Analytical Technologies in Biotechnology
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Module - 4
Electrophoresis
Lecture - 6
Immunoelectrophoresis and capillary electrophoresis

In previous lectures, we have discussed about basic principles of electrophoresis and various techniques concerning the electrophoresis of proteins and nucleic acids. Today in this lecture, we are going to discuss about two more techniques.

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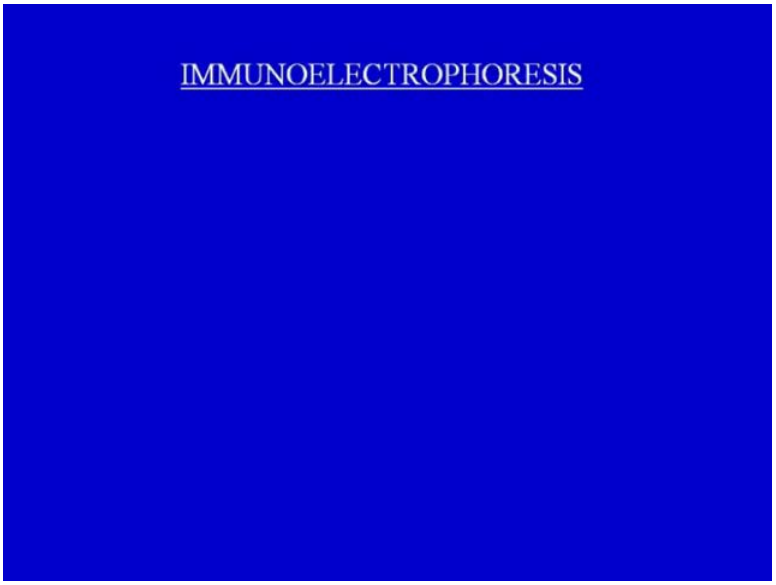

One is immunoelectrophoresis, and another is the capillary electrophoresis.

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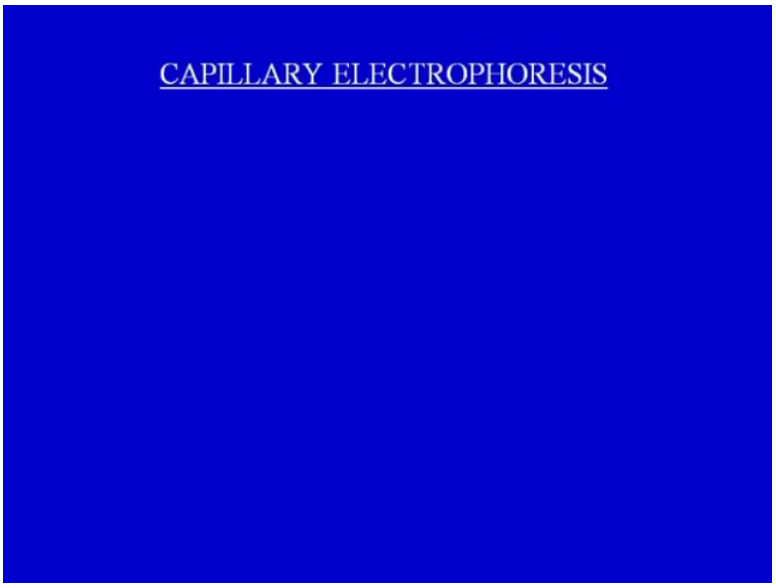

So, we will discuss immunoelectrophoresis first. Now, immunoelectrophoresis is a technique for studying antibody and antigen particularly, their interactions where specificity of immunoprecipitant reactions are combined with, separations of molecules by electrophoresis. So, this technique allows you to see the interaction or to identify the antigen, if the antibodies available. Now, before we go to the technique of immunoelectrophoresis, certain basic things needs to be understood like, we were talking about immunoprecipitant reactions.

