Analytical Technologies in Biotechnology
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Module - 4
Electrophoresis
Lecture - 1
Basis Concept in Electrophoresis

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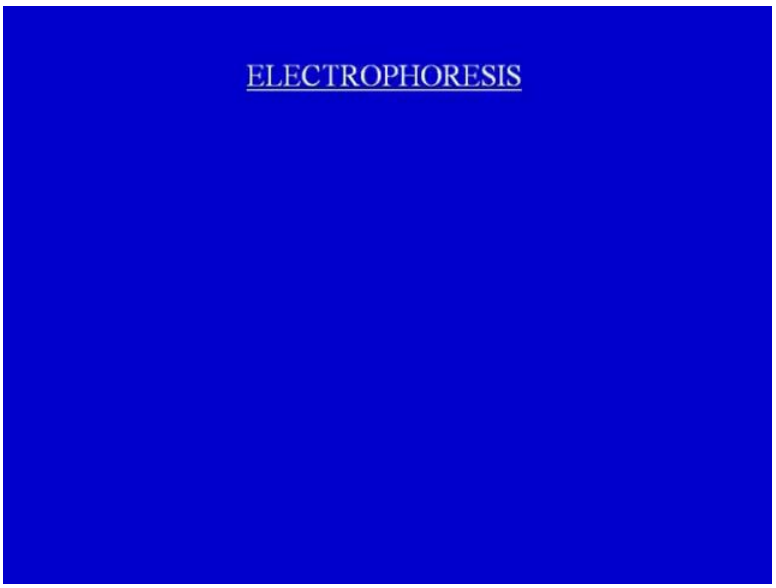

In this lecture, we are going to start a new topic electrophoresis. Now, scientists and area of biotechnology spent a lot of time like biochemists and biophysics in separation and identification of bio molecules, particularly say proteins and nucleic acids. They need to analyze, they need to identify and separate the molecule for different purpose like say you now the function, their physiological and biochemical function of this molecules.

Now, there lot of different properties which are exploited for identification and separation of these by a molecules. For example, if you can recall, you were discussing about say iron exchange chromatography or affinity chromatography or gel filtration chromatography where the properties of charged or affinity or seized for utilized. So, there are lot of differ like charge as a property or charged on the bio molecule can be utilize for separation of these analytes in different techniques.

For example, ion exchange utilizes charge for separation, mass spectrometry analysis utilizes charge for analysis of bio molecules and likewise electrophoresis is very widely used and it is a routine technique which utilize charged for separation. So, electrophoresis is separation technique that is based on the mobility of charged particle under the influence of an electric field. Now, a particular bio molecule could carry either or net positive charge or negative charge. So, positively charged molecule will migrate towards a negative electrode and negatively charged particle will migrate towards a positive electrode.

So, particles have different migration rates depending on their total charge and also on size and shape of the particular molecule to be separated. So, more is the charged at the positive or negative, their migration rate will differ accordingly; as well as if size and shape will also affect the mobility in electric field because the resistance of the friction which is there in a particular medium will be according to the size and shape of molecule.

So, many biological molecules as you know carry charge like, for example, amino acids, peptides, proteins, nucleic, nucleotides and nucleic acids depresses particular charge and depresses analyzable groups. Now, at any given ph, they will adjust as electrically charged species, which could be either as cations or anions according to the ph, they are in. So, there is one called iso electric point or pi value and at which the net charge is zero, but if ph is below the pi, then the molecule will carry positive charge and if it is above, it will carry negative charge in general. Now, under the influence of an electric field, the charged particles will migrate either to cathode or to anode depending on nature of their net charge.

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If you see the development of electrophoresis, it begins with the work of thesalius that is earnst thesalius in 1930s and from then from then on, new separation processes and chemical analysis techniques based on the electrophoresis are continued to develop here even today in 21st century. Now, trysails will support from rock feller foundation developed the trysails operators for moving boundary electrophoresis, which was described in 1937 in a very well known paper, which is a new apparatus for eletrophoretic method of colloidal mixture.

So, it started long back these techniques of electrophoresis. By the 60s that is 1960s, sophisticated gel electrophoresis methods made it possible to separate many biological molecules based on minute physical and chemical differences like charge, size and shape etcetera. This particular development gave a boost to the rise of molecular biology like molecular biology was quiet benefitted and came up as science because of the analytical power, which was provided by the technique of electrophoresis. Acryl amide gels and denaturing agents like STS were introduced and it enabled the separation and analysis of one of the micro molecule that is protein molecules.

In 1970, lamely separated of components of t four phase using stacking gel and STS as a detergent. Then the development of two dimensional gels and iso electric focusing happened around 1975 and then sequencing gel would develop around 77. Also, later agro gels were introduced here. So, a lot of development took place in those times. In at

1980S, the first field gel electrophoreses was developed in 1983, which enabled the separation of very large DNA molecules. Also, the the technique of capillary electrophoresis was developed at the same time. So, a lot of new techniques are still being developed, which are benefitting in different ways. Alright, as we go into the detail of this technique.

