**Bio – Microelectromechanical Systems**

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**Module No. # 01**

**Lecture No. # 17**

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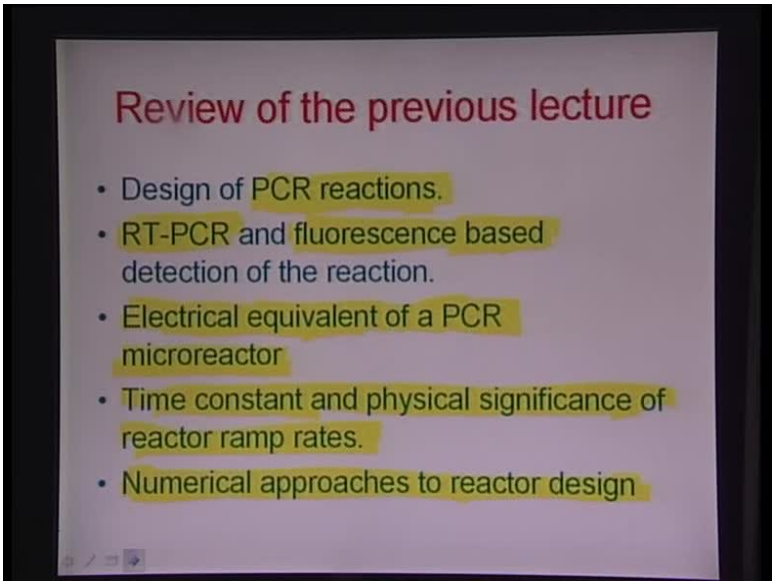


Hello and welcome back to the 17th lecture on bio microeletcromechanical systems. Let us just do quick preview of the last lecture before proceeding ahead and the last topics covered in the last lecture. We discussed about the very famous PCR reaction. PCR is polymerase chain reaction; it is a DNA amplification technique which is done in-vitro by the use of an enzyme and some of these other different reactants like oligonucleotides and different primers. This reaction takes place under certain pH condition.

We also talked about real time PCR which is a snapshot by snapshot view of how the amplification reaction proceeds. It is done by using fluorescence read out methods wherein some fluorescence ties are used to either intercalate or are used or are paired with some other di-molecule or some quencher molecule in a manner that when the

reaction proceeds, the distance between the primary fluorophore and the secondary fluorophore or the quencher changes as a result of which there is an increase in fluorescence. You can detect whether successfully the reaction is being carried out or not.

Real time PCR gives you an idea of what is the current amplification rate of a certain strand of DNA molecule as the reaction proceeds. We also tried to design PCR micro reactors; PCR because of the rapid thermal cycling issue associated with the process can be very easily translated on to a microchip level and there are lots of benefits to do such a react. One of them is rapidity because you are trying to heat up a very little mass of fluid in such microchip architecture by going into a thin surface film.

So, you are spreading out a volume or a bulk into a thin surface film. You are using the surface area for promoting higher heat transfer rates, and thus quicken the ramp-up and ramp-down times, so that the overall cycle time can get reduced because of that and the PCR reaction happens much more quicker in the micro reactor mode.

We talked about developing an RC modeling where we took thermal capacitance, thermal resistance and tried to see by deriving an analogy between the electrical parameters like voltage with physical parameters like temperature which drives the process. We also looked at how the charge in an electrical circuit can be equalized to or made equivalent to the heat flow in a thermal circuit and then tried to develop the time constant from that circuit point of view.

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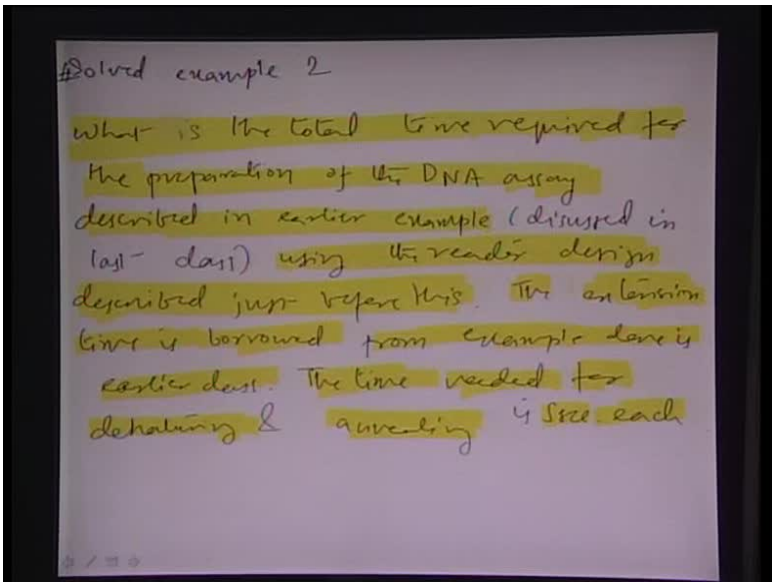


At the end of the day, take home message that we had is that the ramp-up, ramp-down time is independent of the reactor volume especially at the scales. There is a certain finite time constant which has to be used or spent in order for the reactor to get to a certain level of temperature that is equal to three times of RC or three times of the time constant value.

We tried these numerical approaches on one PCR reactor design example. We will do some more today and then move on to our next topic wherein we will study a little bit more about how this DNA polymerase within a cell tries to replicate DNA molecules. Let us do another example on designing a whole PCR process by using this time constant. In this particular example, we want to find out the total time required for the whole assay to get completed.

We assume the constant from the earlier example wherein we already found out that the time constant for the process was about 0.28 seconds. Using the reactor design fundamentals, we found out that the total time that it would take for the temperature to hit the 90 percent mark would be about three times such time constant which is 0.84 seconds.

We assume the extension time again from the earlier examples wherein we assume that 1000 base pair DNA was the template DNA. The extension rate of the enzyme was about 50 base pairs per second which makes the extension time the longest time step about 20

seconds. We further assumed the denaturing and the annealing times to be 5 seconds each and in this case we want to find out the total time needed for the PCR assay. Let us try solving this problem by assuming a certain time constant of 0.28 as had been indicated before.

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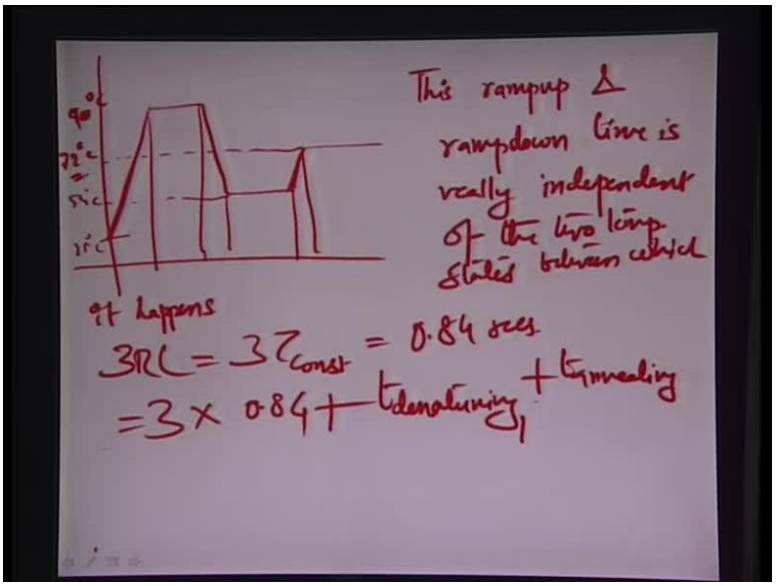


In this particular case, the total time which the total cycle of the PCR could be represented by three temperature steps here. You go from let us say about 25 degree Celsius all the way to about 90 degree Celsius; stay here for a little bit, go down to about 54 degree Celsius, stay here for little bit and then again go up all the way to about 72 degree Celsius. In one cycle, you are doing this heating, cooling mechanism thrice. This is the first time you are doing the ramp-up and you are ramping down and then again you are ramping up to all the way to about 72 degree Celsius.