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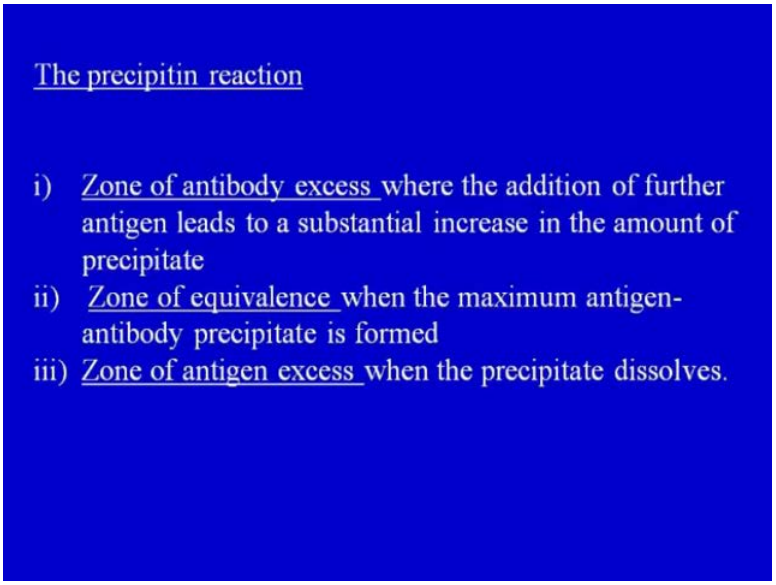

Now, what are these precipitant reactions so let us understand this. Now, precipitant reactions are supposing if increasing amount of an antigen say, human serum albumen or some other protein, is mixed with a suitable fixed amount of antibody solution. Say, rabbit anti human serum albumen or for that matter against any particular antigen, what will happen initially amount of the antibody antigen precipitate increases. As you increase the antigen concentration now, as they, you are adding what is happening is, one thing has to be understood is, many times there are two sides on the same antigen, where the antibody can bind.

It means you have more than one antigenic determinant so for example, there are lots of repetitions or certain proteins or membrane proteins or receptors, which are present. They are specific which are present which can act as determinants so when antigen interacts with antibody, antibody can bind to two antigen molecules. And what will happen is, if there is certain concentration antibody antigen at particularly, at equivalence then they form a large lactase sort of structure, which leads to visible precipitation. It means that the structure becomes so large that, you can see it as a precipitate. I will show you that and that precipitate is called precipitant.

So, in precipitant reaction at certain point, when you add antigen to a suitable amount of antibody or a fixed amount of antibody, at the certain point, when concentration of antigen and antibody reach equivalence or equal. Then there is lot of interactions and large lactase structure is forms, which leads to the visible precipitate. Now, when you are adding, I will show you this in a little by while so as you add antigen, as I said lot of precipitate is formed and the visible precipitate, which is seen is formed. But what happens is, as you keep on adding the antigen, a sharp plateau indicating that all of the antibody had been precipitated, is not obtained here.

And rather precipitate apparently dissolves, at a higher concentration of antigen and this is due to the solubility of antigen antibody complex, containing a single antigen molecule. Even if the antibody molecule is bound to every antibody determinants, the result in precipitant curve will contain so what is happening here is, that at excess antigen the complex, which is visible precipitate is will dissolve. So, if you see the dynamics of this or precipitant curve as we can call it, it contains three zones actually.

One is zone of antibody excess, where the addition of further antigen leads to a substantial increase in the amount of precipitate, as you start adding the antigen from a small quantity then there will be antibody access. Second part of the curve is zone of equivalence where, when the maximum antibody precipitate is formed. And third part of the curve is the antigen access where, when you add more antigen, that is after equivalence the precipitate dissolves actually.

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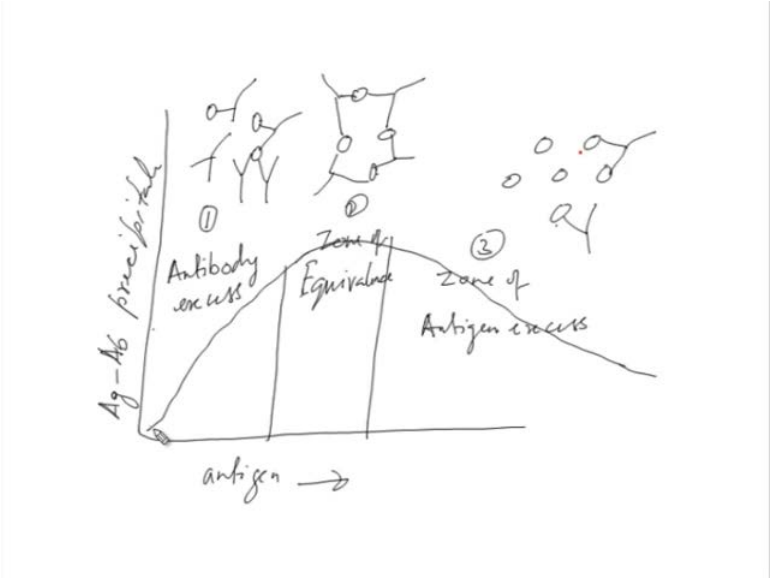

