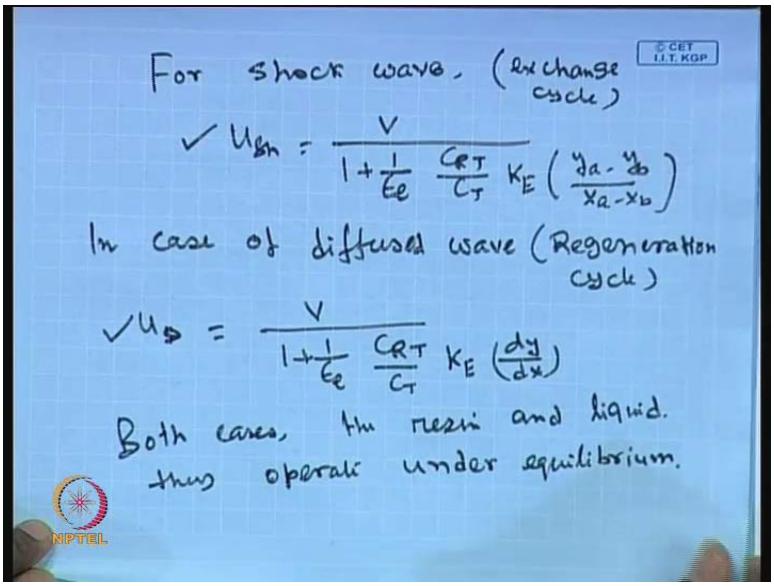

So, in case of shock wave this is basically exchange cycle or basically absorption cycle. u s h is equal to v divided by 1 plus 1 over epsilon e, epsilon is the inter particle porosity C R T divided by C T multiplied by K E y a minus y b divided by x a minus x b. Where the subscript a refers to after and b refers to before the shock wave. And in case of diffused wave 1 will getting the diffused wave, 1 will be getting the regeneration cycle, cycle u s u defuse to itself defuse to velocity divided by 1 plus 1 over epsilon e C R T dived by C T multiplied by K E d y d x.

So, this will be the velocities fine will be working with the shock wave that means, sting cycle and this is the velocity over talking about diffused out or the regeneration cycle. And both cases the resin and liquid sting, they operate under equilibrium that means, the solute concentration in the resin face or in the most liquid face both over the dictate by the equilibrium relationship. Now, will I would just solve a problem for ion exchange column living done look in to an example.

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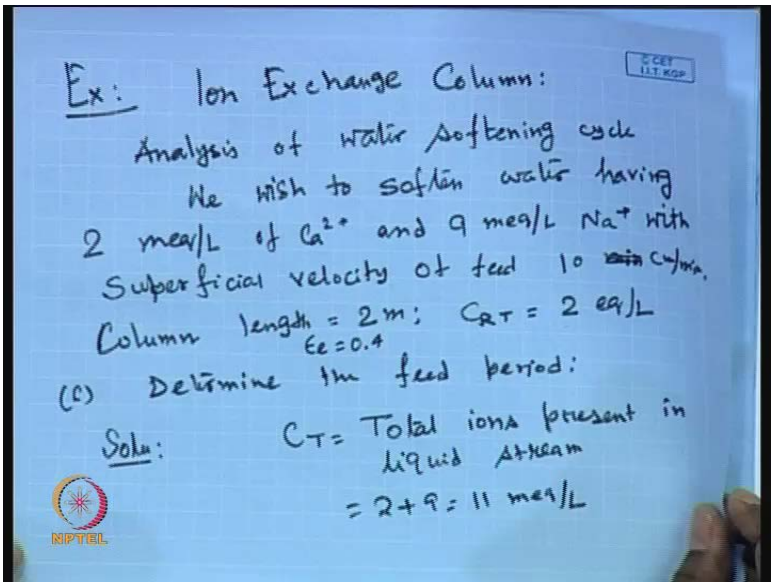

Ion exchange column it is basically an analysis of water softening cycle by ion is basically we wish to soften water that means, you are you would like to remove calcium ion from the water having 2 milli equivalent by liter of calcium ion. And 9 milli equivalent per liter Na plus with superficial velocity of feed as 10 minute 10 centimeter per minute. Now, well using a 2 meter column, column length is 2 meter and C R T is given that our total ions present in the resin is 2 equivalent per liter.

Then first we have to find out determine the feed period that means, for how long will be that you will be basically in running the feed. That you have to find out let us look in to the solution, the C Total is the total ions present in the liquid stream that you can find out. In liquid stream that will be 2 plus 9 equal to 11 milli equivalent per liter and epsilon e is given as 0.4 that is the inter particle positive that is given as 0.4 in this particular problem.

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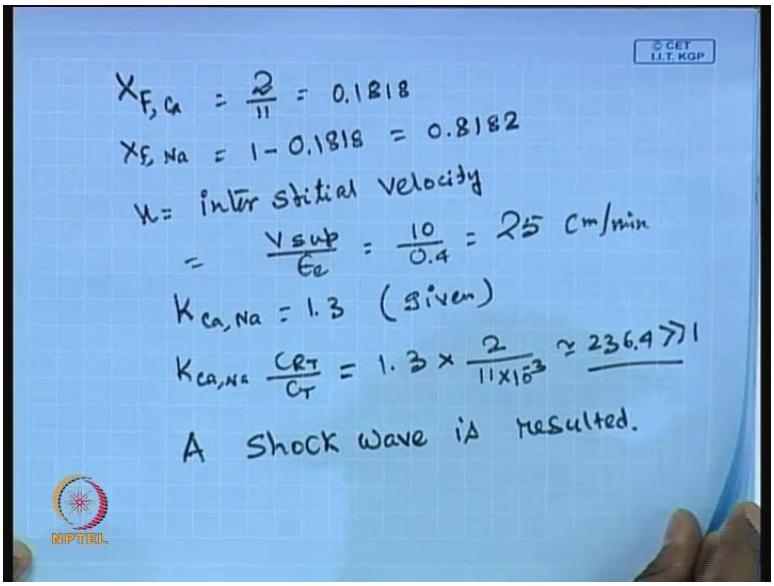

So, once you get the total ion concentration have present in the liquid stream you will position to get the mole fraction of calcium ion in the feed and that becomes 2 by 11 that is 0.1 8 1 8. And mole fraction of sodium in the feed would be 1 minus that so, it will be 1 minus 1 8; 1 minus 0.1 8 1 8 it will be 0.8 1 8 2. Now, v is the interstitial velocity velocity that is given as superficial velocity u, right you talk about u interstitial velocity used by divided by epsilon e and this turns out to 10 divided by 0.4 is equal to 25 centimeter per minute.