As we know from our last illustration that this ramp-up and ramp-down time is independent of the two temperature states between which it happens. So, it is dependent on the reactor volume. In this particular case, the 3RC or the 3 time constant was found out to be 0.84 seconds and in a whole PCR cycle you are seeing that this time constant has to be executed three times because there are two heating and one cooling step.

So, there is one heating here, one cooling and then there is another heating up here; so it gets repeated three times - this time constant plus you have the independent the extension annealing and denaturing step- so you have t denaturing t annealing plus t extension.

This is a very important concept to understand. I would just like to point your attention to this fantastic problem wherein it is really independent of the temperature in between which this body or this reactor is oscillating irrespective of whatever the two temperature points are. In this case for instance, you see that you have to go in one instance from 25 degrees to about 90 degree Celsius and then you have to come down from 90 to 54 and from 54 back to 72.

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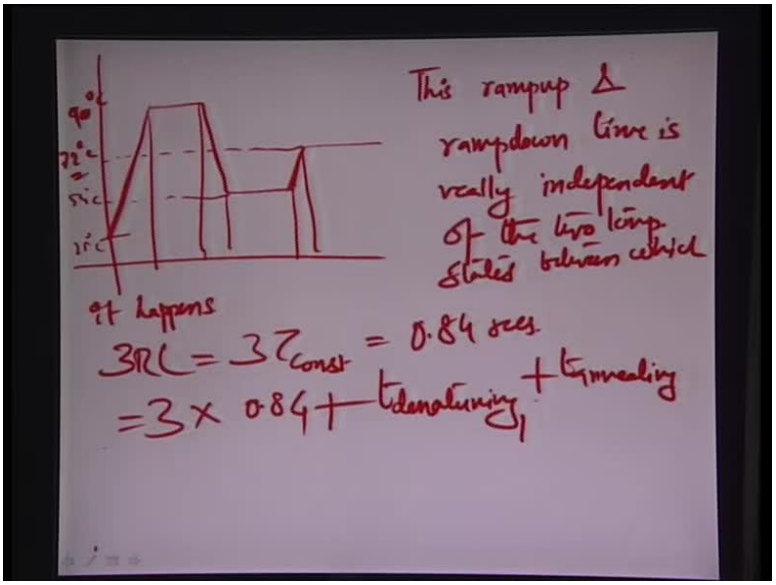


The delta t is varying in this case; but we assumed that the time constant, the amount of time that it would take for the fluid or the particular volume of fluid inside the chamber to reach the temperature of the walls of the chamber would be constant. It will depend on the properties of the thermal mass. Now, if you are to assume that there is a surface where we can rapidly change the temperatures without having to for the temperature of the surface on which this reactor is placed to reach the particular temperature point, in that case the only time that is needed assuming the temperature of 90s just about met or the temperature of 72 is just about met -the only time needed is to heat up the bulk which again is constant; it is not a function of the two temperature states but just the volume to area ratio which determines the time constant in that particular case.

If you can have a control on the ramp-up, ramp-down of the material that is involved in the heating process like for example, the remaining portion of the chip over which the heaters are placed then controlling from the surface which is in close proximity to the

solution is independent of the two temperature states. This is a very important concept to be understood at this stage.

In microchip architectures, the advantage which such architecture would provide is the fact that such a surface which is the surface wall holding the bulk or holding the fluid - that surface is essentially very small. It has its own thermal mass which is effectively very small and therefore, it can rapidly reach a certain temperature point from another point whether it is cooling or heating also because the surface area is more prominent it happens rapidly.

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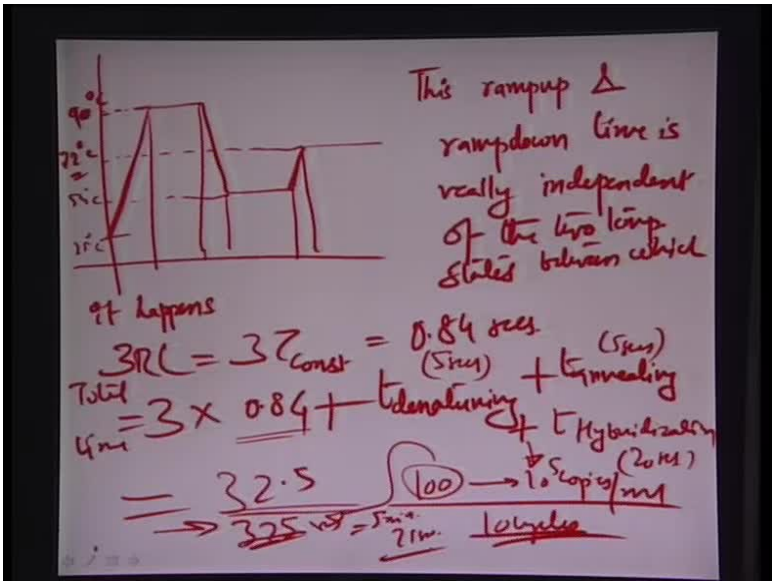


This issue is of extreme importance. Once this temperature is reached, we do not need to bother about what is going on in terms of heat transfer within the bulk of the particular solution. You have the total time of the reaction; in this case as 3 times of 0.84 seconds which is the time constant for the PCR volume or for the micro reactor volume from the last question and plus denaturing time plus annealing time plus hybridization or extension time.

We already know that the annealing and the denaturing times are all 5 seconds as illustrated in the question itself. From the earlier example where we calculated 1000 base pair DNA and extension rate of about 50 base pair per second, we found out the total time hybridization is about 20 seconds. Therefore, the total time that one cycle would need is about 32 seconds in this case. If you were to assume that you had to produce 10

to the power 5 copies per mill from about 100 copies per mill of the particular solution then assuming that about 10 cycles would be needed to do this, you will be left with about 325 odd seconds - by the by this is actually 32.5 is about 2.52, so, 32.5 here. So, it is about 325 seconds that you would take for cycling this for about 10 times so that you could produce about 10 to the power 5 copies of DNA from 100 - assuming the efficiencies that we had discussed in the last lecture in the last example.

So, the total time for the whole reaction to have 10 to the power 5 copies per mill starting with about 100 copies per mill is about 325 seconds or about 5 minutes and 25 seconds. In a macro scale reactor thermal cycler where there is a huge aluminum blog this would go as high as about close to 2 to 3 hours. There is definitely an advantage that micro scale PCR has to offer.

