Transport Phenomena in Biological Systems
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Lecture-61
Electrophoresis

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Welcome, let us begin or let us we are continuing the same situation here simultaneous concentration gradient and electrical potential gradient. We are going to look at a process that is highly relevant for biological engineers, the process of electrophoresis, movement of ions or separation of ions in an electric field, we use it for protein separations DNA, nucleic acid separations and so on so forth.

Routinely in the lab and it has very many different applications, the electro focusing has applications in downstream processing and so on so forth. So, we are going to look at some principles of this we are trying to understand this a little better for better design operation and so on so forth.

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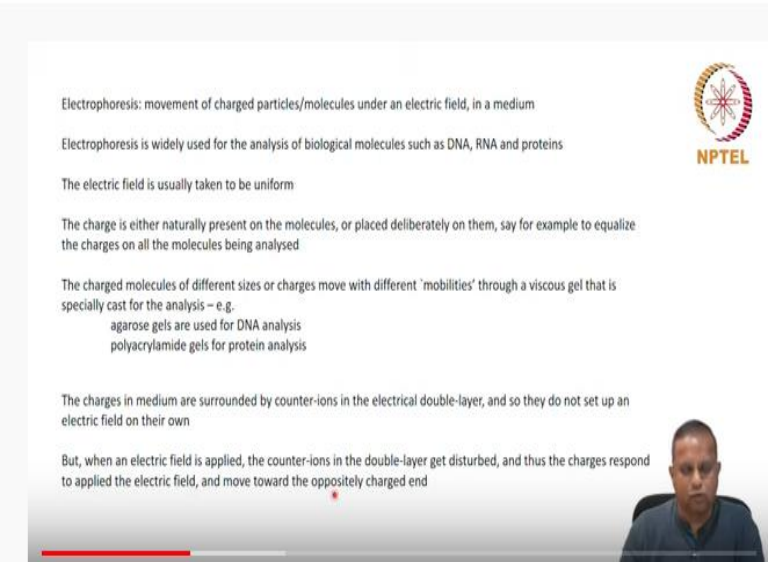

Electrophoresis is nothing but the movement of charged particles or molecules under an electric field in a medium, we know this. Electrophoresis is widely used for the analysis of biological molecules it has DNA, RNA proteins, nucleic acids proteins. The electric field is usually taken to be uniform at you know the design is made in such a way, that this assumption is valid electric field is uniform.

The charge is either naturally present on the molecules or placed deliberately on them say for example to equalize the charges on all molecules being analyzed. These are typical conditions under which this process takes place in the lab separation of biological molecules in the industry it happens in a slightly different fashion. The charged molecules of different sizes or charges move with different mobilities through again mobility is coming in here through a viscous gel that is specially cast for the analysis.

For example agarose gels are used for DNA analysis, polyacrylamide gels for protein analysis and so on. The charges in medium are surrounded by counter ions in the electrical double layer we have already seen this the charges in the medium are surrounded by the oppositely charged ions the electrical double layer it is called and therefore they normally do not set up an electric field on their own.

However, when an electric field is applied the counter ions in the double layer get disturbed and thus the charges respond to the electric field, the charges are able to see the electric field because the disruption of the electrical double layer and move towards the oppositely charged end of the device.

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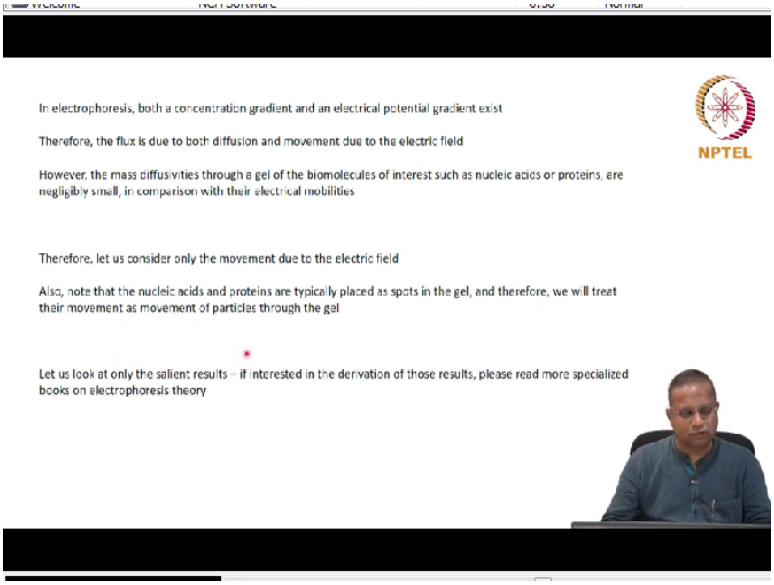