The equilibrium constant of calcium and sodium that is given as 1.3 and K c a N a C R T divided by C T can be calculated so, this is a given data a equilibrium constant given. So, once you get the data then you can calculate this quantity this turns out to be 1.3 into 2 divided by 11 into 10 to the power minus 3. So, this re equivalent and this is will be equivalent. So, you have to covert to equivalent and this sterms out to be 236.4 which is much greater than 1. So, talking about shock wave that is so, based on this criteria it to the conclusion that the shock wave is resulted.

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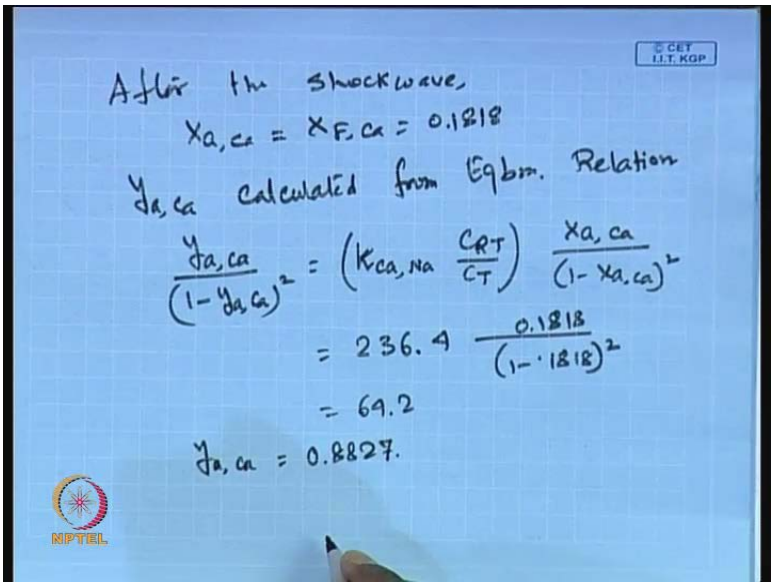

Now, let us do the further calculations after the shock wave X a c a will be the constant mole fraction of calcium will be same as that our feed concentration. So, it will be same as X F C a that is 0.1 8 1 8; that means, after the shock wave you should get the same feed concentration that we has going into the system. And y a c a is therefore, calculated on the equilibrium relation; calculated from equilibrium relation now if you been look into equilibrium relation this becomes y a c a divided by 1 minus y a c a squared y a c a is basically a mole fraction of calcium after the shock wave in the resin face. So, this will be assume that K c a N a C R T divided by C T x a c a divided by 1 minus x a c a square of that.

So, these turns out to be just put the values 2 236. 4, 0.1 8 1 8 1 minus 0.1 8 1 8 square of that and this is 64.2 and now you can calculate y a c a it turns out to 0.8 8 2 7 now will be in a position to calculate the things.

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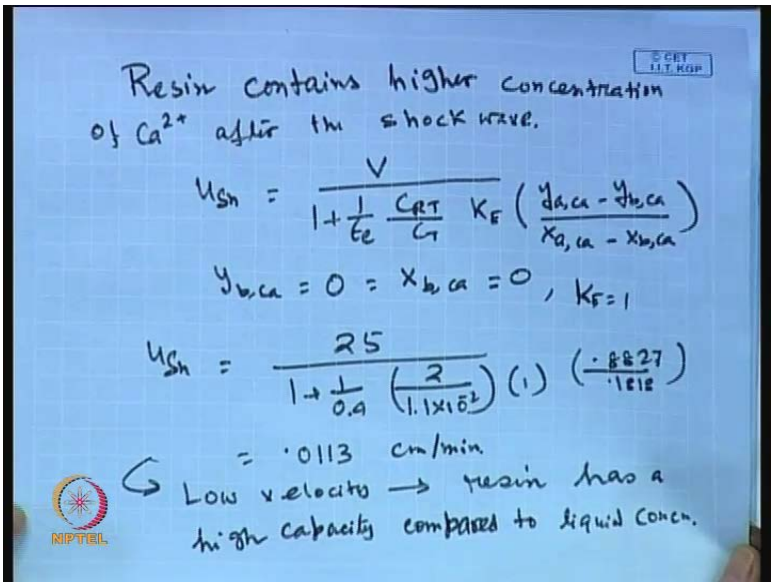

So, resin contains higher concentration of so, therefore, the resin must be the containing the higher concentration of calcium after the shock wave. Now, you can get the hole velocity divided by 1 plus 1 over epsilon e C R T divided by C T K E y a c a minus y b c a divided by X a c a minus X b c a. And y b c a is before the shock wave the concentration of calcium in the resin face, but as nothing while before the shock wave the resin had been contain in the calcium. After the after you will pass the solution it will be exchanging the calcium so, before then to be having an value 0.

And X b c a; that means, the concentration of calcium after the concentration of calcium in the liquid stream it has also equal to 0. So, you can now if you can if you put the values these becomes 25 divided by 1 plus 1.4 into 2 1.1 into 10 to the power minus 2 multiplied by 1 K E equal to 1 and there is 0.8 8 2 7 divided by 0.1 8 1 8 and it turn out to be 0.0 1 1 3 centimeter per minute. Now, the low velocity these velocity is extremely low this, low velocity of the hole ion hole is basically because resin has a very high capacity compare to liquid concentration and it is selected for c a 2 plus.