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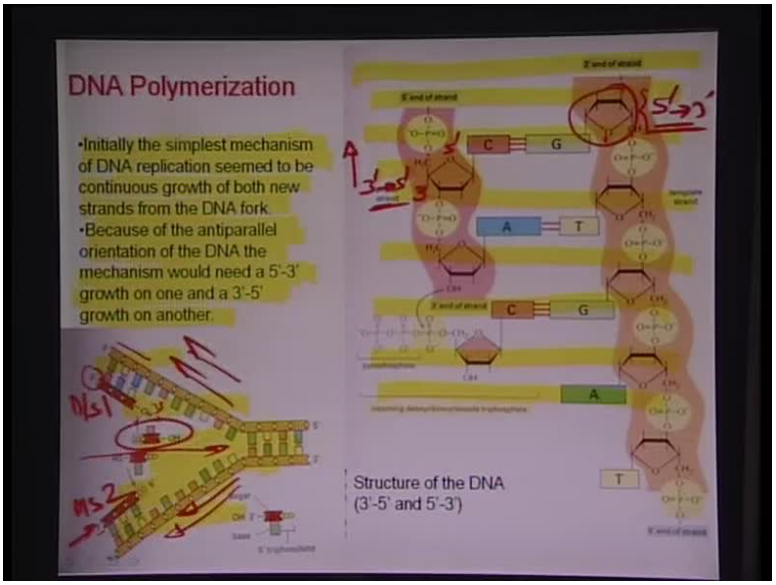


After these designing problems are over, let us try to understand this process of DNA polymerization, which is extremely important process for understanding of the basic kinetics or kinematics of the way the DNA is reconstructed between cells as they split and get reconstructed.

If you look at the DNA structure and closely evaluate it - the way that the sugar phosphate backbone is being laid out is opposite to each other. Here, in this particular illustration as you can see shows how if you look at this particular side - let us say this side, the sugar phosphate or the sugar bond or the sugar molecule is pointing from the 3

prime to 5 prime side. If you may remember from our earlier discussed nomenclature for DNA molecule, this is the third carbon; it is called 3 prime, this is the 5th carbon of the sugar, it is called 5 prime.

This direction is from 3 prime to 5 prime very amazingly the other strand of the DNA has an opposite direction of the sugar molecules. It is starting from the 5 prime end and going up to let us say the 3 prime end of the earlier strand. As you see here, the sugar molecule, the 5 prime is pointing downwards as opposed to this with the 5 prime is pointing upwards. So, the two strands of DNA are just opposite to each other in terms of the way that sugar molecule is aligned or oriented. It is very interesting phenomena because that defines the way the DNA polymer is able to replicate these independent moieties especially well within cells.

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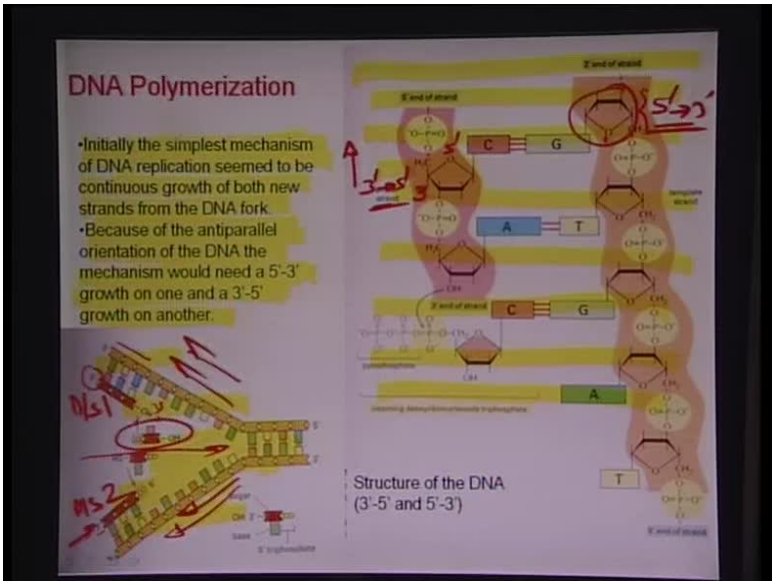


The simplest mechanism of DNA replication seems to be continuous growth of both daughter strands from the DNA fork. This right here is the DNA molecule as you can see here and if you see this closely there is an unzipping action. There is an unzipping action here and there is an opening of these two strands in both directions like this and thereafter there is a continuous growth of both the new strands from the DNA fork.

So, essentially people used to think that when these two daughter strands have been isolated; one in this direction, one this other direction. They would automatically replicate from the moieties which are present in the solution that is the triphosphates and

the kind of DNTPs or triphosphates and the enzymes which do the suturement. So, people used to think that as for the fork is being separated and as the new daughter strands generate and the suturement kind of happens parallely and therefore, you get this red train here of this red portion of the molecule is the suturements portion.

This is the artificially sutured portion. Just because the DNA molecule has a base which is antiparallel to each other and you know in the last point discussed - so just because the orientation of the DNA has one sugar pointing from 5 prime to 3 prime on one flank and another sugar molecule pointing from 3 prime to 5 prime or another flank.

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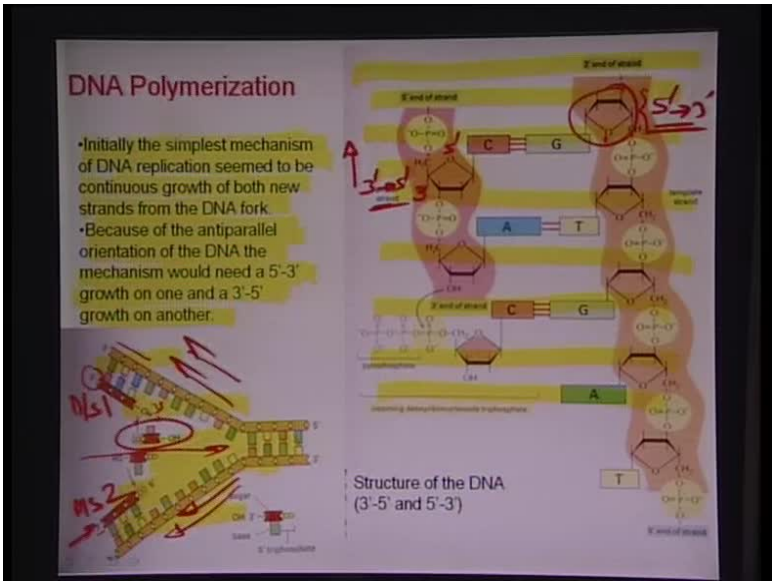


They can be said that they are antiparallel. Therefore, there really needs to be a growth for these daughter strands which are emanating out here in the antiparallel direction. Let us say - daughter strand 1, this is daughter strand 2 they are emanating and getting unzipped and getting reconstructed and the red portion is the reconstruction on the particular strand on both ends. But in that case, just for the sake of maintaining the antiparallel nature, one of the suturements should be starting from 5 prime to 3 prime which is opposite to this 3 prime to 5 prime, is antiparallel to this other flank 3 prime to 5 prime and another should be from 3 prime to 5 prime which is antiparallel to the flank 5 prime to 3 prime.

This concept needs to be a little clear; just because the two strands on the DNA on both sides or two flanks on both sides are antiparallel in nature, it automatically means that

the suturement should be from 5 prime to 3 prime on one side and from 3 prime to 5 prime on another side.