Let me show you this whole thing on the screen, what you have is if, I show you the precipitant curve, precipitant curve will look something like this. You have antigen on this axis and you have antigen antibody Ag for antigen, Ab for antibody precipitate or you can say, precipitate which is kind off seen, as you add the antigen. So, what you see is the, curve kind off looks like some sort of this kind of pattern, where we have certain regions actually. And this region, if we call them this one, that is the first one is the zone of antibody access. This is antibody access, this is zone of equivalence and here when you have added sufficient quantity of antigen, then it is zone of antigen access.

So, what does that means actually this means like, if you see in first set you will have lots of antibodies, which are around and they will be certain interactions, but and as you are increasing, there will be antibody access. But as you increase the antigen some precipitate will be seen on increasing concentration of, antigen reasonable precipitate will be more. Now, zone of equivalence large lattice structures are formed like I said, if

we have lot of these equivalent integer molecules just like, you will have lot of these kinds of structures.

Which kind of are in a long range and they will be formed in a very long range and they will be like a visible precipitate, which could be centrifuged and measured also, could be obtained. In zone of antigen access, you will have lot of antigens as compared to number of antibody molecules and there could be like lot of antigen ridges, because you have fixed amount of antibody. So, this is a curve or we call it precipitant curve now, how do you see this curve as you go along, there are lots of different methods to see this curve. And there is one process, through which see the antigen antibody interaction is immunodiffusion.

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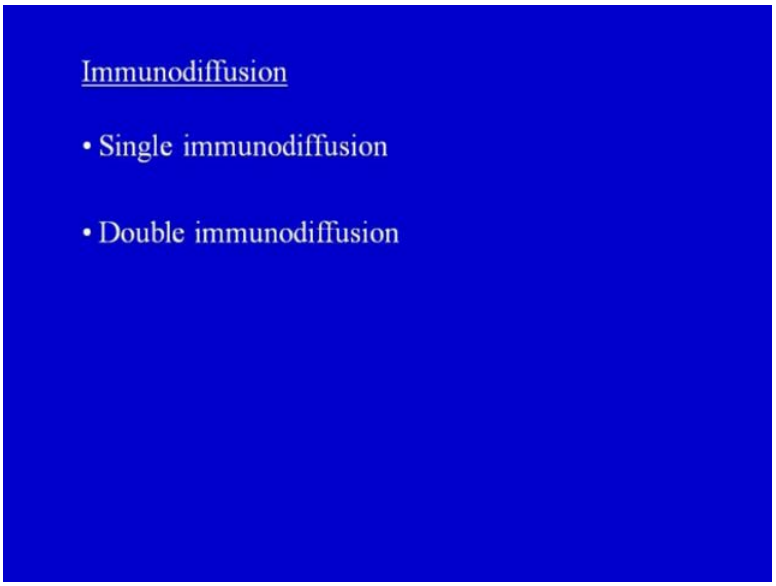

So, there could be different forms of immunodiffusion which could be single immunodiffusion and it could be double immunodiffusion. Single immunodiffusion means that, only antigen is moving and antibody is immobilized and it could be in one dimension or it could be in two dimensions. In double immunodiffusion both antigen and antibody moves towards each other and interacts to form the precipitant lines. And that could also be in one direction or in two dimensions.

So, let us see that the phenomenon of immunodiffusion, that we show you on your screens actually. So, the immunodiffusion one is like I was saying, it could be simple immunodiffusion. Single immunodiffusion, where only one of the reactant that is the

antigen moves towards antibody, it could be like I said, it could be one dimension or it could be in two dimension.