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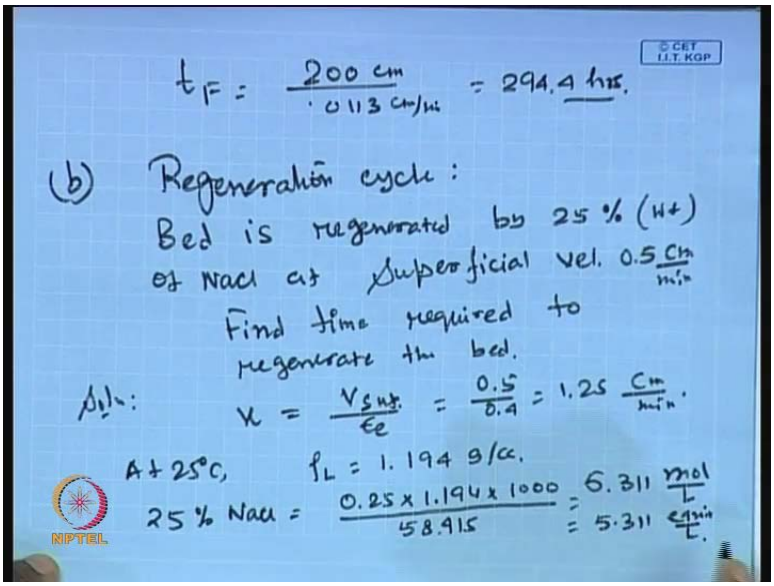

So, once you get the velocity now you will be in the position to calculate the retention time. The retention time for the feed will be therefore, 2 meter so, it will 200 centimeter divided by 0.0 1 1 3 centimeter per minute and it turns out to be 294.4 hours. So, it is quite high turn so that so, thus if you can avoid the food and you will be getting a concentration of an calcium after the shock wave that will be almost equal to 0. Now, the second part will be bed is regeneration cycle the bed is regenerated by 25 percent by weight of N a c l at superficial velocity 0.5 centimeter per minute.

So, you have to find a time required to regenerate the bed let us look in to a solution, once you get the superficial velocity you can convert into the interstitial velocity why v superficial divided by epsilon e. So, it turns out to be 1.2 5 centimeter per minute and rho 1 is given as a 25 degree centigrade, rho liquid is given as 1.1 9 4 grand per c c. So, you can convert 25 percent N a c l to equivalent per meter equivalent per liter. So, 25 percent N a c l it means 0.2 5 into 1.1 9 4 into 1000 you will see density of liquid at 25, 25 percent of N a c l divided by 58.4 1 4 5 that is the molecular weight of sodium chloride. So, it turn out to be 5.3 1 1 mole per liter and this will be 5.3 1 1 equivalent per liter.

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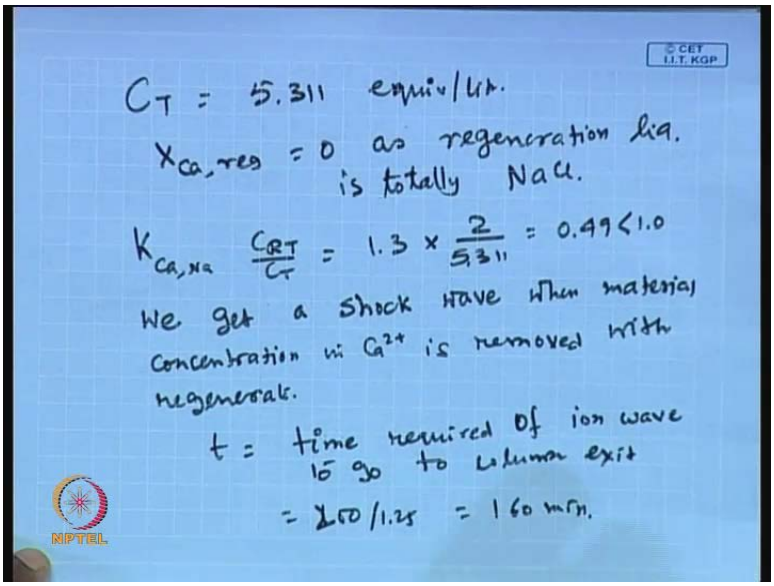

So, you can get an estimate of C T that C T turns out to be 5.3 1 1 equivalent per liter. And mole fraction of calcium is equal to 0 as regeneration liquid is called N a C l is totally N a C l. You can this value K c a N a C R T by C T in this particular case it turns out to be 1.3 into 2 divided by 5.3 1 1 and this will be 0.4 9 which is less than one. So, you will be obtaining when you get a shock wave when material concentrated in C a 2 plus regenerated is removed with regenerated. The time required for going to time required of ion wave to go to the column exist can be calculated go to column exit is 200 divided by 1.2 5 that is around 160 minutes.

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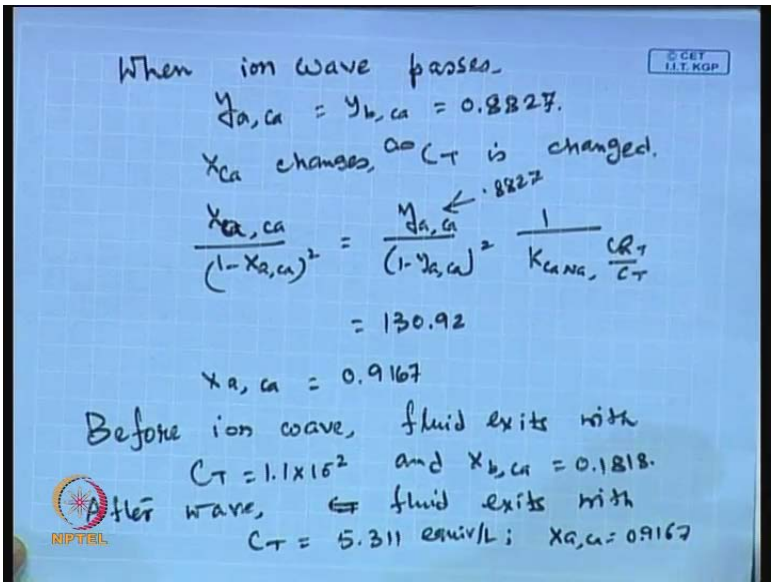

Now, y a ion wave it passes y a c a is equal to y b c a is 0.8 8 2 7 and X c a changes because my C T is no changed. So, you can calculate X c a X a c a divided by 1 minus X a c a square of that equal to y a c a divided by 1 minus y a c a square 1 over K c a N a C R T divided by C T. Now, if you put all the value this turns out to be 130.9 2 you put 0.8 8 this 0.8 8 2 7 and this early over be computed. So, X a c a becomes 0.9 1 6 7; that means, the liquids stream that will be coming out of the column will be reach in calcium and it will be in the mole fraction calcium will be 91 percent. So, before ion wave fluid exits with C T equal to 1.1 into 10 to the power minus 2equivalent per liter and X b c a 0.1 8 1 8. Now, after immediately after the wave the fluid exit with C T 5. 3 1 1 equivalent per liter and mole fraction is 0. 9 1 6 7. Now, these continue until the shock wave are exceeds to 0.