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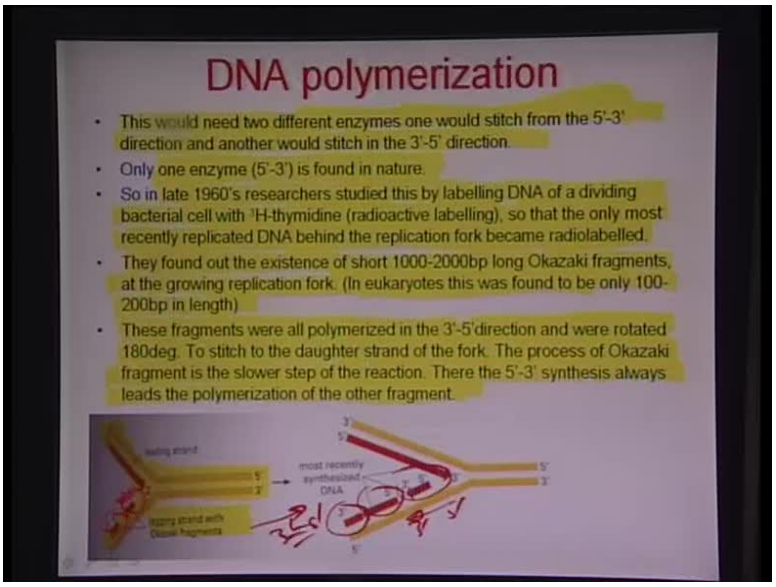


Effectively this would need 2 different enzymes, but in nature if you look at what really does the job there is only a single type of enzyme which does this job. So, then effectively there should not be any stitching in one of the directions. Here the need would be of two different enzymes one would stitch from the 5 prime to 3 prime another would start stitching from the 3 prime to 5 prime direction but only one enzyme has been isolated so far in nature which can only do the suturement from the 5 prime end of the DNA molecule to the 3 prime end of the molecule.

So therefore, there has to be a mechanism which can accommodate this very fact that the presence of only a single enzyme would also let the strands grow in a manner that is well suited and still it develops this antiparallel nature or characteristics.

In the late 1960s, researchers for the first time studied this by labeling DNA of dividing bacterial cell with this radioactive die, H-thymidine and this labeling was essentially used to ascertain what is going on especially in along the 3 prime to 5 prime direction. We already know that the enzyme that exists in nature does a suturement only from the 5 prime to 3 prime direction.

This labeling was done in a way that only the most recently labeled DNA - replicated DNA behind the replication for became suddenly radiolabelled. So, you have a fork let say and it is opening up like this and if I somehow able to label this particular DNA behind this replication fork - that means if this is the fork which is opening behind it, that means this parent DNA which is able to split up into two if I can suddenly radiolabel it.

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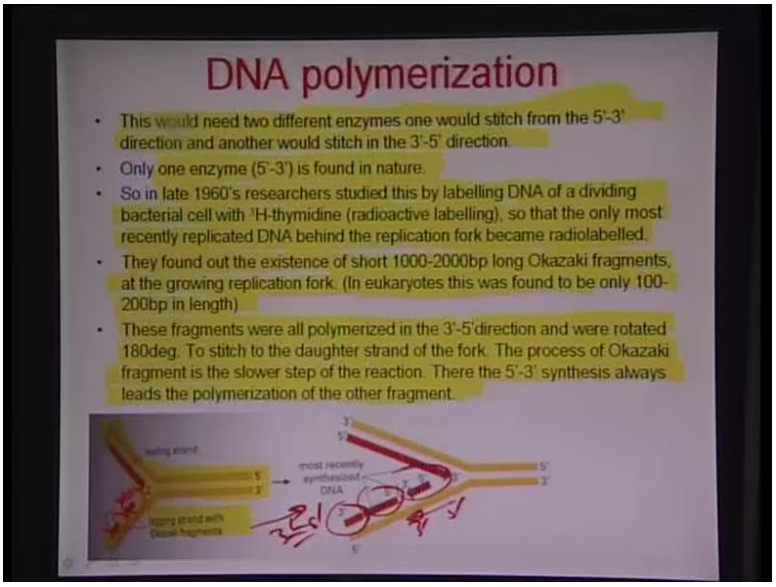


I have an idea of what is going on how this label changes from one parent DNA to the daughter DNA. From all these, we do have information or we have some very good idea about what is going on particularly in the 3 prime to 5 prime direction. Scientist found out that in the 3 prime to 5 prime direction, it is really small fragments of about 1000 to 2000 base pairs which get first separately made in the 5 prime to 3 prime direction and then suturement happens in the 5 prime to 3 prime direction of these groups or fragments not single base pair by single base pair as normally happens in the 5 to 3 prime direction.

Let me just reiterate again what I said - that by labeling techniques by labeling the DNA which is just trying to open into a replication fork,, people have tried to find out that particularly in the 3 to 5 prime direction as there is no existence of an enzyme they have found that there are these growth of short fragments about 1000 2000 base pair long which are essential sutured from the 5 to 3 prime direction and they are replicated also in the 5 to 3 prime direction, but then instead of doing a single base pair at a time what happens in the 5 to 3 prime direction normally- in the 3 prime to 5 prime plane or the 3

prime to 5 prime portion of the fork, it is the group by group suturement of this fragment by fragment suturement which happens and which can cause the 5 prime to 3 prime enzyme to replicate on a 3 prime to 5 prime fragment or strand or flank of the DNA. These fragments are known as essentially okazaki fragments on the name of the guy who found these fragments out first.

Therefore, they found out this existence of short 1000 to 2000 base pair long okazaki fragments on the growing replication fork. Eukaryotes was found to be only about 100 200 base pairs whereas in prokaryotes this would be as high as about 1000 to 2000 base pairs. These fragments would then attach in bulk as fragment by fragment rather than individual base pair by base pair. This is also illustrated in this figure here.

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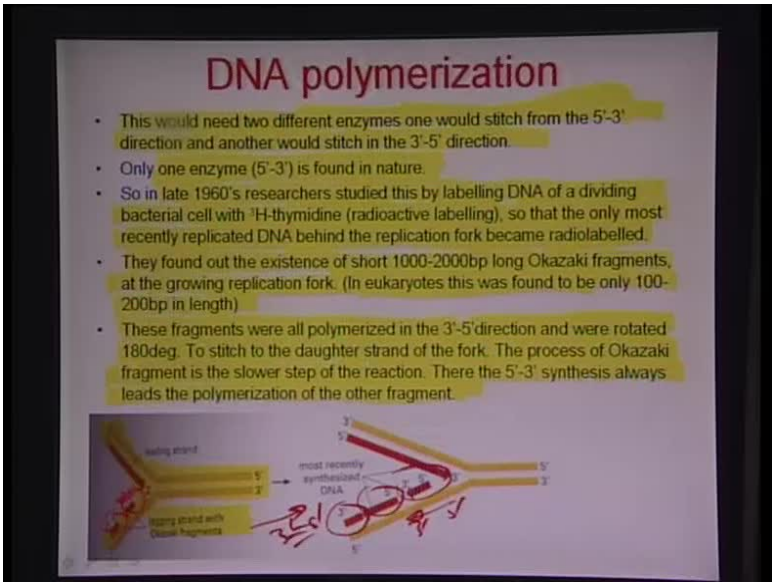


If you see this particular example, this is the fork which is radiolabelled and this the DNA which is radiolabelled and it is opening up as a fork and slowly one portion is getting replicated from the 5 to 3 prime direction which is perfectly ok, for the other portion getting replicated from the 3 to 5 prime, the replication actually happens in the reverse direction, it happens in the 5 to 3 prime direction.

However, it happens through the short segments which get generated and the segments get connected to each other rather than individual base pairs. You can see it lot more bigger here may be. You can see these segments getting created and these segments which are actually able to get sutured also in the same direction- the 5 to 3 prime

direction even though they are on a flank which needs to go 3 to 5. So, that is what okazaki fragments are.

In a nutshell, these fragments were all polymerized in 3 to 5 prime and were rotated 180 degrees and to stitch the daughter strand of the fork and the process of okazaki fragment is slower step of the reaction. So, it defines the overall reaction time as well. So, there the 5 prime to 3 prime synthesis always leads the polymerization of other fragments.