In electrophoresis, both a concentration gradient and an electrical potential gradient exists, that is you know essentially what we have been discussing so far and that is the reason why we are looking at electrophoresis. Now therefore the flux of these ions is due to both diffusion concentration gradient and movement due to the electric field, it is due to both of these. However, the mass diffusivity is through the gel of the biomolecules of interest such as nucleic acids and proteins are negligibly small in comparison or their electrical mobility okay.

It is only a comparison that we are looking at due to the effect of the electric field they are going to move much, much faster compared to the diffusive moment through this viscous chip. Therefore, we just need to consider only the moment due to the electric field although the moment due to the concentration gradient well exist but it is so small the fluxes are so small in comparison, that they are negligible.

Something like if you have 1000 the other one to be a 1 whether the final number as a 1000 or a 1001 it does not matter right, still number is a 1001 you need to add both a 1001 so 1000 is so

close to a 1000 that even if you ignore the 1 it does not matter, that is the essential idea here. Also note that the nucleic acids and proteins are typically placed as spots in the gel and therefore we will treat their moment as molecules or particles through this gel.

And let us look at only the salient results and we are not going to derive much of anything here in this lecture, if you are interested in the derivation of the results, please read more specialized books on electrophoresis and so on ok. We are not going to get into the complexities involved and there quite a lot of complexities involved in the derivation. If you are interested please read advance books.

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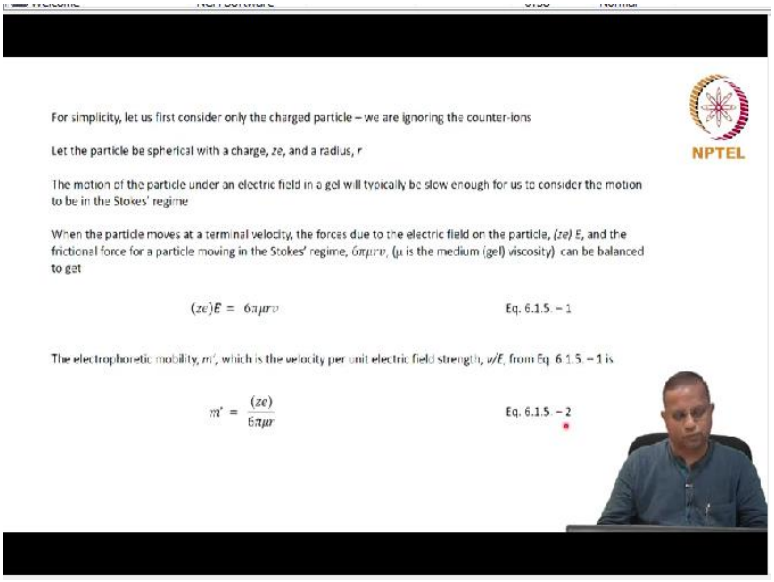

Now for simplicity let us consider or let us first consider only the charged particle, we are going to ignore the counter ions and so on so forth. We will assume that the counter ions are sufficiently disrupted that the charged particle behaves as if it exists on its own. Let the particle be spherical where the charge is ze okay ze the valency e is electronic chance, ze and a radius r okay. So, we are going to consider them as particulate charges the spherical particle.

The motion of the particle under an electric field in a gel will typically be slow enough for us to consider the motion to be in the Stokes regime right. So, when the Reynolds numbers are low then we know that the flow the movement of the particle in a fluid occurs in the Stokes regime, so the

motion of the particle under electric field in a gel is going to be very slow usually keep it overnight and so on so forth.

For reasonable separation to occur right, if you recall your gel electrophoresis and therefore we can consider the motion of the particle to be in these Stokes regime. Stokes regime, terminal velocity you have to recall that. So, when the particle moves at terminal velocity the forces due to the electric field on the particle which is ze times e right, ze is the charge of the particle times the electrical field intensity (ze)E.