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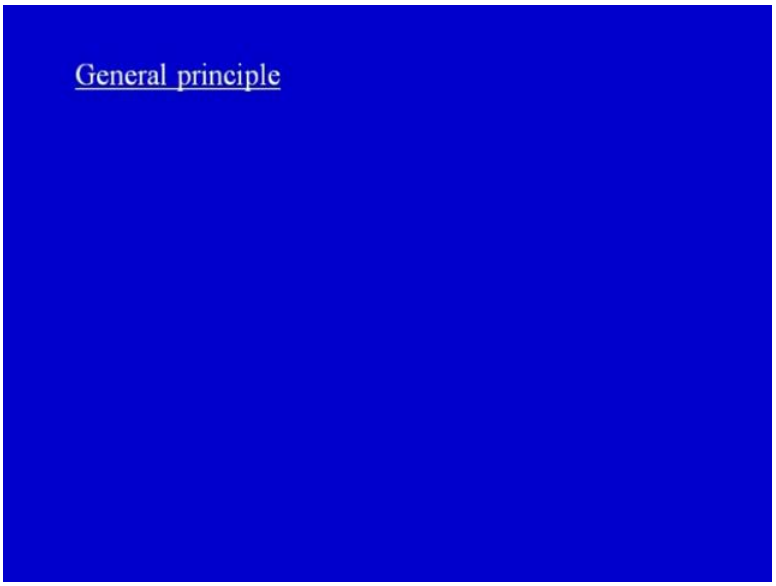

let us first little bit understand the general principle and let me you give you an overview of this technique and what we are going to discuss in this section. So, let me bring you to the screen. So, now let us first little bit understand before we go into details about the electrophoresis technique. So, what we were saying is a charged species under the influence of electric field moves to the respective electrode.

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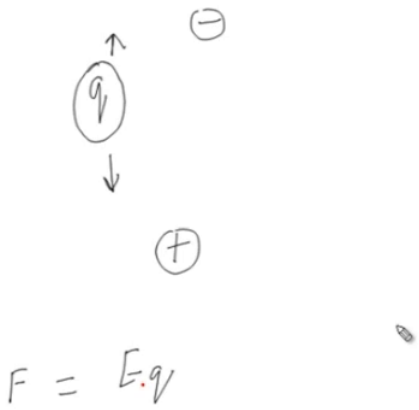

So, if I say that there is a charged species, say the charged species we just denoted it by q and this charged species, which could be any molecule, which could be say, protein molecule or d n a molecule, now as you apply and there are two electrodes. It is kept in under two electrodes, which is we say, there are two electrodes here, positively electrode on one side, negatively charged electrode on another side.

Now, as you applied the electric field, there will be a particular force will be applied on to the charged molecule and current will flow. This charged molecule also moves in a particular force. So, if I say it is positively charged, then it will move in towards negatively charged electrode; if it is negatively charged, then it will move towards positively charged electrode. Now, earlier when this electrophoresis started with the work of trysallius, it was mostly done in free solutions. So, there was a lot of problem of diffusion and other things.

So, we will come into that little later. So, as this charged molecule moves, it experience a particular force and this force can be given by the electric field E that is applied and the charge, so E q. So, if I say E applied electric field and q is the charge, so force which is is experienced by the charged particle under the influence of the electric field is E q. Now, there is another force when a particle moves, like we are talking about size and shape here that is the frictional force. That frictional force opposes the motion of the particle as

it moves through a particular medium and that force is you can also call it a viscous drag and which can be donated by F.

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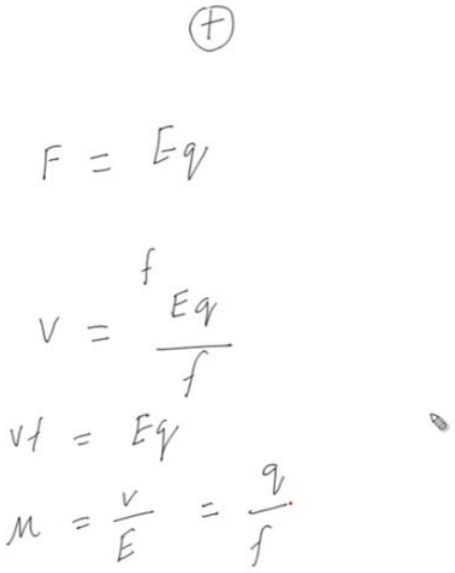

So, the velocity if I say, velocity of the particle, which kind of moves under the influence of electric field will be given by E q by f that is frictional force or we can also say it is v f equals E q. That is viscous drag after certain period of time, the force which is forward moving force E q equals the viscous drag, which depends on the velocity of the molecule that is v f. There is another term used, which is more popular term is electrophoretic mobility and this electrophoretic mobility is given by velocity per unit field or you can say that is q upon fictional force.

So, that is the electrophoretic mobility, which is more commonly used. The important part is like I said let us take an over view here, most of these experiments starts in free solutions and the separation is done, may be free solution and then paper and other mediums. In free solution, there were a lot of problems about say diffusion, convective currents and all those things. So, the separations were not as good and later on gel electrophoresis were agarose and poly acryl amide was utilized.

Now, these gels could be handy in the sense that first the separation was much better to the sieving effect. There was less of diffusion, the band where the diffusion was minimized, the heating effects were minimized where convection currents are concerned and lot of other improvements took place, which could give a very better separation of

proteins and nucleic acid in particular. Lot of techniques developed afterwards like as we discussing about for is as we discuss the history of electrophoresis that acryl amide gels may introduced and then later on agarose gel for nucleic acids were introduced.

Remember nucleic acids, agarose nucleic acids are large molecules and it was very difficult to run them on larger molecule of DNA. Large size of DNA molecules were difficult to run on poly acryl amide gels as the poor size in a poly acryl amide gels is smaller. So, agarose gels were quite handy in that. Proteins were run on poly acryl amide gels and not agarose gel because the poor the size in agro gel is too large to separate the protein. Later on, a lot of different teachings were developed as we discuss. There was like a continuous buffer system, a discontinuous buffer system like lamely introduced discontinues buffer system with the stacking and the separating gel as STS in electrophoresis.