Irrespective of whatever be the case, the enzyme used is only one, which is 5 to 3 prime and in order to make this process a little quicker, mother nature has given this wonderful ways and means of forming a fragment and the stitching fragment by fragment rather than going individual base pair. This is essentially a very important aspect about the DNA polymerization.

This is an engineering course; I am not going to go into the full details of the way that this enzyme would work, but then we can look at enzymes as a suturing hand. You can look as if there is this kind of palm of mine which I fold like this and then the molecules pass through from one side of this palm and goes out in the other side and this palm actually presses two strands together and there is bonding because of that. This is the nice analogy also thermodynamically this is what happens.

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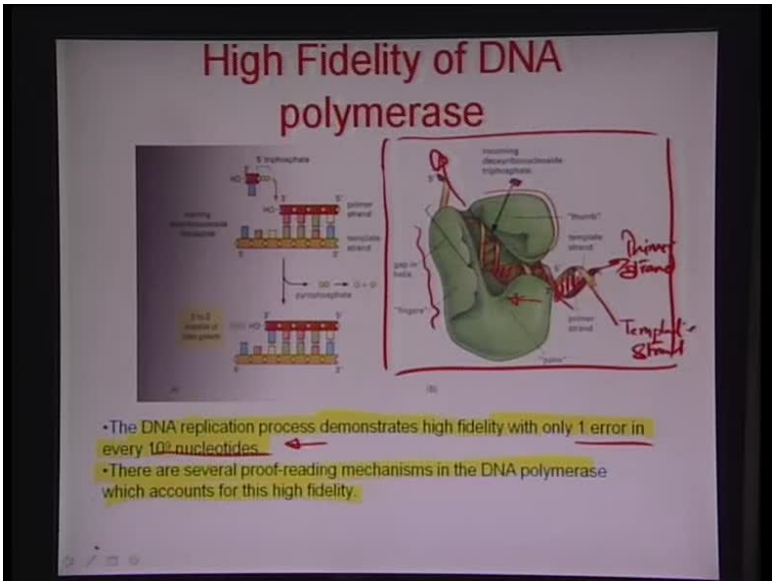

This can be a kind of thermodynamic map of what is going on in the reaction by looking at it as if the molecules are passing like a chain through this gap of a palm of a hand and we are pressing this palm from time to time so that we are suturing different molecules together. Using this model again, you can see what the hand really looks like you know this particular illustration here.

You can assume as if the template strand which is given by this yellow portion and the primer strand which is given by the red portion, they move together and one end of this palm from this end of this palm and they are moving out from this other end of the palm. You can think of as if these are really the fingers which are pressing and this is the thumb which are pressing on the molecules and developing these bonds between the base pairs.

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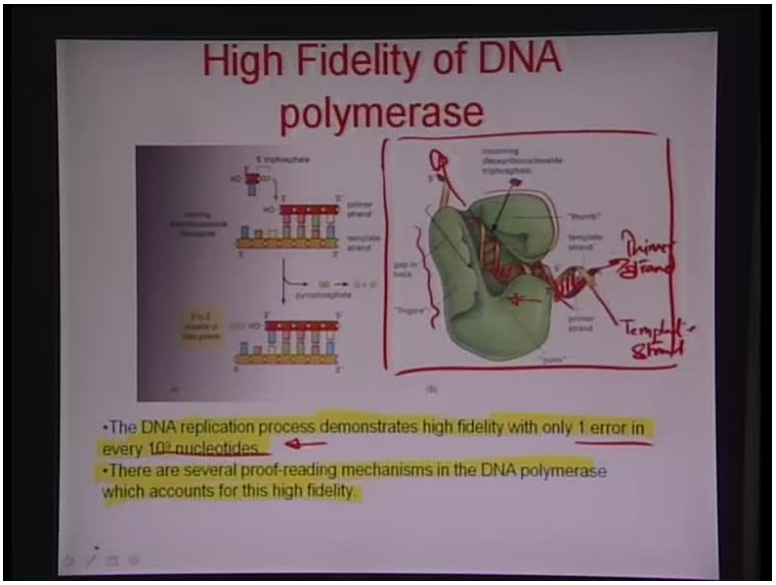


It is also hand drawn by thermodynamic considerations. This is the way the energy would be applied in order to suture the molecules to each other. There is a very good aspect of this DNA polymerase mechanism or this mechanism of suturement by the DNA polymerase that it is a very high fidelity process. There is about one error which demonstrates only in about 10 to the power 9 nucleotides,

It is fantastic because if this error rate were any different or any bigger than this, there would be severe mutations and then we would have a continuous process of changing our outwardly physiological response, behavior, shape, size, everything else with time

but however because of this high fidelity process, there is almost one error in 10 to the power 9 such suturements which this DNA polymerase can ever make and that makes the process of suturement very high fidelity, less error prone process and then there is hardly any mutation or very slow rate of mutation which happens over a considerable amount of time when subjected to a certain stimuli or conditions related to the environment.

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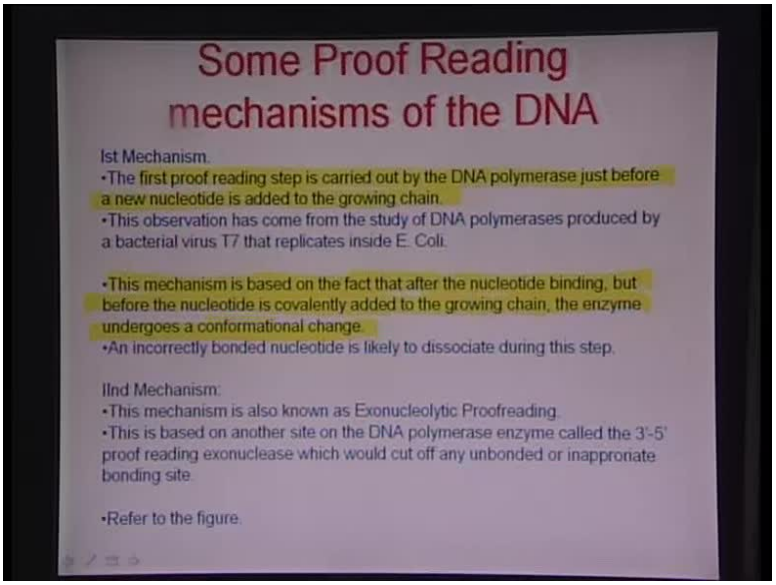


Therefore, the DNA replication in a nutshell is a very high fidelity process, moved about one error in about every 10 to the power 9 nucleotides. There are several proof reading mechanisms of the DNA polymerase which accounts for this high fidelity. Let us look at some of the mechanisms here. The first mechanism is where the first proof reading step is carried by the DNA polymerase just before a new nucleotide is added to the growing chain.

I have an illustration here; when there is a new nucleotide which is added to the growing chain there is a proof reading step, which is carried out. This mechanism is based on the fact that after the nucleotide binding but before the nucleotide is covalently added to the growing chain; the enzyme undergoes a conformational change.