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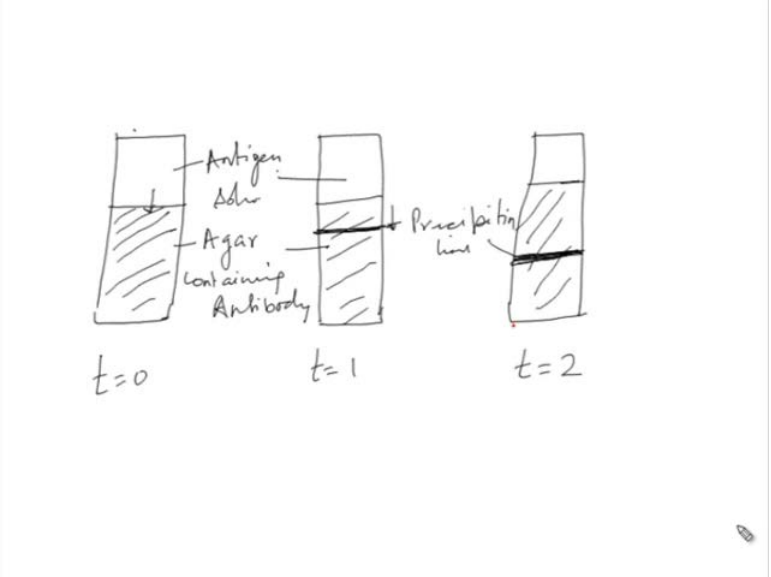

So, say in one dimension if you are saying, if this is particular now, if this is your agar containing, this is your agar containing agar antibody. And this is antigen solution kept in here. Now, antigen solution is kept on top and this is agar, which contains antibody, particular antibody for that antigen at time t0. Now, these things takes immunodiffusion, takes lot of time like days together and so we will not be talking about that, but what we are talking about is simply the phenomenon. So, what happens is, what is the second step so it is the same thing now, this antigen solution and this is agar containing antibody.

Now, what will happen is slowly the antigen will start moving into the agar, which contains antibody. Now, the precipitant line or the curve which I showed you, as the antigen enters antibody access. So, what will happen? As the antigen interacts with the antibody there be a minor precipitate is formed and there will be thin precipitant line could be seen. Now, as antigen enters, more antigen enters into the gel or agar then slowly the antigen concentration increases and then from antibody access, you will find antigen antibody equivalence, at certain point. So, what will happen first we will see a precipitin line, as a visible line because it will be very thin in the beginning.

So, we will see a precipitin line and this precipitin line is seen as antigen concentration increases. Now, what happens is the precipitin line moves in this direction actually, the

precipitin line moves in down here, because as the antigen comes in, the antigen access occurs. And at certain point, the precipitin or the antigen antibody complex dissolves. So, antigen, but antigen concentration ahead is little less, what happens is essentially, the precipitin lines starts from here. But keeps on moving downwards so what you will get is finally, at certain period of time you will see that, the anti precipitin line has moved quite far, depending on the antigen concentration.

Because after sometime, antigen will be done and so precipitin line will move to a certain extent. So, you can see this at say time 1, at three different times say, this is in days actually. You will see that, the precipitin line is formed and then it moves down and at certain point it stabilizes. And that depends on the concentration of the antigen. So, that is how a precipitin lines can be formed and can be seen in single dimension. Now, the, this simple immunodiffusion can be in two dimension also.

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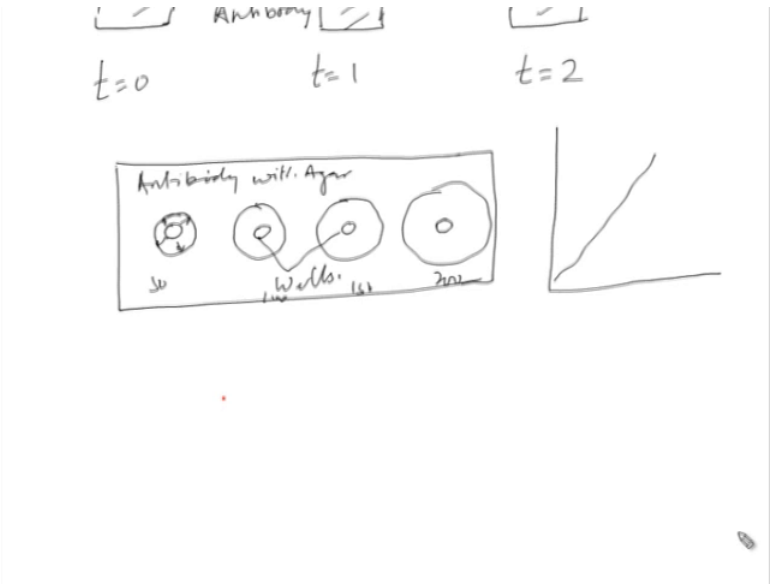