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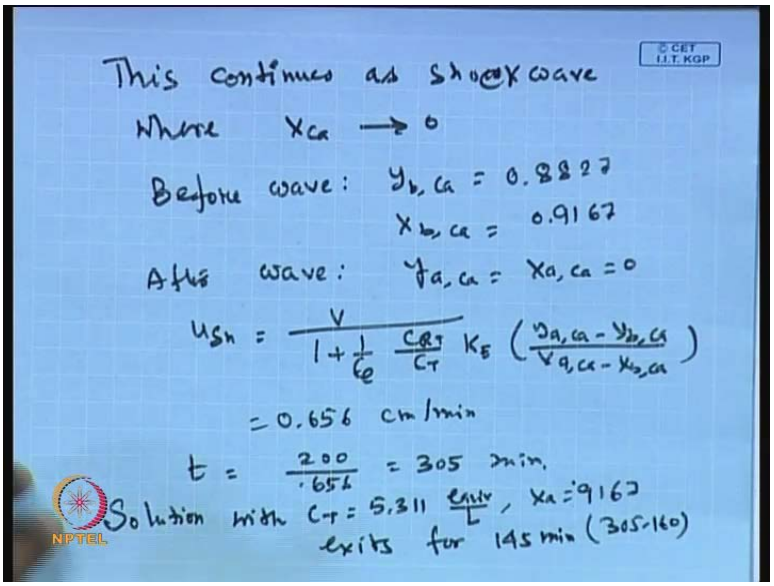

This continues as shock wave where X c a reaches to 0. So, you will be having before wave you having y b c a is 0. 8 8 2 7 and X b c a is 0.9 1 6 7 and after the wave you will be having y a c a equal to X a c a will be equal to 0. So, you can get the value of the hole velocity the interstitial velocity it will be inverse put it in the same formula as you have look in earlier and this turns out to be 0.6 5 6 centimeter per minute and the time required is 200 divided by 0.6 5 6. So, it will be around 305 minute this time is about 145 minutes after the total ion wave exits where as the solution. Solution which C T is equal to 5.3 1 1 equivalent per liter and X a 91.9 1 6 7 exits for 145 minute. How do you get 145? This 305 minus 160, once you do the regeneration cycle then you can go further washing.

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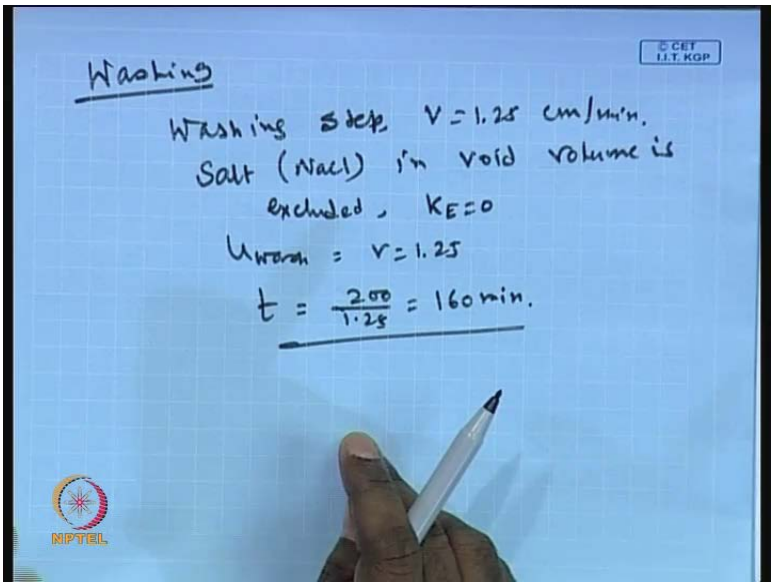

So, in the washing is basically in case of washing you have washing the whatever N a c l that is they are in the raising face. Now, washing step have velocity v is 1.2 5 centimeter per minute since solution found in the void volume is excluded salt is N a C l in void volume excluded. In that case K E inverted to 0 and u wash will be nothing but v that is 1.2 5 and the time required is 200 divided by 1.2 5 equal to 160 minute. Now, come out all the times and that will be giving you total water sulfuring cycle time. So, this gives a fairly good idea about the how the ion exchange column will operate and how the chromatographic column will operate. Now, in the then we move to the next separation process that is quite important and there is a electrophoresis separation method and this method will utilize the charged properties of the bio molecules which would be separated.

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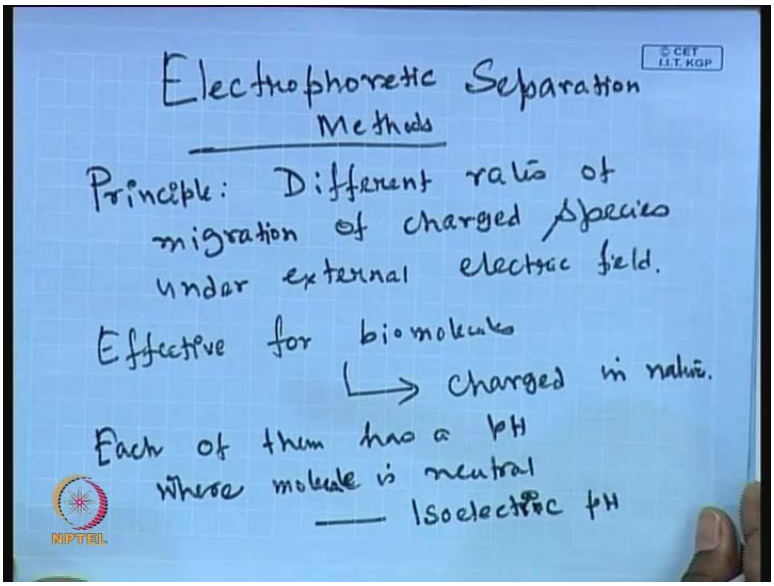

So, let us go to the electrophoretic separation methods and the basic principle deep separation method is that the different rates of migration or different rates of velocity, different rates of migration of charged species under external electric field. So, if you had charged species then we are having different charges so, whenever this electric field is same electric field is operated and then the their velocity is the migration velocity will be different. So, you will be getting at a particular location will be getting that 1 point particular species at a time earlier different, compare to other species there will be coming later you at a particular point.