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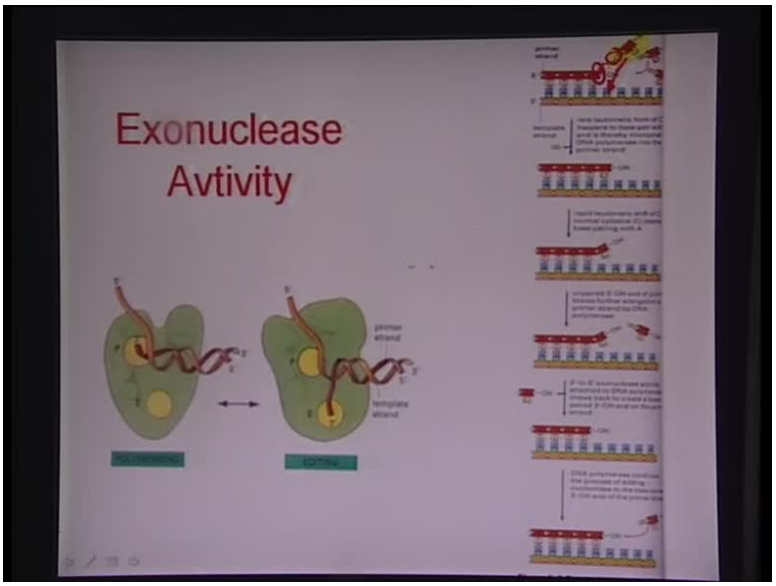


Let us say you have a moiety here, which is coming and is about to bind to the complementary strand which is there on the template and once it binds on to this complementary strand, it has to get attached on to this other portion of the sugar phosphate chain - before this happens, the enzyme if it feels there is a wrong binding by any chance, gives a jerk or a confirmation change and it leads to cleaving this molecule away. So, in the next step enzyme tries to pick up a different molecule and see that is going to work or not.

Before adding this portion of the input molecule to the chain after this base pair has been bonded to the base pair here on the template, enzyme tries to proof read and see if there is an out of shape situation. If there exists an out of situation, it suddenly changes its confirmation and tries to cleave the molecule off from the growing DNA chains. Therefore, there is no question of suturement of the phosphate part on this DNTP, the nucleotide to bond to the remaining part of the DNA chain.

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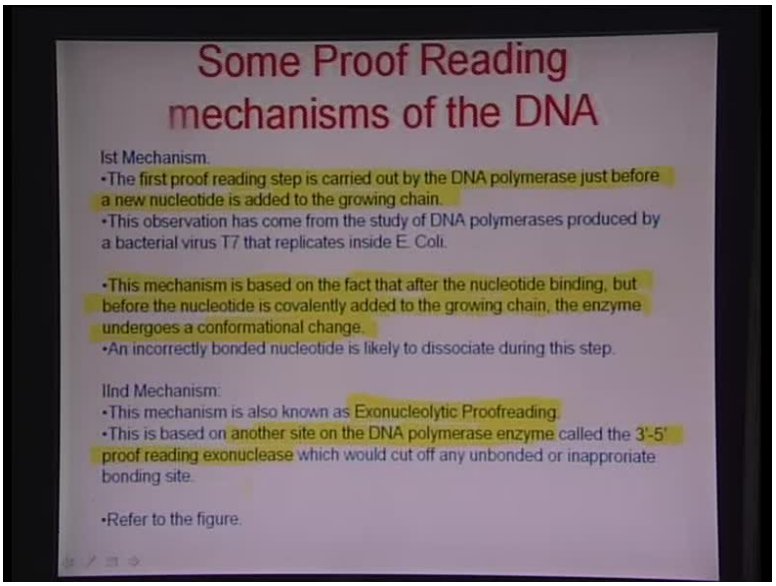


The other mechanism which is of interest is also known as the exonucleolytic proofreading mechanism and this happens because of the presence of another site DNA polymerase enzyme.

This site is also called the 3 prime to 5 prime proof reading exonuclease. It would cut off any unbonded or inappropriate bonding sites. This is very finely illustrated here and this is the site as you can see of the exonucleolytic proof reading mechanism. This is the primer strand, this is the template strand and they are all going into this hand like feature and going out. The moment here the DNA molecule of this hand sensors of this enzyme senses that there is a misfitment of two base pairs along each other, it redirects the primer strand towards the exonuclease site on the enzyme and it does not let the primer portion which is inappropriate bond to the other template portion. The template portion in this case is coming from this end and the primer portion in this case is getting sutured here.

So, the moment there is a difference in the base pair and it does not match up very well, the molecule changes direction and goes to this exonuclease site which causes the particular complementary base pair on the template strand to go unbonded. These are the two mechanisms on DNA polymerase enzyme which leads to accurately and with high fidelity produce repeatably a whole DNA chain.

Basically that is probably all about how the DNA polymerase would try to do the proofreading and suturing of the different strands to complete the whole DNA molecule.

This is of course in-vivo process and this is done inside the cell and that is a pathway to the daughter strands to split equally and go in to the two daughter cells from a parent cell which splits up.

After doing this, another interesting concept which is relevant for the diagnostics industry and that is concerned with the detection of DNA is electrophoresis. As we have already discussed before in the whole classification of electro kinetics flows, there are different kinds of flows where in one case an external EMF is applied to cause a flow; in another case, there is a pressure driven flow which causes an EMF to happen.

In the first category, the two different processes that we discussed are electrophoresis and electroosmosis. Electroosmosis we have discussed in much detail. Electrophoresis we just left with the basic definition which is that of the motion of charge molecule inside a medium and an electric field.

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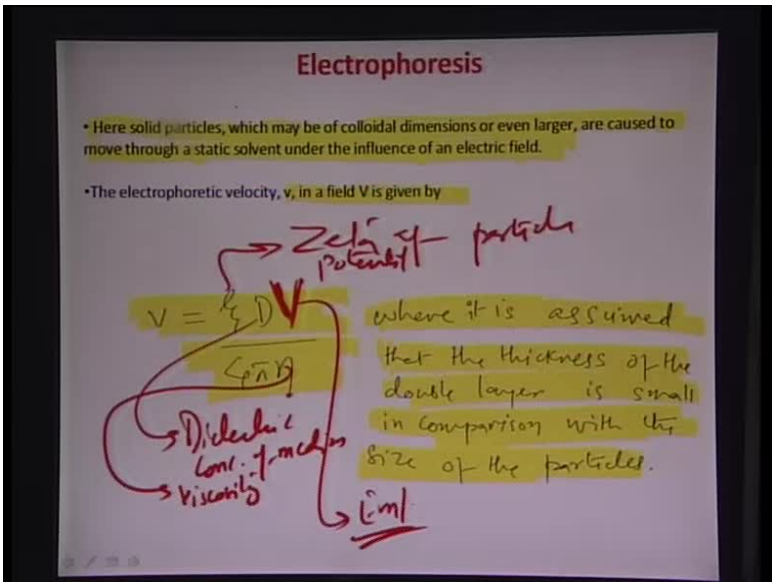


Here solid particles which may be of colloidal dimensions or even larger are caused to move through static solvent under the influence of an external electric field. The velocity of the field is given by again zeta DV by 4 pi n and where it is assumed that the thickness of the double layer is small in comparison with the size of the particle. We assume that there are no effects related to the double layer close to the surface or at least there are no tangible effects of the surface double layer. The only effect that can happen is in terms of

reduced charge and increase diameter by the counter ionic cloud for an ion that is moving towards the certain electron.

In this equation, v is the electrophoretic velocity, zeta here is the zeta potential of the particle with respect to the medium, D is the dielectric constant of the medium, these are viscosity, and this is also the applied field, this is really the applied EMF - external EMF.

Electrophoresis is done particularly for molecules like DNA and it gives a lot of utility to find out of the overall base pair numbers of size of the molecule. The way it can be done is through porous networks comparing with the size of the DNA - few 100 nanometers of pore sizes probably.

So, if you push the DNA molecule by virtue of a dragging electric field within such a sequence of force or a network of force, it is going to straighten up the molecule and if these pores are randomly oriented, these molecules would tend to move in the serpent form within those pores towards the positive electrode.