And the frictional force for a particle moving in the Stokes regime which is nothing but 6ηπrv 6μπrv μ is used for the viscosity here 6μπrv is the general viscosity, they can be balanced okay. So, they balance out that is why the terminal velocity results there is no acceleration there, the forces are balanced.

The electrophoretic mobility okay which we are going to represent as m', it is nothing but the velocity per unit electric field strength okay, you go, this is the way we are defining it the electrophoretic mobility m which is the velocity per unit field strength, unit electric field strength

> The electrophoretic mobility m' which is the velocity per unit electric field strength v/E, can be written from Eq. 6.1.5-1 as
>
> $$m' = \frac{(ze)}{6\pi\mu r} \qquad (6.1.5\text{-}2)$$

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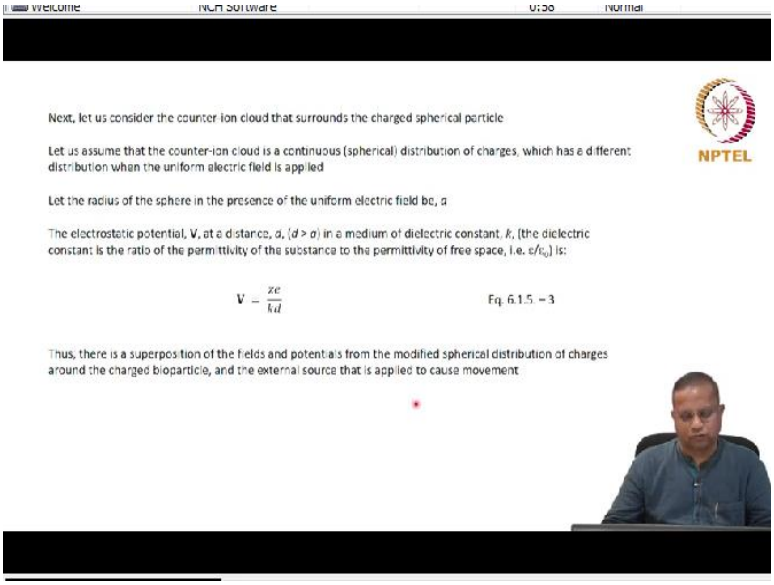

Now let us consider the counter ion cloud that surrounds the charged spherical particle. If we do that it is assumed that the counter ion cloud is continuous or this is a spherically distribution of charges which has a different distribution when the uniform electric field is applied. Then the radius of the sphere in the presence of the uniform electric field ba, this is for the counter ion cloud okay, there is the charge that we considered as a particle.

Then now we consider in the counter ion cloud. The electrostatic potential V at a distance d and of course being beyond the sphere of influence or the cloud radius d greater than a in a medium of dielectric constant k, dielectric constant is the ratio of permittivity of the substance to the permittivity in the free space we know this ε by ε_0 from our earlier classes is the dielectric constant.

So, the electrostatic potential at a distance d greater than a in a medium of dielectric constant k is **V = ze/(kd) 6.1.5 - 3** and therefore there is a superposition of the fields and potentials from the modified spherical distribution of charges around the charged by a particle okay. So, we looked at just the particle known then we looked at the ion cloud both of these exist okay. So, now we are looking at both of them together. And the external source that is applied to cause movement. The superposition all these fields superpose.

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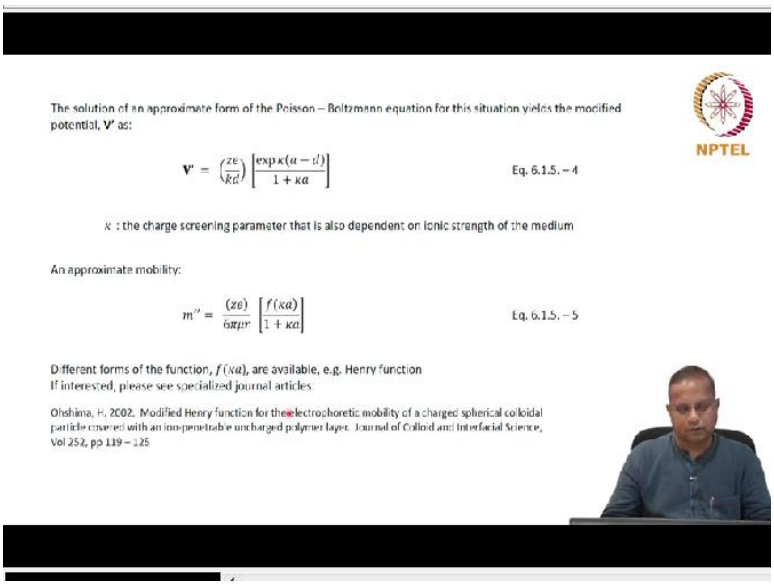