Then, there were a lot of techniques for DNA sequencing, which is now it is not utilized, but earlier poly acryl amide gels were utilized for separation of sequencing the DNA and remember poly acryl amide can run normally smaller fragments of DNA. Later on, iso electro focusing, which was based on the pi values, was developed. There was pulsed field gel of electrophoresis for separation of very large like say 60 kb or large DNA molecule was introduced. Likewise, capillary electrophoresis and other techniques were introduced as we will be discussing them as we go along. So, let us come back to our discussion here. So, what we were discussing about like I gave you the over view of this in a very short about electrophoresis**.**

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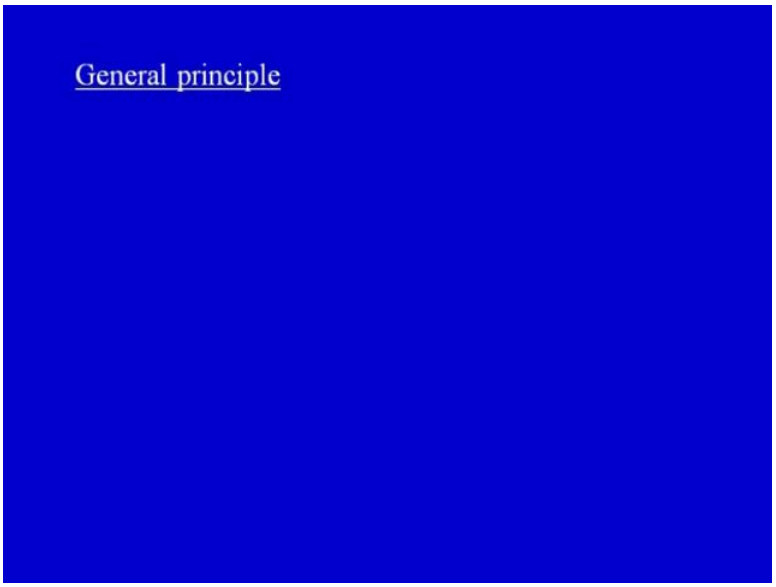

Now, general principal as I was discussing that there is the force and which is experienced by particular analyte or charged species. So, the electrophoresis is as such as the migration of charge analytic under the influence of an externally applied electric field. As we said positive charged moves to the cathode and negatively species move to the anode, so the efficiency of electrophoretic separation as we will be discussing will depend or will be affected by three factors, mainly three factors. There might be other factors also. One is electrophoretic mobility of particular charged species. Another important factor, which we are going to discuss, is the heating which is called a joule, ohmic or resistive heating and which also affects the electrophoresis separation.

Then, electro osmotic flow, which is kind of quiet suppressed in gel electrophoresis, but it could be a problem in say electrophoresis were the medium or say capillaries, capillary glass or other mediums can carry charges by themselves and can interfere in the electrophoresis run. So, this is like in support media. So, electrophoresis can be performed. So, these are the three factors and then electrophoresis like I said can be performed in free solutions or in support medium, which is gel electrophoresis, which we are going to discuss. This can act as anti connective and sieving medium, actually this is support medium.

Gel electrophoresis is the most popular form of electrophoresis and routinely used in different research and teaching laboratories. It is a very important tool in the labs, which

are working on proteins and nucleic acids and they are routinely used for different applications. So, let us get into little bit of basic concept or basic principal of electrophoresis and little bit overview we have given. What happens is when potential difference is applied across the electrodes, what it does is it generates a gradient.

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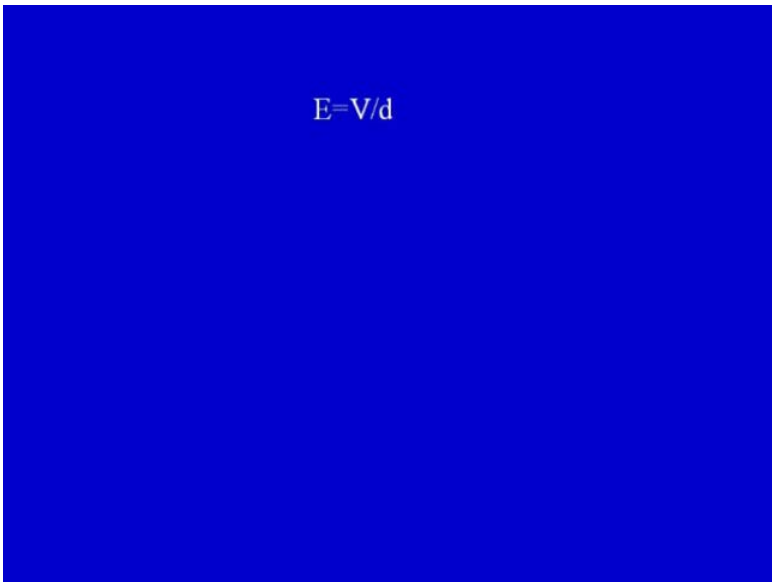

It could be given by E, which is applied voltage actually divided by difference of the electrodes. So, this potential gradient is the applied voltage divided by the distance between the electrodes, which is E equals V upon d, the voltage upon distance between the electrodes. Now, when the potential gradient is applied, force on a molecule that is we can say a charged species contains q columns of charge, q coulombs of charge is E q Newton. This force drives the charged molecules towards the electrode. So, this is a particular force, which will be experience by the charged species. Now, but when it moves through medium, however there will be also another force, which is the resistive force or a frictional resistance. This frictional resistance will retard the movement of charged molecule.