So, you can collect the particular species earlier compared to the other species thats why you can separate. Now, these are effective by most of the bio molecules. Why this is effective from the bio molecules? Because most of the bio molecules are charged in nature. Each of this molecule will be high in a characteristic pH, where the charged molecule is neutral of them has the pH where molecule is neutral, that particular pH is known as the isolated pH. Now, the 1 can control the operating pH by adding the H c l or N a O H. So, you can you can add a few drops of H C l that pH of the solution will go down in the acidic region, if you can you have some drops of sodium hydroxide the pH of a solution can grow up in the alkaline region.

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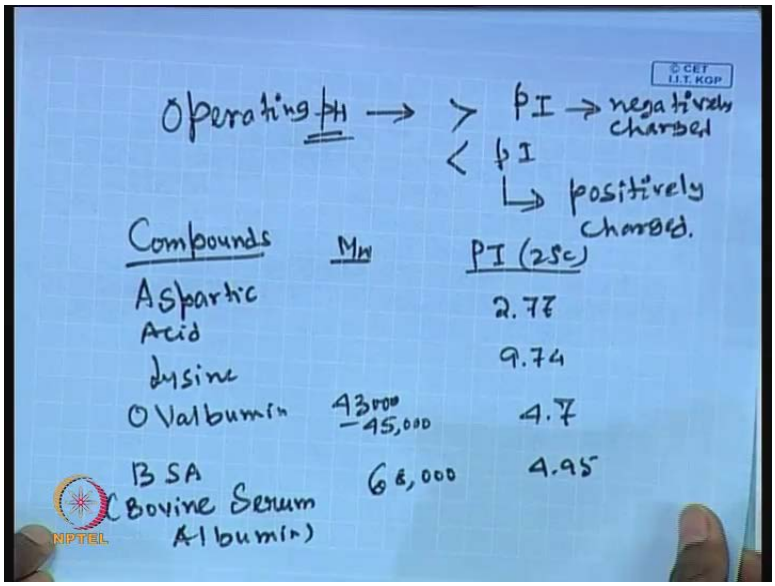

So therefore, 1 can control operating pH and above the isoelectric point or bellow the isoelectric point. The operating pH will be above isoelectric point we call P I it can be below the isoelectric point as well. Now, if the operating pH is above the isoelectric point pH is negatively charged, on the other hand if the operating pH is less than isoelectric point the molecule is positively charged. Now, based on this principle you can make them charged so, if you have a mixer and if you have an isoelectric having a isoelectric point with know in the oral range of pH rates from 2 to 7. Then if you settled a around 5 or 6 then you can have some molecules positively charged, some molecules negatively charged.

Now, if you apply extreme electric field then you 1 particular port or cathode or anod some molecules will be going and the other port some molecules will be attracted it. Now, I will give you some of the some compounds list of the compounds and molecular weight and isoelectric pH at 25 degree centigrade. Aspartic acid this will be having a isoelectric pH 2.7 7, Lysine 9.7 4, Ovalbumin it is a protein it has a molecular weight in the range of 43000 to 45000 it will be having a isoelectric 4. 7, B S A Bovine Serum Albumin it is molecular weight it 68000 and it has isoelectric point around 4.95.

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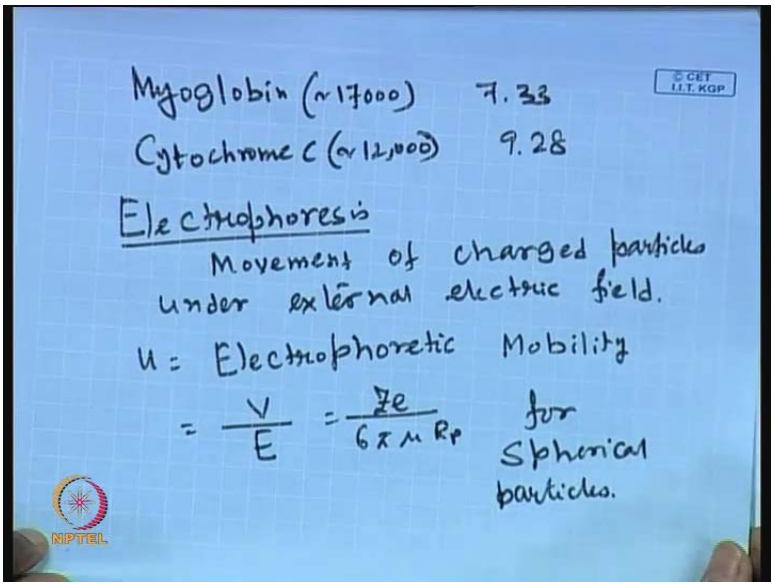

And Myoglobin another put in which will be having a molecular around 17000 that has an isoelectric pH 7.3 3, Cytochrome c it will be having a molecular weight 12000 and that will be having an isoelectric pH around 9.2 8. Now, movement of the charge species under the external electric field as we have already discussed earlier, this phenomena is known as the electrophoresis. So, electrophoresis is the known electro kinetic effect which is responsible for this electrophoresis separation method. The moment of charged particle particles under external electric field now you can calculate the electrophoresis mobility. And the electrophoresis mobility will be electrophorotic velocity carrying it is full strength and if you learned out it is Z e divided by 6 pi mu R p for spherical particles.