Now, how does it happen in two dimensions now, what is done is in two dimensions this is done on a simple microscopic slide actually. Now, here what you do is, you punch different you can say holes or slots for loading the antigen solution. So, these are the wells which are being cut, these are wells actually and these wells have been cut in here for putting in the antigen solution. Now, here different concentration say 50, 100, 150, 200 increasing concentration of antigen is loaded in here, just to see the effect here. So,

what we will see is that, there will be radial diffusion in all directions, in all the wells once we have loaded the sample.

So, it will, but it is simple diffusion since only antigen is moving and this agar, this antibody is contained in agar actually. So, antibody with agar is put in here now, when the movement occurs then what will happen precipitin lines will be formed in here. Now, as the concentration increases so is the size of the or the diameter of the precipitin lines will be. So, what you have seen at 50 micro gram say for example, just for example, there is a precipitant line not farer from the centered here. And as the concentration increases, the precipitin line also moves so this clearly shows that, more is the antigen concentration farther, the precipitin line will be formed.

As we have seen here also, simple first simple immune diffusion also in one dimension. So, what you can do is in here, you can always make a standard curve here and this standard curve can be used for knowing the concentration of unknown sample. So, this is simple immunodiffusion and you can get precipitant lines, which can be visualized. So, this was simple or single immunodiffusion now, same thing can happen with double immunodiffusion. Now, let us see how double immunodiffusion will occur, in double immunodiffusion what you are going to see is that, rather than like simple this antigen moving, both things will move.

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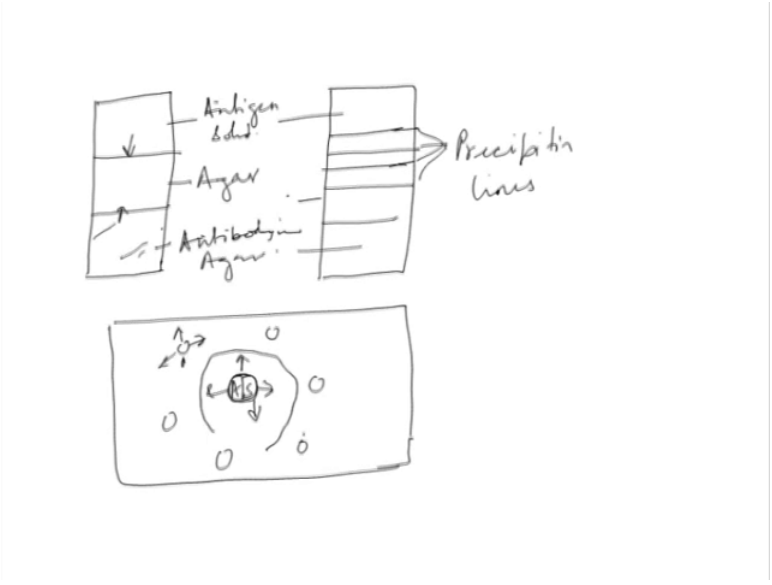

So, what we have if it is a single dimension, in a single dimension what they will have is, there will be antigen solution and then there will be agar, which does not contain anything. And there will be agar containing anti body so anti body in agar so here what will happen here, this is not immobilized anti body, the anti body in here is not immobilized. So, what will happen antibody will move towards this side and antigen will move in toward the antibody and antibody will move towards the antigen. So, what is going to happen now that, as it as moves, as both things move towards each other, you will get and as we have seen you will get lot of precipitin lines, depending on.

So, these are what you call precipitin lines now, you will see only one precipitin line if there is only single and one antiserum for that. You will see multiple precipitant lines, if it is a complex mixture of antigens and corresponding antibodies are available in that antiserum. So, this is double immune diffusion, that is in single dimension now, this could also be in like I said two dimensions, in two dimensions what is done, it is a very simple way like there will be agar on this microscopic slide. You can punch a centre hole, where you can put anti serum and you can have slots for placing, you can have slots for placing antigen.