Now, in free solution, the frictional force is linearly proportional to the velocity of charged analyte and the magnitude of this viscous drag that occurs is F v or frictional force into the velocity of the charged particle. So, the frictional force is a measure of the hydrodynamic size and shape of the molecule, then the pore size of the medium where the electrophoresis is being performed or taking place and the viscosity of the buffer. So,

therefore the viscosity, velocity of the charged molecule in electric field will be given by…

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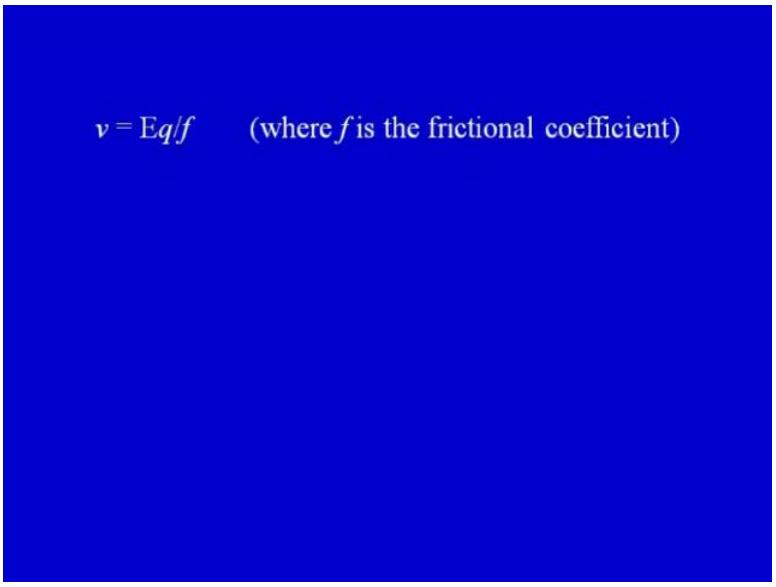

v equals E q upon frictional force or frictional coefficient, so that is the velocity a particular molecule will experience or will have when it moves through the, under the influence of electric field through a particular medium. Now, like I said, the more common term for the migration of charged particle is electrophoretic mobility.

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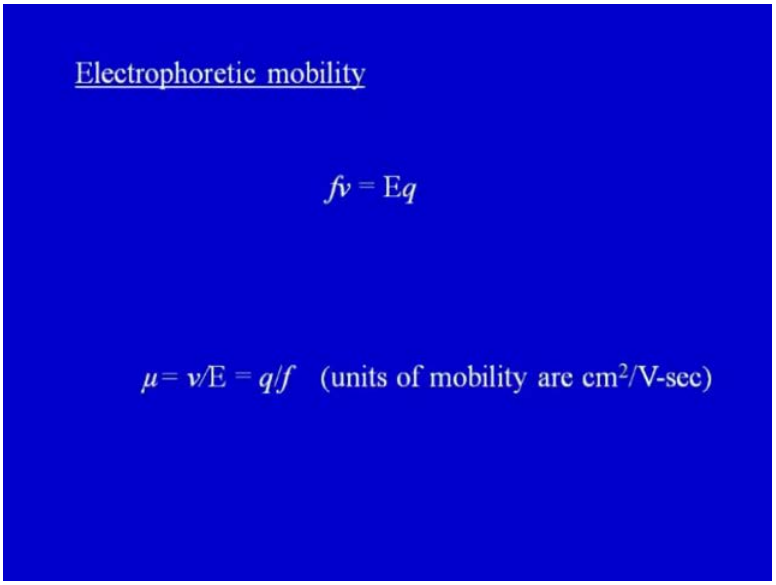

So, as the ion moves or accelerates, a frictional opposes the forward motion. So, in the beginning, the charged particle is put under the influence of the electric field, it will move towards the opposite electrode and it accelerates as it moves through medium. As it accelerates, the frictional force comes into existence and this frictional force will oppose the forward motion of the ion or the charged species.

Now, what will happen is that the molecule which is rapidly moving reaches a steady velocity, which is steady state terminal velocity when two opposing forces that is the forced molecule on the charged molecule and due to the electric field E q and the viscous drag or the frictional drag f v becomes equal. So, this has to be understood that at the field, the field is applied, the charged the particle will accelerate through the medium, but soon frictional force catches as soon as the force pulls very quickly, the two forces become equal.

Then, the particle or the charged species moves with a constant velocity and that is given, here that two forces become equal. Here, this is electric phobic mobility here of a charged analyte or a particle is more commonly used term and this will be defined as the steady state velocity of analyte per unit field. So, when charged species has acquired a constant velocity, then the electrophoresis mobility of that will be defined by the velocity per unit field or by another term called the charge upon the frictional force. So, that is the electrophoretic mobility.

Now, electrophoretic mobility is the fundamental parameter, which determines the efficiency of separation based on charged to size ratio. So, remember this is very important. Depending on say a particular charged species or of say two analytes might carry the same charge, but different sizes, so what will happen? The one which will have a bigger size will experience more frictional force and will move slower than the one which is smaller, but carrying the same charge.

Likewise, if two analytes are there of same size, but they carry different charges, so one carrying more charge will move faster than the one carrying the less charge, but of same size. Likewise, shape will also play a role depending on the shape like for example, a spherical shape will move faster as compared to elongated shape molecule.

Now, the change in ph effectively alters the charge on the ions and their electrophoresis mobility. Like I said, most bio molecules will have a pi value that is iso electric point. As

you move away from the iso electric point, you will, the analyte will carry more charge. So, if you are very close, the ph is very close to the pi or at pi, and then the net charge carried by the analyst or the molecule is zero. But, as it moves either and below the pi or above pi, the charge will increase as the ph is farthest from the pi.

So, depending on the ph, the analyte will carry a particular charge, which will be more or less and depending on that, how much charge is being carried the molecule carries, the electrophoretic mobility will be determined. So, ph will certainly affect the electrophoresis mobility.