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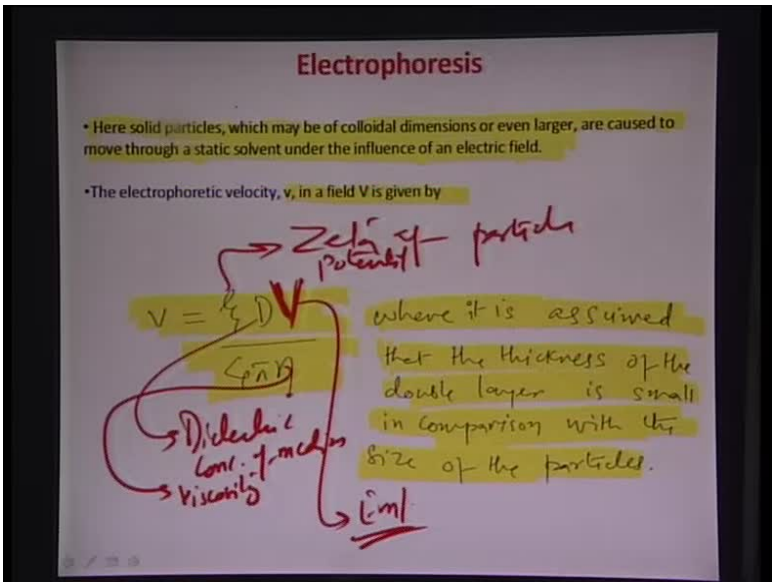


In the process, they would face a couple of different forces which would lead them to move in a particular direction. Of course, there is a force of attraction that the positive electrode would provide on the negatively charged DNA and there is force which is associated with the friction that the DNA would undergo while moving through such a set of pores or sieves. The balance between the two forces - that is the frictional forces

probably in the backward direction is the drag force, it tries to stop the DNA or hinder the DNA from moving through this porous network and the electric field driven forces are forward force, it is the driving force for moving the DNA.

So, the balance between these two determines at what rate the DNA would move or what would be the velocity of the DNA. Assuming that you have a bunch of different lengths that you want to separate and you jumbled them all together in a solution and try to use an electric field and inject this through the series of pores or sieves in the gel. It is almost always true that the shorter the strand is, the lesser is the frictional effect and it starts moving faster in comparison to the longer sizes or lengths of the DNA. Therefore, there is always a fractionation effect which comes in to picture with the longer ones moving slower and the shorter ones moving faster and after a while you can see these independently - assuming that you have intercalated some fluorescence molecules to make them visually distinctive you could see them as different bands or ladders across a DNA gel, if you can illuminate such a gel with the UV excitation frequency for the particular di-molecule.

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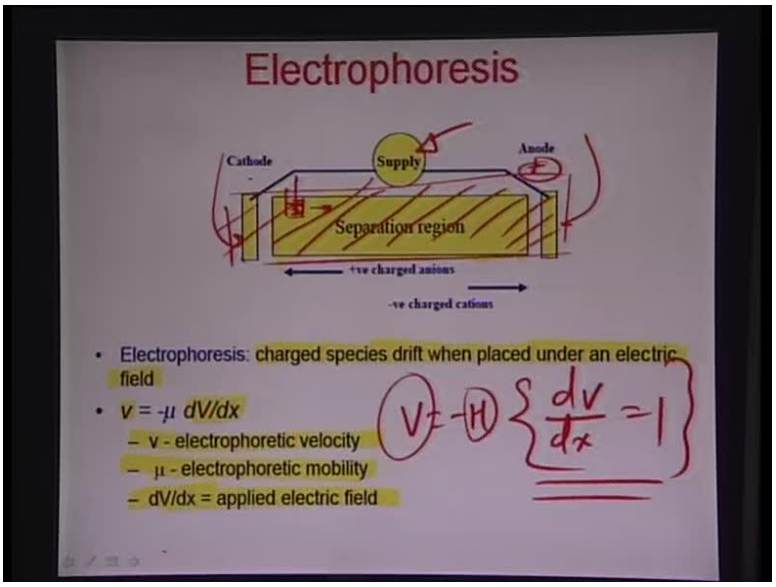


Here the following things are required - you have a separation region as you can see in this figure here- this separation region is the matrix and the sieve in the porous matrix or the porous sieve, you put the DNA somewhere inside this matrix by making a pocket and just simple poring. You put the DNA here somewhere and then you apply a field wherein

you make one side, the cathode and another, the anode, which is the positive electrode and then dip this whole configuration in a buffer solution, an ionic buffer solution.

As you apply a voltage onto this electrode through this external supply, there is a tendency of the negatively charged DNA to move through this gel network through towards the anode of the positive charge. What it does is, it gets injected into the gel material.

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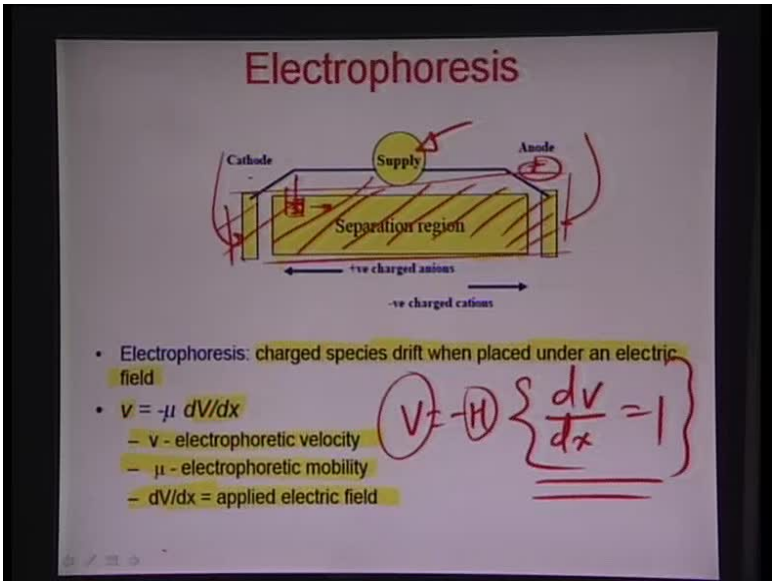


So, by definition, electrophoresis is the drift of charged species when placed under an external electric field and the velocity related to electrophoresis of the electrophoretic velocity is proportional to the applied electric field or the voltage gradient. So, dV by dx. Mu here is the constant of proportionality which is an estimation of the velocity per unit electric field applied and it is a constant of proportionality between the electrophoretic velocity and the electric field.

So, mu equals v as dV by dx equals one -its velocity per unit electric field that we are talking about in terms of the mobility. Let us try to see at single particle level what happens, identical to what we did before in terms of electroosmosis over a particular electrode where we tried to estimate the double layer the diffuse layer and then how the dynamics between the double layer -on one side it is being pulled through the externally applied perpendicular electric field on another side it is being held back by the sheer

forces of the viscous forces, how the dynamics works there - we did all these things in detail.