And these antigens could be in different concentration now, depending on the concentration like I said, more is the concentration farther will be the precipitin lines. Accordingly you will get a pattern, which will be seen in here that is precipitin lines will be seen as per the concentration. And this is double immunodiffusion, here antigen like these are moving in all directions likewise, antibodies also moving in all directions.

And at certain points they will be in equivalence and they will form a visible precipitin lines. So, that the double immunodiffusion now, there is another important part of double immunodiffusion is that, how does they look like, there are reaction of identity, partial identity or nonidentity are very important. For example, if you have same antigenic determinants, what happens if there are partial resemblances in antigenic determinants or there are no resemblances in antigenic determinant.

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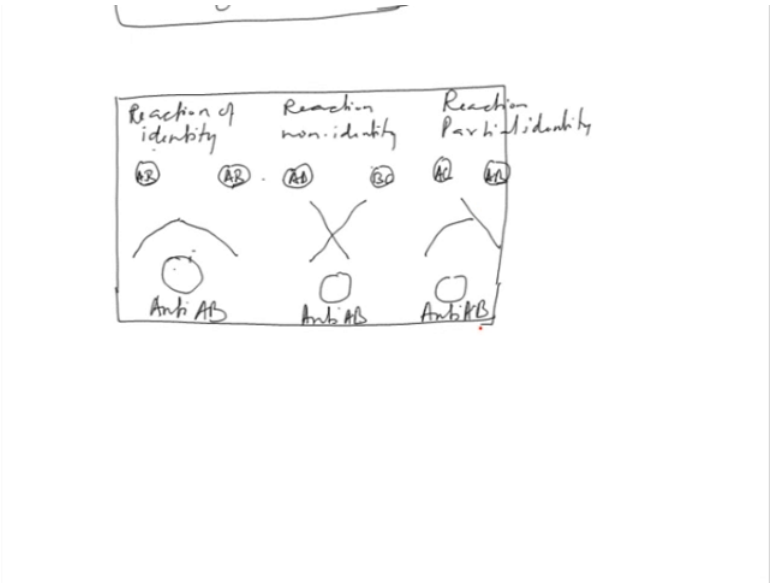

So, let us see what is that now, everything is same like you have a plate, microscopic plate you have agar, containing things. So, what is done is to, see this particular phenomena that is the reaction of identity, one is that you have like I said, like here it is antigen. And there it is antibody, that is anti Ab now, see here both have same antigenic determinant Ab, Ab. So, what will happen this is reaction of identity and how does this particular precipitin line will look like, that is what we are interested in. So, precipitin lines this case will kind of merge actually, that is the precipitin line of interaction between these two and precipitin line of the interaction between the, both wells, they will merge with each other.

And this is called the reaction of identity, where you have identical antigenic determinants, second case could be where you have nonidentity. That is both antigenic determinants are so second one could be reaction of nonidentity. Now, in this case like I said, there could be different antigenic determinants like I say, these are two antigenic determinants, you have only anti Ab. So, what you have is you do not have common antigenic determinant so what will be the resultant, you will find a kind of a pattern which crosses each other, they are not identical.

And third case could be partial identity, the reaction of partial identity, in partial identity at least there is one common antigenic determinant like say, if I say this is Ac and Ab. And you have anti Ab, same antibody or anti sera then what will happen since there is

only partial identity, it will look something this sort of pattern will be seen. That is, in case of at least one antigenic determinant is common so this is how this will look like. There is another important or interesting aspect of immunodiffusion in over here and that is, you can determine or you can have the idea of the molecular weight of a particular antigen. That is the effect of the molecular weight of the precipitin lines actually so that is also very interesting to see.