So, different analysts, which separate electrophoresis are due to the differences in electrophoretic motilities and that is how they are separated. Like I said, analytes and charge species having similar charge will separate if there molecular size differs or shape differs and as there will be an experience of different friction force actually. So, even the pore size of the medium and other things will also effect. Like for example, for some molecules, the pore size may be as big that they do not experience any hindrances as compared to other molecules. So, these factors will determine the electrophoretic mobility. So, we in equation term, say we summarize this.

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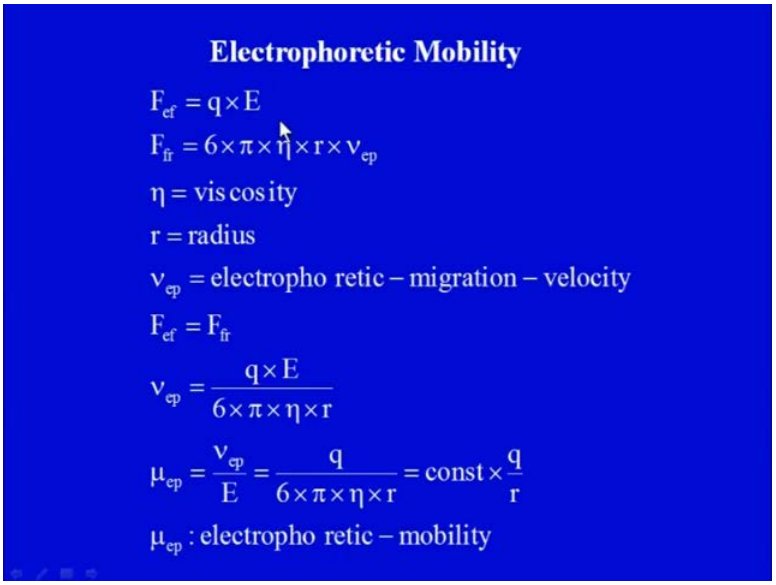

Then, you will have a force on a molecule which is q E, there is a frictional force, which is given by this equation, viscosity of the medium will play an important role because more the viscosity, more will be the friction, radius of the of the particle will play. So,

electrophoretic migration velocity the will be effected by all these factors and as we said electrophoretic mobility is force divided by the friction, this will determine like as we have already discussed, this is equation gives the electrophoretic mobility of a particular analyst or charge species in all the factors like viscosity, particle radius and all this things have been taken into account here.

So, one factor was the electrophoretic mobility. Now, another factor which is important in the migration of the charged species or electrophoresis run is the heating, which is called joule heating or ohmic heating or resistive heating. Now, when the current in the solution between the electrodes is conducted mainly by the buffer ions, a small proportion is being conducted by sample ions. Then ohms law expresses the relationship between the current, voltage and resistance. which is given by particular equation.

Now, the distance migrated by charged molecule will be proportional to the current flowing and the time. The current can be increased by increasing the applied voltage and in principle, electrophoretic separation could be accelerated. So, when certainly when you increase the current or when you increase the voltage, the current will increase and certainly the force experienced by the molecule will be higher. So, the electrophoretic separation could be accelerated. The distance migrated will depend on current and the time for which this is being done. But, there is a one very big problem here as which comes out as you increase the applied voltage and as you observe this whole phenomenon. That is the generation of heat in here in the electrophoresis apparatus or so where ever you are carrying this.

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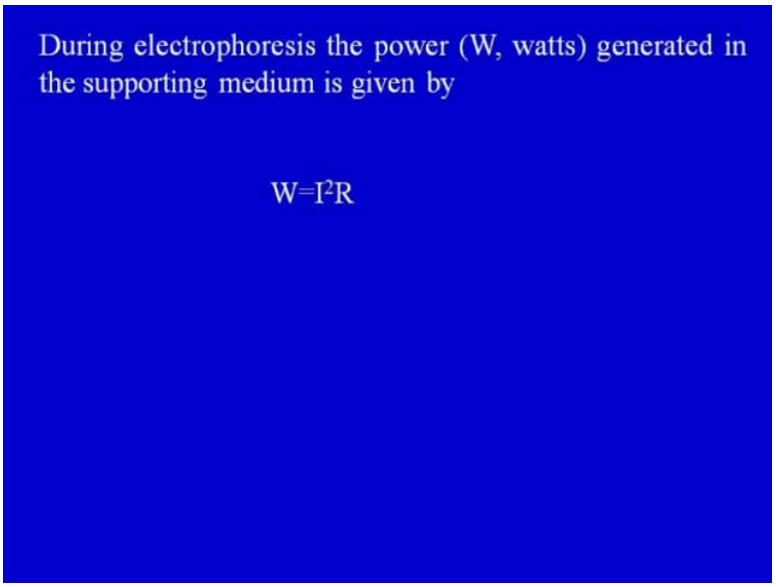

So, during electrophoresis, the power generated in the supporting medium will be given by this particular equation that is W I square R. The most of the power, which is generated here, is dissipated as heat. So, what is the problem here because of heat? Now, heating of electrophoretic medium or apparatus has lot of effects actually, several effects which are important here.

Now, what will happen when heating is done or heat this apparatus or the medium is heated? One is that an increased rate of diffusion of sample and buffer rise will occur as heating is increased and which will lead to the peak broadening of the separating the separated analytes. So, as the heat increase take place, as the current is increased, whatever mean and as the time goes by on an electrophoretic apparatus, this will certainly lead to diffusion of sample.