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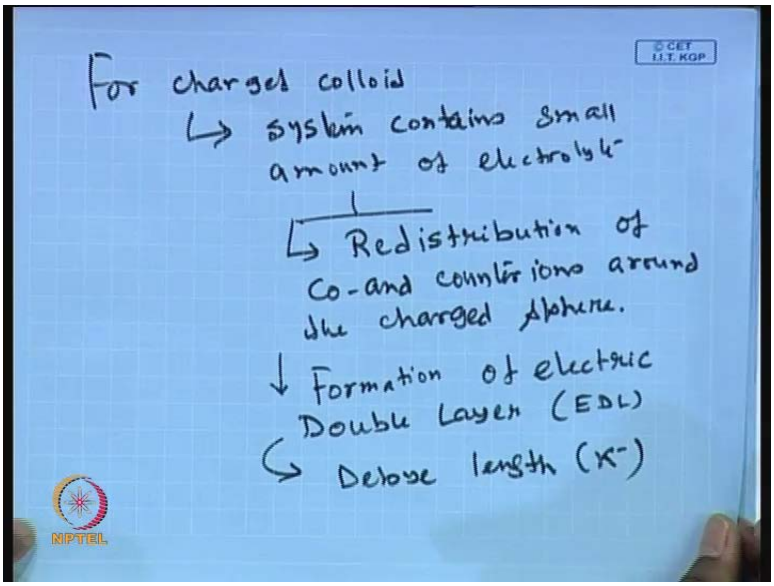

And in case of charged colloid for charged colloid the system contains a small amount of electrolytes. Dilute concentration of electrolyte solution they carry appreciable current and therefore, there is a redistribution of the charges around a charge feed redistribution of co and counter ions around the charged sphere. So, then we have face the formation of electric double layer E D L. Now, depending on the value of I know once you get the electric double layer formation you can find out the Debye length let over you have been done all the calculation all layer. Debye length corresponding to the concentration of electro light present in the solution.

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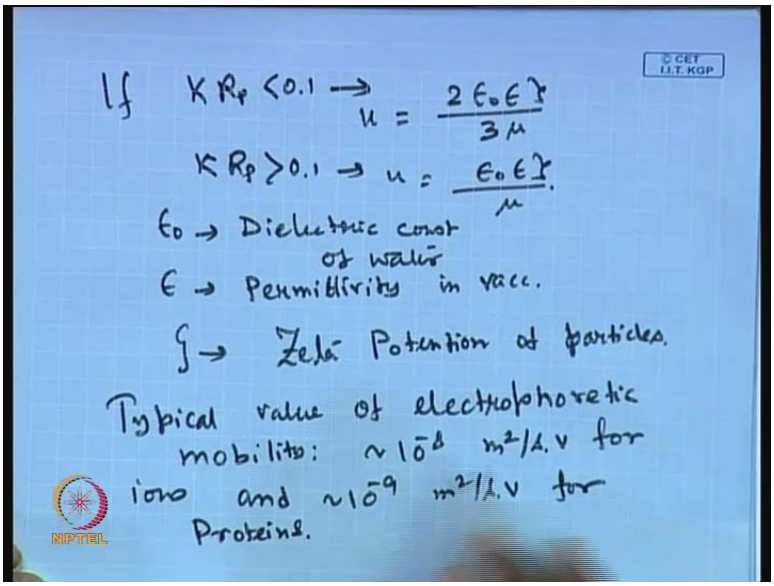

Now if kappa R P is less than 0.1 have p is the particle radius then electrophoretic mobility is given as 2 epsilon naught epsilon zeta divided by 3 mu and, on the other hand if K R P is greater than 0.1 when u is given as epsilon 0 epsilon zeta divided by mu is epsilon 0 is basically dielectric constant of water this value around (( )). And epsilon is the permittivity in vacuum and mu is the solution viscosity and zeta is the zeta potential of that particle. We already define the definition of zeta potential and how they will be place in a liquid solution.

Now, typical value of the electrophoretic mobility in feed solution would be in the order of 10 to the minus 8. So, this is the typical value electrophoresis mobility may be it will be in the order of 10 to the power minus 8 meter square per second v for ions. And it will be less for proteins, 10 to the power minus 9 meter square per second 0.5 for proteins. Why it will be less? Because of proteins will be having larger size compare to the ions. So therefore, there will be less mobile so, it will be tilde at least one of the magnitude less.

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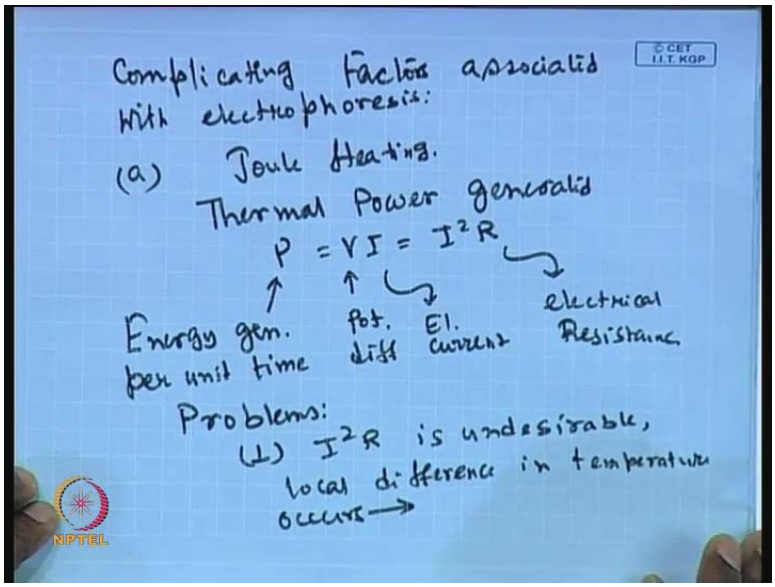

Now, it has look in to that liners arrow complicating factors those are associated with the electrophoresis.