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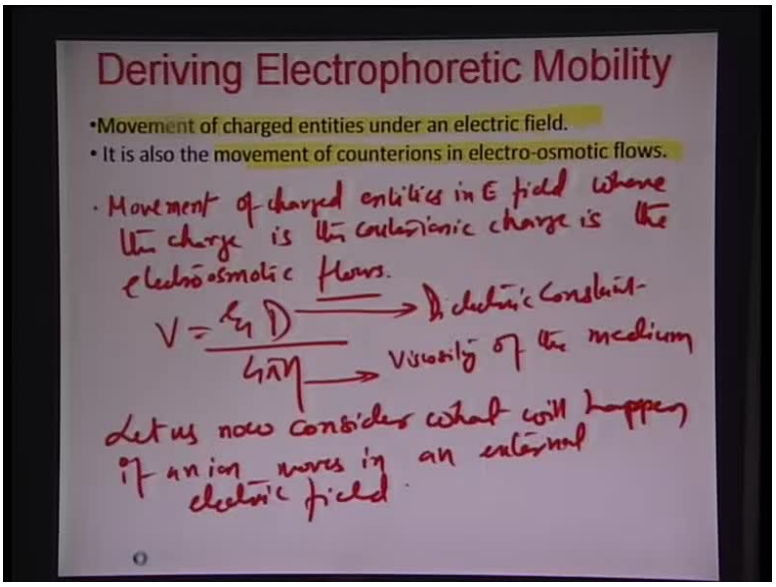


Here we would try to build an identical model for charge particle by deriving what the electrophoretic mobility of that particle will be once you take it through a medium or through some kind of an ionic fluid. Electrophoresis is the movement of charged entities under an electric field and also the movement of counter ions that is in electroosmotic flows. Couple of important things we would like to illustrate here - one is that the movement of charged entities in E field where the charge is the counter ionic charge is the electroosmatic effect. If you remember if there is a surface which is primarily having a negative charge it is a counter ion which would comprise the bulk flow in case of electroosmotic flow. Here it is just the opposite, although the velocity V would still be defined as zeta D by 4 pi eta D essentially is the dielectric constant of the medium. I am borrowing this equation from the electroosmosis case. It really does not make much of difference as far as the equations of motion for the charge goes, only the charge type varies in this particular case, eta is the viscosity of the medium.

Let us now consider what will happen if an ion moves in an externally applied electric field. The first instance that comes to our mind is Stokes's law - you have a particle which is moving inside a fluid, there is always going to be a drag force so assume the particle moves in the direction from left to right so the drag force would be in the

opposite direction. This drag force can also be found out from Stokes's law. This is the frictional force that the particle faces once it flows through a medium.

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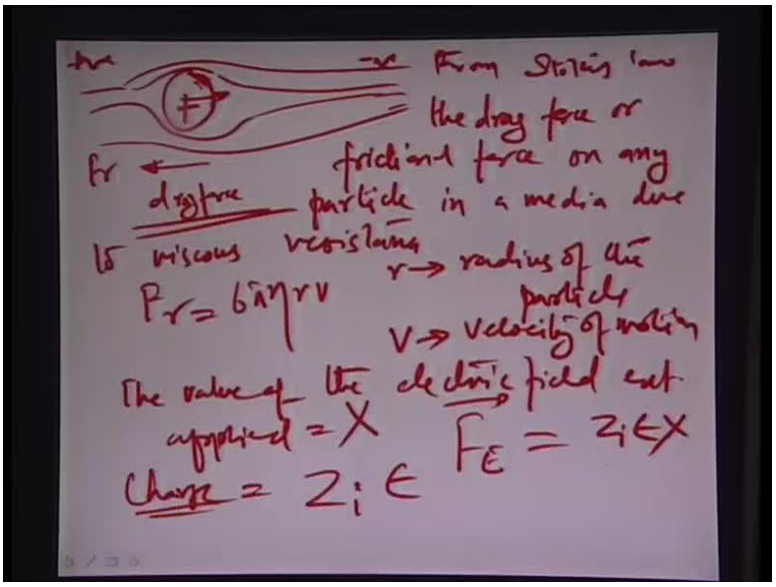


Frictional force on any particle in a media due to viscous friction or viscous resistance - the drag force F r is represented by 6 pi eta r times V, r is the radius of the particle, V is the velocity of motion. Now, let us suppose that this is a positive charge it goes towards the negative electrode here, so, we suppose that the value of the electric field externally applied in this particular instance is given by the term X. If our charge here has a valence Z i, E being the electronic charge, epsilon being the electronic charge, the total charge on the particle here which moves is about Z i E and the total force that the particle would face in the electric field is also given by Z i Epsilon X.

So the particle will only be moving in the solution if these two forces are equal causing the particle to have a uniform, a rectilinear motion without any acceleration or forces. Of course, there are going to be buoyancy driven forces, sedimentation forces, and gravity driven forces which happen to the particles. Assuming that the particle size is small and we are talking about a bunch of different ions, we hardly get any of these effects. We can be safe as far as the gravity effects of the particles.

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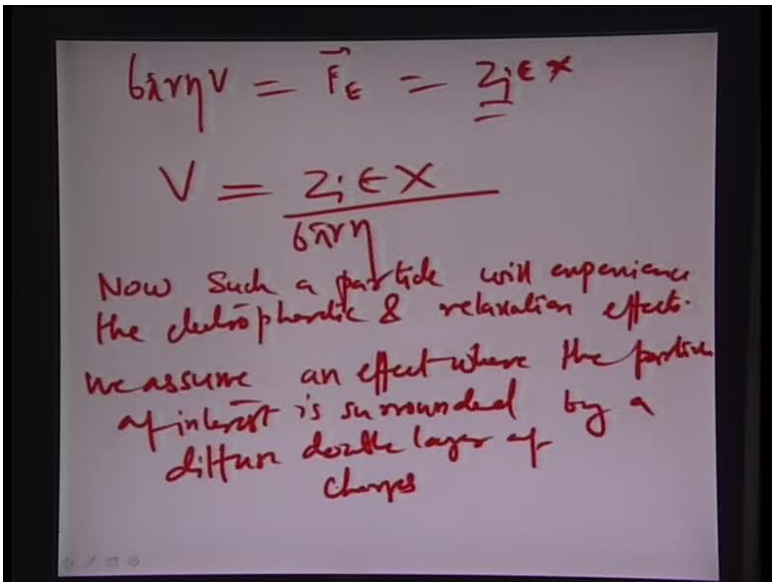


But then, the very fact is that the drag force only if equalized to 6 pi r eta V is equalized to this other electrical force F E, would create a situation where the particle would move through the solution on applying the electric field. So, 6 pi r eta v, the drag force is also equal to the electric force which is Z i E X. X is the external electric field epsilon is the charge on one electron and Z i is essentially the valency of the ion that we are considering in this particular example. V here, the velocity of the particle in the medium would be given by the term Z i E X by 6 pi r eta.

This is a just plain case of the particle does not face any other restrictions from the counter ionic cloud that it is in. However it is of utmost important to mention here the particle is not alone; there is a tendency of this particle to drag bunch of different counter ions and the solution around it and the effective charge of the particle is shielded because of the counter ion cloud or the ion density which is around it. Number two, another very important point is that the overall diameter of the particle also increases notionally because of the availability of this counter ionic charge.

So, if we involve the charge differential and also the diameter differential of this particle core with an ion cloud surrounding going through or being dragged through the solution by the external field, the equations of the motion will slightly change. Let us look at some of the aspects if we consider this particular relaxation effect. We call this particular effect of counter ionic cloud coming in surrounding the particle of interest, the

electrophoretic relaxation or the relaxation effect. Such a particle will experience the electrophoretic and relaxation effects. We assume an effect where the particle of interest is surrounded by a diffuse double layer of charges.

Now, this diffuse layer thickness is a function of the concentration of the ions in the solution. This will go up as the concentration is more. So, higher concentration will typically have more number of charges on the counter ion cloud. We will stop here and try to look into the whole derivational aspect of this relaxation effect on electrophoretic in our next lecture. Thank you!