Therefore, there the resolution or you can say peak broadening will take place and the separation will not be ideal or not be good. So, this is one major problem. Due to temperature rise, there will be temperature gradient may be formed and the formation of the convection currents leading to the mixing of separate samples would occur. That is another problem due to convection currents.

This is quite suppressed like I said if you compare free solutions to the gels, there will be a less problem of convection current. Then there is thermal instability of analysts. Now, many analytes, which are of biological origin, are sensitive to heating. As the

temperature rises, it may be like a few do not want to need denature of enzymes or proteins, but at a higher temperature, certainly these, many of these enzymes and proteins will denature. So, that is another problem that if you want them in a native form then and if there is a heat generation, then and certainly thermal instability will be a big problem for analysis of these samples.

Then, there will be a decrease in viscosity buffer as the temperature rises or heat is generated. As the viscosity decreases, there will be a reduction in the resistance of the medium. So, there are certain alike these several effects which will certainly occur when heating occurs. They will and there will affect the electrophoresis run.

Now, there are methods for decreasing this heating. So, when electrophoresis is being performed, it could be performed at constant power rather than constant voltage where current increases, and therefore heat output is increased. Now, running particular electrophoresis on constant power can eliminate not the heating, but the fluctuations in the heating. So, even when you run it at a constant power, there will be generation of heat, excess constant heat generation will take place and still it will be a problem. So, how to solve this?

One is that one you can do is you can run the electrophoresis at a very low power or a current or applied voltage, but there what will happen? You will certainly increase the analysis time. So, when you do it at lower power or low current or low applied voltage, the analysis times will increase and this will result certainly in poor separations. So, then again, this does not solve the problem.

So, what has to be done? A balance in reasonable power setting for appropriate separation times needs to be used. So, you have to somehow work out a reasonable power setting and what analysis time, so that right analysis time obtained for separation and your resolution does not go down. Then what you can do is another method could be to dissipate heat by cooling apparatus or cooling system.

So, if cooling systems could be employed where chilled water could be circulated, or in cold room, you can run this to remove the liberated heat. So, another thing could be done is to dissipate heat is thin gel or small diameter capillaries, which have large volume ratio can be used and their electrical resistances are high. This current flow is reduced for a given voltage.

Now, the problem with all though these are good solutions, but for say problem with even doing in say like small diameter capillaries or say slab gel, many times you can control the things at the center, but at the edges, there might be differences in the heat because heat is being dissipated at the edges and not in the center. So, there will be certain convection currents or diffuse things might occur or the charges species of different species might not experience same force and due to the heating there might be a problem. So, that are certain problems which need to be solved, but certainly you can reduce them by these measures.

So, the two factors which we have discussed is electrophoretic mobility for successful electrophoresis system, another is the heating problem and this could be solved by a balancing the power supply or like a power setting and analysis times. Now, third important factor which affects your electrophoresis run is that is electro osmotic flow or electro endosmosis EOF, which we call it. Now, electro osmotic, electro endosmosis or electro osmotic flow refers to migration of the bulk liquid towards the cathode. The phenomenon happens because of formation of double layer at the wall of the capillary.

How does it happen? What happens is it occurs due to the presence of charged groups on the surface of the support medium. For example, paper will carry a carboxyl group, glass and silica, silica will carry a silanol group, agarose carries, if it not purified a suffrage group and likewise, these surface chargers will certainly disrupt the normal flow actually. So, the phenomenon is quite well explained in case of capillary tubes.

Now, in fused silica capillary tubes, the silanol groups are deprecated or you can say they are ionized at ph higher than 3 and these ionized groups create an electrical, double layer at the capillary wall or the capillary wall electrolyte inter phase. Now, what will happen? The positive irons in solution will migrate to the wall and a double layer is developed at the wall of the capillary. Let me show you this on the screen actually.

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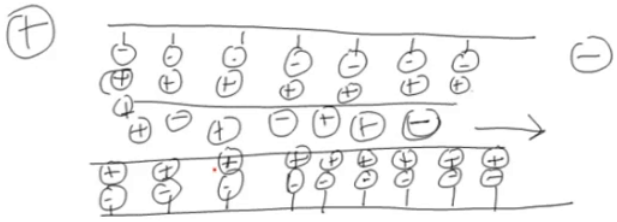

You will have capillary, which is defused silica or other capillaries. Now, like I said above ph 3, this silicone group is ionized and they carry net negative charge. Of course, as you increase the ph, these groups will be charged, more groups will be charged may be at ph 3 or say there is certain less number of groups charged or not complete charge pattern has taken place. But, what is going to happen is as you increase the ph, many more groups will be charged.

Now, so what you have at the surface, inner surface of the capillary wall? You have negative charge. Now, there is a particular electrolyte in the capillary like if you say it is free solution you are utilizing here and then will that particular electrolyte, a particular solution which is inside this capillary will carry both positive and negative charge. Now, what will happen? This positive charge will be attracted towards the negative charge here. So, what are you going to see is on both sides of the wall that is inner side of the wall, you have a layer being created which is we call a double layer of negative and positive charge. On both sides, you have these positively charged ions from the electrolyte and from the solutions are moving towards negatively charged particles.

Now, these are positively charged. Then there is another positively charge, negatively charge ions which are here in between actually. So, these are in between here. Now, what happen are these negatively charge will be aligning here and when voltage is applied, then this positively charged ions will moves towards to the cathode or negatively charged

electrodes. So, which are attaching to the wall, when they are applied, the voltage is applied, they all move towards and some positive charge, they will move towards the cathodes.

Now, essentially what should happen? The positively charged ions should move towards negatively charged cathodes and negatively charged ions like these ones should moves towards the positively charged electrodes. But, because of this formation of double layer and their interactions, the whole flow, the electrolytic flow occurs towards the cathode. This flow carries the kind of carries the every thing toward the cathode.