Electrophoresis now the first composition that arises out of joule heating, the power thermal power that is generated because of a flow of current is delegated by the joule heating is P equal to v times I effective multiply the current. So, it will nothing but I

squared R it will nothing but I square R where R is the resistance electrical resistant v is the potential difference. P Is the energy generated per unit time is the power v is the potential electric potential difference, I is the electrical current and R is the electrical resistance. Now, in this particular case the joule heating will be having 2 forms of problem.

The problems those will be occurring because of joule heating first is that I squared R is undesirable because it helps to local fluctuation in the temperature. Local difference in temperature occurs so, if local difference in temperature occurs there is a local difference in density vacuum constancy you have frank function of the temperature this result to density difference. If the density difference is quite significant the convection will occur like you have a free convectional occur right. So, convection occurs so therefore, the movement of the transport of that particle will be disturbed by dilute the electro photon mobility will be disturbed by the convectional convective you know motion.

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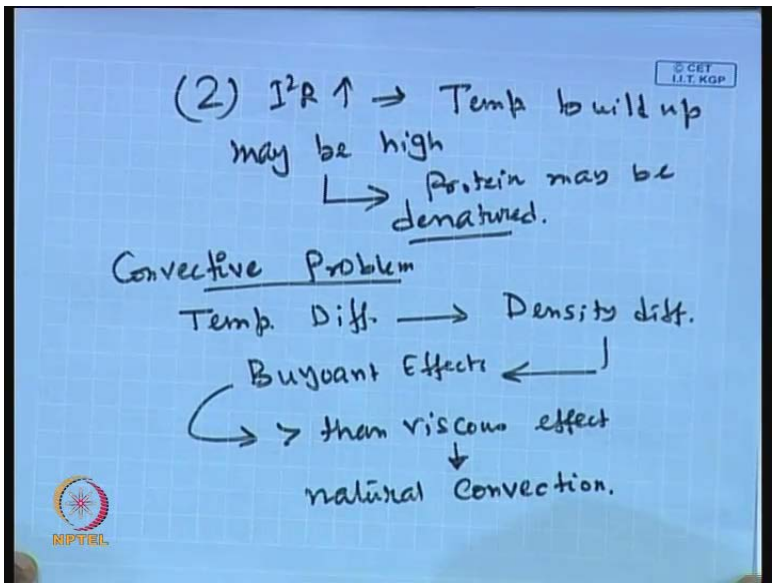

Secondly, by second point that you will be getting out full that at high temperature. If I square R of joule heating is high the temperature difference may be high a temperature build up may be high enough and at that high temperature the protein will be denatured may be denatured. Now, say these toward the other problem competing factors during the electrophoresis and it was look into the convictive problem much a detail. Now, temperature difference, local temperature difference due to joule heating it leads to

density difference. If the density difference is there that leads to the Buyoant and whenever if that Buyoant effects the here come the viscous effects then you will be having a natural conviction right. If it is more than viscous effect then you will be getting a natural convection effect type of circulation. So, density difference can be written as.

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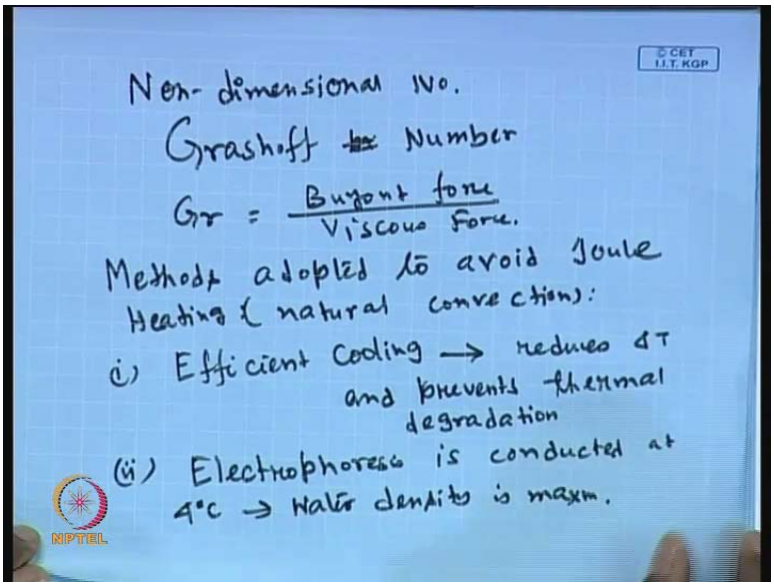

So, this will be basically lead to a non dimensional number. A non dimensional number Grashoff number become very important becomes very important and by looking into that value of Grashoff number one can say by that the natural convection is dominant or whether the natural convection is not dominant. So, Grashoff number is defined as Buyout force divided by the viscous force here buyout force becomes more combative viscous forces in the natural convection will be dominant. Now, there are several you know methods are used to avoid natural convection because if you use the electric field occurs a 2 plant when you will be having a totaling solution in presents electrolytes there will be parallel that will generated.

So, I square R type of heating a joule heating you cannot avoid. So, some kind of joule heating will be always occurring so, in order to avoid the dangerous effect of joule heating there are some methods to be adopted. Methods adopted to avoid joule type of heatings are natural convection. Number 1 efficient cooling reduces delta T and prevents thermal degradation. So, you can have an efficient cooling in your system that basically leads to reduces delta T and prevents thermal degradation.

Secondly, electrophoresis is conducted at 4 degree centigrade where water density is maximum. So, if you conduct the experimental 4 degree centigrade is the density is maximum by changing density will not be much in that case you can calm down or control the convectional effect.