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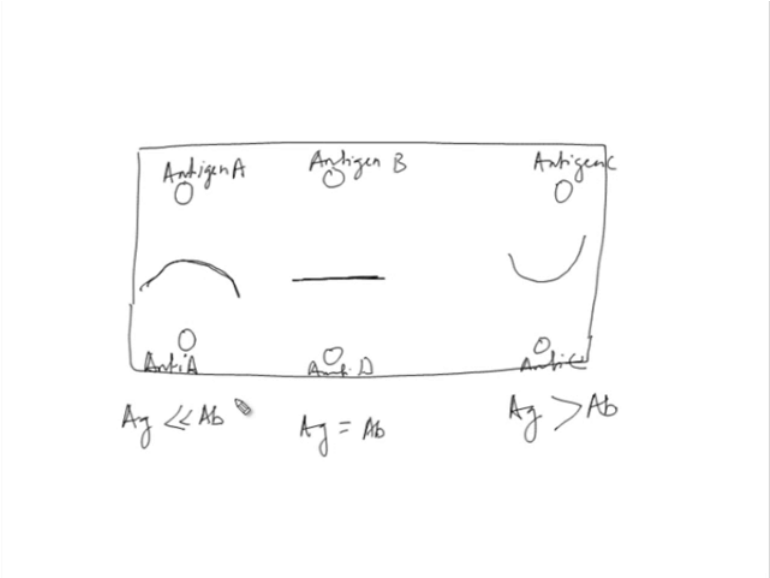

So, we can, let me show you this here. So, again this is a microscopic slide where agar is there and you have punched two holes. So, let us see first in the case of, this is antigen say, this is antigen A and this is anti A, that is antibody A or antiserum you can say, against antigen A. Now, to just like it will not give you say, what is the molecular weight, but it will give you an idea about molecular weight, in terms of whether it is bigger than antibody or it is smaller than antibody. In case of wherein, antigen has smaller molecular weight as compared to antibody then what you get, you get a pattern something like this.

That is you have like a pattern where, precipitin line will be towards like, it will curve towards the antibody. There could be another one that is say, antigen B and there is anti B, these are the slots we have in the agar plate, this is agar plate. It facilitates the fusion because its large pore size is there, it is not a separation as such. Now say, antigen equals antibody, molecular weight equals then you will see a straight line here rather than, a

curved line. If you have, I think you must have guessed the third scenario say, there is an antigen C and anti C, the third scenario would be that if say antigen is bigger than or antigen, molecular weight is more than the antibody.

Then, there should be a curve, which is different or opposite to the curve which, where antigen molecular weight was lower. So, these kinds of patterns will be seen and could be given idea on, in this particular experiment of immunodiffusion. So, let us go back to our discussion so we were discussing about immunodiffusion and precipitin lines. Now, let us get back to the immunoelectrophoresis so electro immunoelectrophoresis is the extension of what we are discussing.

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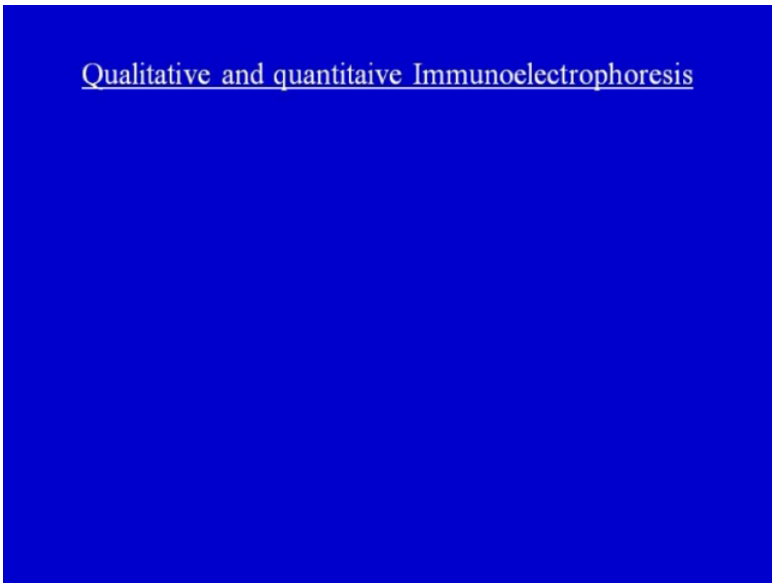

It could be two kinds, qualitative and quantitative immunoelectrophoresis. Now, first step in immunoelectrophoresis is to, separate the antigen sample into their component part by, electrophoresis. So, that is why it is immunoelectrophoresis because you are performing the electrophoresis to, separate the mixture of antigens, on agarose gel. Second step, it is similar to immunodiffusion what is done is, the separated components are ((Refer Time: 25:22)) with antibodies in the gels and the specificity of antibodies facilitates the, identification of antigen in given sample.