So, that is the electro osmotic flow and which will occur only when there is support media like in capillary, there is charges like I said there could be on agarose, there could be on, there could be on paper or same acryl amide, might these could be done. So, now what is happening is that positively lines solution migrate to the wall and the double layer as we have shown you is developed at the wall of capillary.

Now, there is an external layer, which is layer of positively charged moves compensates for the negative charge on the surface as I have shown you in the inner capillary wall. There is a diffused layer which is adjacent to that, at the external layer which is mobile cations actually. So, what happens when potential established or applied? The cations or positive charge in the electrolyte near the capillary wall will migrate towards the cathodes. And what they will do is as they migrate towards the cathode, all these interactions, they will pull all the cathodes, they will all have electrolyte solution with them towards the cathode creating a net electro osmotic flow towards the cathodes. So, here you have a particular situation where electro flow kind of dominates and even when this negatively charged species might be trying to go towards other side, but they are not able because of the electro osmotic flow.

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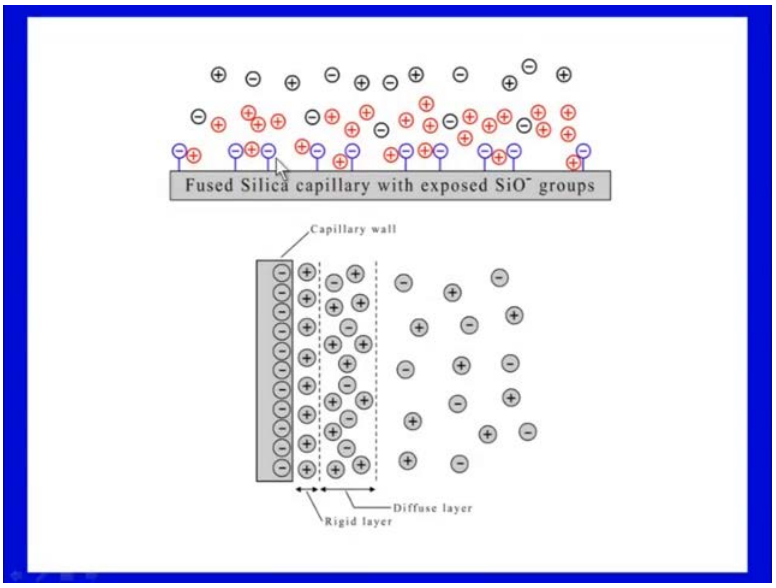

So, this figure kind of explains what I have already explained that there are fused silica capillary with negative charges. I said as I said at different ph, it will be charged differently. There is an external layer here which is shown here that which is like the cations compensating for negative charge on capillary wall. There is diffused layer which is here and as the applied voltage is applied, everything moves towards cathode. So, that is the phenomenon which is electro osmotic flow.

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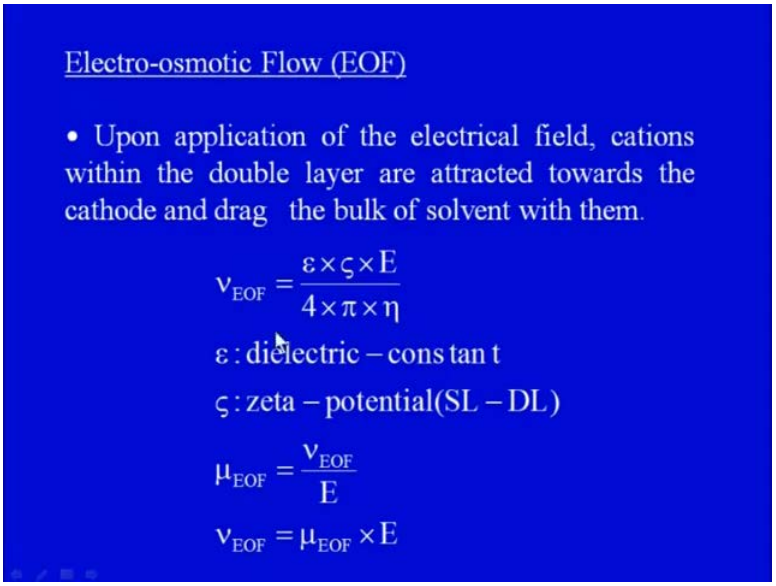

Now, so what is happening is dragging the wall of solvent towards the cathode and these are the equations here for electro osmotic flow which is dielectric constant, that is zeta potential, the applied electric field and so these things which are here and viscosity gives you around what will be the electrophoretic mobility of electro osmotic flow and that the velocity in electro osmotic flow by E that is applied electric field. So, electro osmotic flow is like I said is suppressed in gel electrophoresis in another, but it is quite utilized as in capillary electrophoresis as we discuss in later classes, as we will be discussing in subsequent lecture here.

Now, electro osmotic flow in opposite direction to analyte that is electrophoretic flow in general. Then electro osmotic flow practically is equal to across the capillary and so the band broadening is minimal here. Then they are not reproducible. So, many times separation efficiencies are low. Since, they are reproducible and you cannot really rely on them if you try to do it again and again. So, in gel electrophoresis, this thing is quite separated and it is not adjusted, but in capillary electrophoresis, it is being used for separation.