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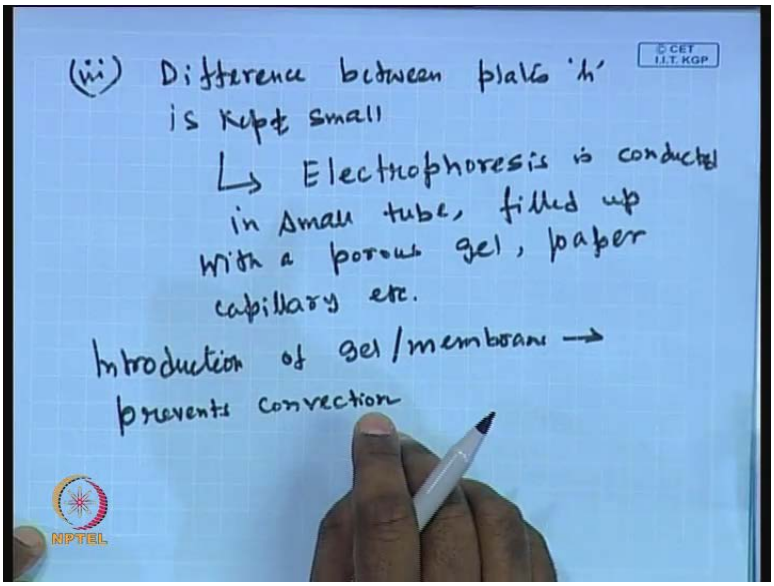

The third method is generally used is difference plates difference between plates h is kept small. So, if this differences kept small then the natural circulation because of natural convection can be prevented to some extent. So therefore, electrophoresis is conducted in small tube or gel or membrane. So, electrophoresis is conducted in small tube or the material inside the tube is filled up by a porous gel or porous membrane. So, that the natural convection would be will be controlled filled up with a porous gel or sometimes porous will call paper capillary, capillary tube; that means, it is very small diameter etcetera. Now, introduction of gel or membrane what it does it prevent convection now second complication that occurs in the case of electrophoresis is occurrence of electro osmosis.

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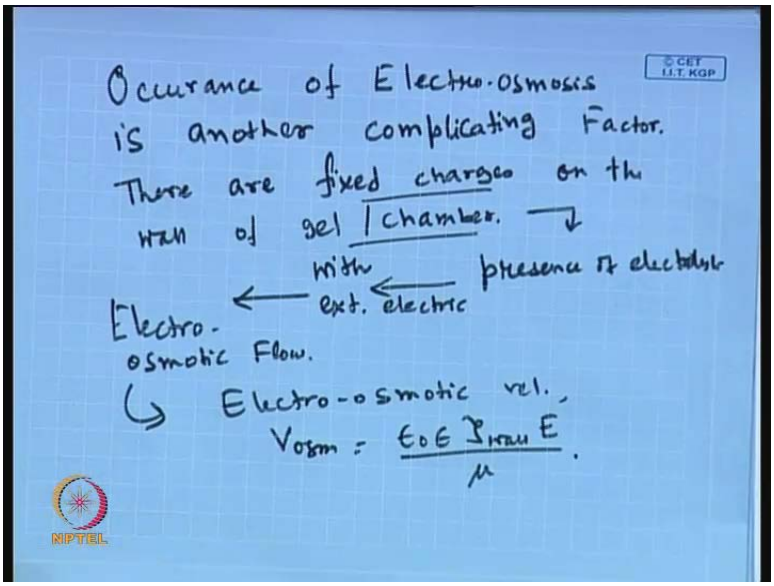

Occurrence of electro osmosis is another complication complicating factor. Now, there are fixed charges on the well of the gel or the chamber whatever it is and this because it is a fixed charges and presents of electrolyte now with an external electric field it leads to electro osmotic flow. In fact, we have already define how the electro osmosis occur earlier and the flow movement by electro osmotic flow by electro osmotic velocity. There will be defined velocity will be v electro osmotic will be epsilon 0 epsilon zeta of the wall or the gel divided by E multiplied by E divided by mu. Mu is the electro osmotic velocity and the electro osmotic velocity is it reduces the separation because it added with the electrophoretic velocity. So, the velocity becomes more so, if the velocity becomes more then the residence time between a 2 particle will be less. So, it hint us so, the hole what is a problem that will be arising out of cross.

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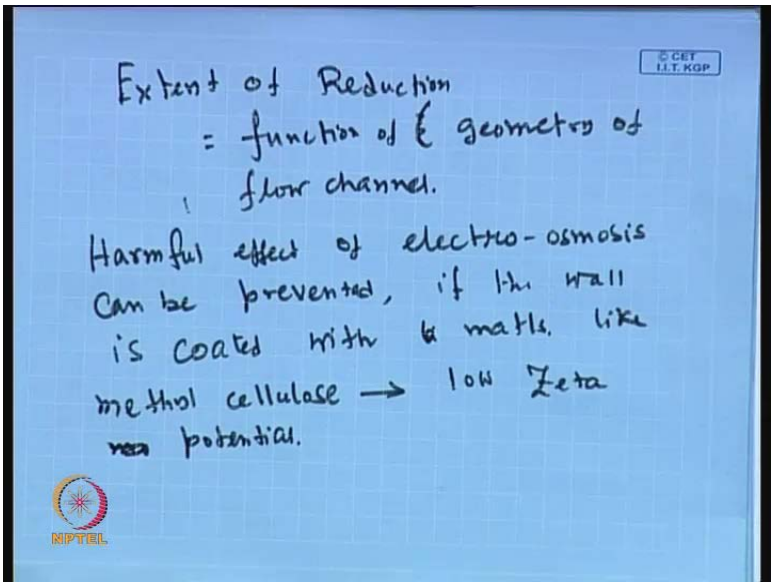

Motive velocity reduction of separation will becomes a result. And this reduction extent have reduction is a function of geometry of the flow channel. Extent of reduction it becomes a function of you know geometry of the flow channel and of course, the harmful of electro osmosis can be prevented. If the wall is quoted with a material in some material that will reduce the zeta potential, if the zeta potential the wall is reduced in the electro osmotic velocity will also reduced. And how does the material may be methyl cellulose or some neutral material you just not charged.

So, harmful effective electro osmosis can be prevent by if the wall is coated with neutral materials like materials like Menthyl cellulose which was really in a resulting in to a low zeta potential. So therefore, the mobility due to the zeta potential the electro osmosis mobile electro osmosis can be minimized. Now, in order to get this idea there are several configuration of electrophoresis ion occur for example, gel membrane and paper electrophoresis, gel electrophoresis, membrane electrophoresis, paper electrophoresis and there is something called page electrophoresis.

So, polyelectromide enhanced new electro or electrophoresis we will be looking into some kind of normal electrophonic method separation in the next class I will be solving some problems also. So, that we will see how the separations occur during electrophoretic method thank you.