Under these conditions complex situation, that is the reason why I am not getting into it in this introductory course.

$$\mathbf{V}' = \left(\frac{ze}{kd}\right)\left[\frac{\exp \kappa(a-d)}{1+\kappa a}\right] \qquad (6.1.5\text{-}4)$$

where κ is the charge screening parameter that is also dependent on the ionic strength of the medium. An approximate mobility in this situation is given by

$$m'' = \frac{(ze)}{6\pi\mu r}\left[\frac{f(\kappa a)}{1+\kappa a}\right] \qquad (6.1.5\text{-}5)$$

That is also dependent on the ionic strength of the medium.

And different forms of the function if the f(κa) are available. For example Henry function is available and so if you are interested you could see some specialized journal articles for these functions, various functions are being used a couple of them are here or one of them is here Ohshima 2002 modified Henry function for the electrophoretic mobility of a charged spherical colloidal particle covered with an ion penetrable uncharged polymer layer.

This gives you an idea, do not worry too much about it published in Journal of colloid and interface science in 2002 okay. So, if you are interested in such things you need to go to these papers okay.

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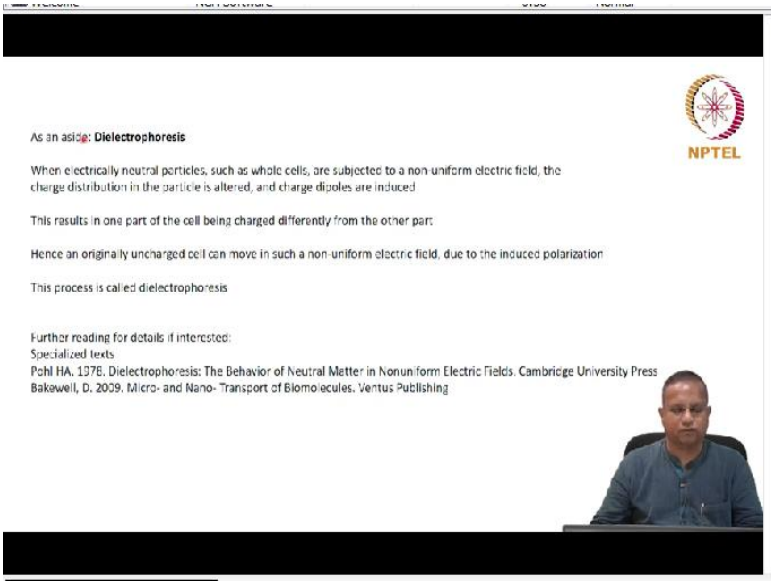

So, that was electrophoresis, let me also mention something about dielectrophoresis which the biological engineers can come across. When electrically neutral particles such as whole cells are subjected to a non-uniform electric field. The charge distribution the particle itself is altered okay and the charge dipoles are induced. So, typically uncharged neutral particles contain charges but they are all balanced out.

When this is placed in an electric field this the charges that are already there in the particle but they are all balancing each other out, they move to a different distribution or a different distribution is created and that causes charge dipoles to be induced. Dipole is nothing but charges separated by distance you know this. So, this results in one part of the cell being charged differently from the other part as you would expect.

Hence an originally uncharged cell can move in such non-uniform electric fields due to induced polarization, even though it is not charged it starts moving because a charge or dipoles are induced in initially uncharged species and this process is called dielectrophoresis and if you are interested you could read these specialized texts Pohl 1978, it is called dielectrophoresis. The behavior of neutral matter in non-uniform electric field is published by the Cambridge University Press in 1978.

And Blakewell micro and nano transport of biomolecules published in 2009 by Ventus publishing, these talk about di-electrophoresis okay. That is all I have I think with this we complete the aspect of mass transport under a simultaneous effect of 2 driving forces that is concentration gradient driving force and an electrical potential gradient driving force. We spend quite a bit of time on this, but it is very relevant and therefore we spent the time. In the next class when we meet up we will take up another aspect of multiple driving forces inducing a certain flux, see you.