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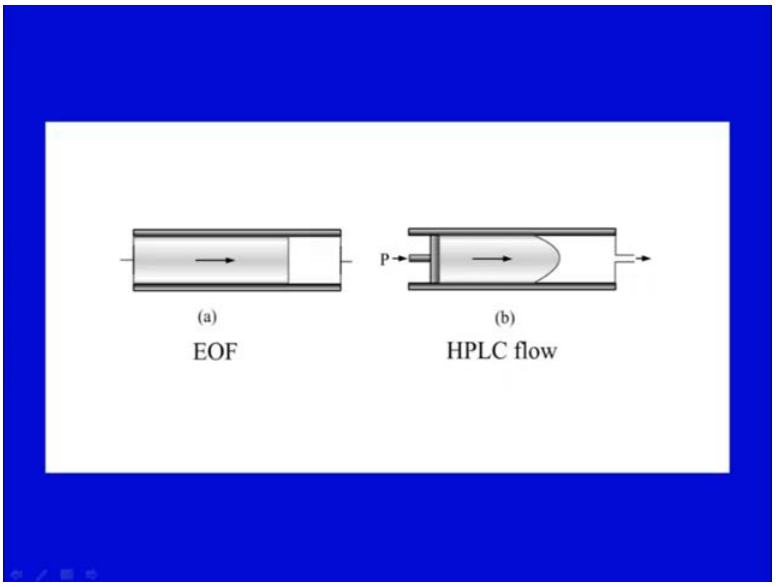

This shows here, how electro osmotic flow front will looks like as compared to HPLC flows or other flows. Now, when you have electro osmotic flow, what is the movement of the charge species under this influence actually?

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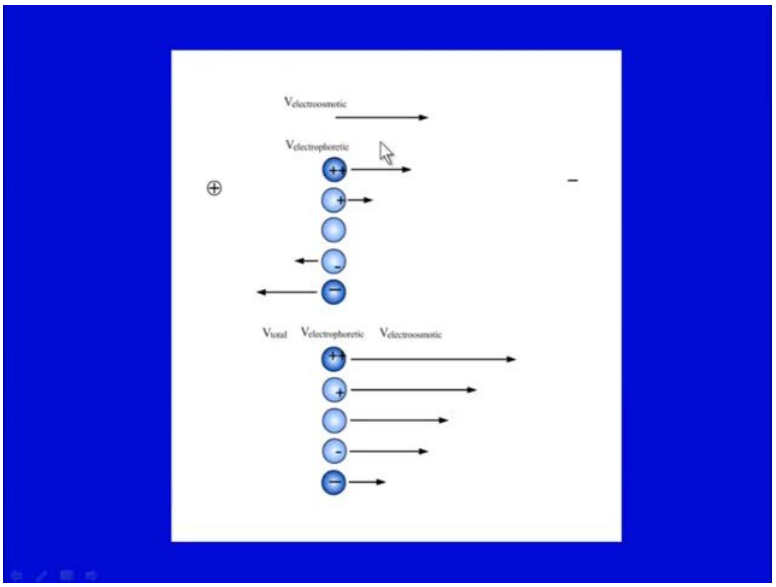

You see in this figure here, the velocity of electro osmotic flow like how does the charge species move. If you can see here, there is a comparison here that if you have a positive charge, then they will like if you can see here, there is like one flow towards this that is positive charge. More is the positive charge, more rapidly it will move towards the cathode, less is like charge, it will move. So, relative, it is a relative movement or migration depending on the charge being carried by the species. Likewise, neutral species will somehow will just move through, but not as rapidly as the positively charge species.

Then, negatively charged species though it is well experienced, electro osmotic flow will move towards other side that is towards an out and upper depending on how much charge is carried. So, what is net flow here? The net flow is that the positively charged species in the electro osmotic flow will move much faster for at highest rate or then followed by another positively charged species, but carries less of the positive charge. Then it will followed by neutral, then negatively charge species and then less more negative charge.

So, less negative charge will follow neutral, more negative charge resist a electro osmotic flow, but still it will move towards the cathode only with the bulk solvent that is the drag of bulk solvent. These were the three important factors, which kind of which is

very important to for an electrophoresis to run. These are like quite like say for example, joule heating.

There are a lot of things have be to improved the electrophoresis to control the electro osmotic flow in nonexistent and gel electrophoresis. Likewise, joule heating could be suppressed by cooling systems and other means like you can run chilled water through the plates where the, where this particular heat is been generated and electrophoretic mobility likewise could be improved by taking correct measures.

Now, so in this lecture, to summaries this lecture here what we have done is we have taken, we have discussed about the electrophoresis in terms of general principle of electrophoresis where we have seen how charged species moves through a medium when it is under two electrodes, when applied field the field is applied actually, a voltage is applied. There are three factors which are important that is electrophoretic mobility, joule heating and the electro osmotic flow. Now, like I said electro osmotic flow is only like applicable certain capillary electrophoresis or if other medium, then that will certainly effect the electrophoretic movement.

So, these are factors which are important. In next lecture, we are going to discuss about the components of electrophoresis system. Just to introduce a bit basic components which include electrophoresis apparatus, the place where you would be and we are going to discuss mostly about gel electrophoresis. So, what is the matrix being used here? Gel apparatus, where gel is been cast, how to load the samples, there are different matrices like agarose and poly acryl amide matrices. Agarose is run mostly in horizontal apparatus and acryl amide gel is run on vertical apertures.

Then, there are like lot of factors say you require a power supply apparatus and how these gels are cast, how this whole processes of electrophoresis is conducted, we are going to discuss that in the next lecture. Likewise, we will be discussing different techniques like discontinuous buffer system or continuous buffer systems, how proteins and how nucleic acids are separated on different gel systems and what are the different variations, and electrophoresis techniques. For example, we were discussing about iso electric focusing or capillary electrophoresis, STS phase electro force and what are their applications, utility like in various research labs, and in and identification and analysis of various components or bio molecules. Thank you.