So, what is done is a very thin say, 1 to 2 meters agarose gel it could be say around 1 percent, gel is cast on a glass plate or microscopic slide. And antigen samples are placed in small circular wells, as I have shown you, which could be 2 to 4 millimeter in

diameter, it is punched in the gel. Now, gel is placed between the electrode chamber in horizontal electrophoresis set up.

Now, mostly it is anodic migration, that is from negative to positive electrode now, gel is in contact with electrophoresis buffer through, buffer saturated filter paper wicks. A suitable immunoelectrophoresis buffer, which can be like 0.08 molar trigs, 0.02 molar tristine, 0.3 milli molar calcium lactate ph 8.6 or there could be other buffers will be utilized. And the electric field is applied in the order of say, 5 volt per centimeter now, what is done it is like, simple electrophoresis.

After electrophoresis is complete then antigen mixtures will be separated as per their charge and then anti sera will be placed in longitudinal troughs, which are 1 to 2 millimeter wide, I will show you this, which are again cut into the same gel and parallel to the migration path and one trough is cut on the each side of the sample lane. Like if you have two sample lanes then you can cut two, if it is one then you can cut one trough. And different anti sera will be placed in there, like if there are two lanes or three lanes, you have to have anti sera for each. Now, gels are then incubated over night at room temperature in humid chamber, remember all these experiments of immunodiffusion are done in humid chamber.

The antigens diffuse readily and antibodies will diffuse laterally, because they are in longitudinal troughs towards each other and resulting into antigen antibody interaction and precipitin arcs. Where, antigen and antibody have appropriate concentrations, that is equivalence actually. Now, the precipitin arcs can be visualized directly or they could be seen by staining like say, presence of precipitin arc is evidence for both antigen in the sample and the antibody in the antiserum. So, this is how simple qualitative immunoelectrophoresis will be performed. So, let me give you an idea about how the whole procedure is being done, but as we have already done this in earlier just to give you an idea.

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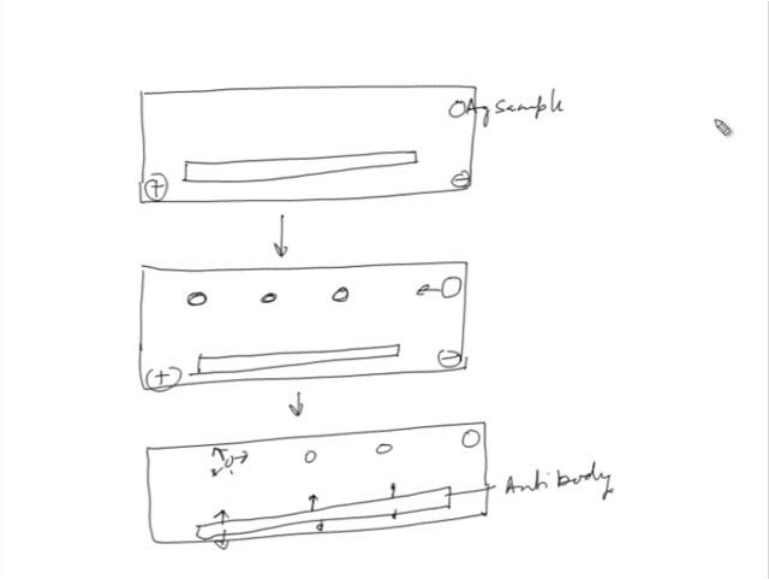

So, what you have is a simple microscopic glass slide and this glass slide contains, if I say particularly, what you have is, and you have say particular antigen sample, particular antigen mixture. Now there will be longitudinal trough here, which is cut in the gel only. So, what will be the next step, the next step will be the electrophoresis, in electrophoresis what will happen, you have. So, your sample will run remember since, it is from cathode to anode, those samples which carry a negative charge, will run towards this side. So, that has to be, experiments have to be planned accordingly, so when it runs what will happen, there will be migration.

And you will find as it runs here, you will find different bands in here, that is separated antigen from components from the mixture. Now, what so once you have run the electrophoresis, as we have discussed, the next step would be to fill this next step would be so what you have is, you have these components from the antigen mixture. And you have, what you will fill in antisera for that particular antibody solution or that particular antigen. And this contains antibodies from each of these samples or for one sample like say, you are looking for identify certain sample then it might contain anti body for that. So, what will happen now, there will be lateral diffusion like I said from the well and they will be radial diffusion from these in all directions.

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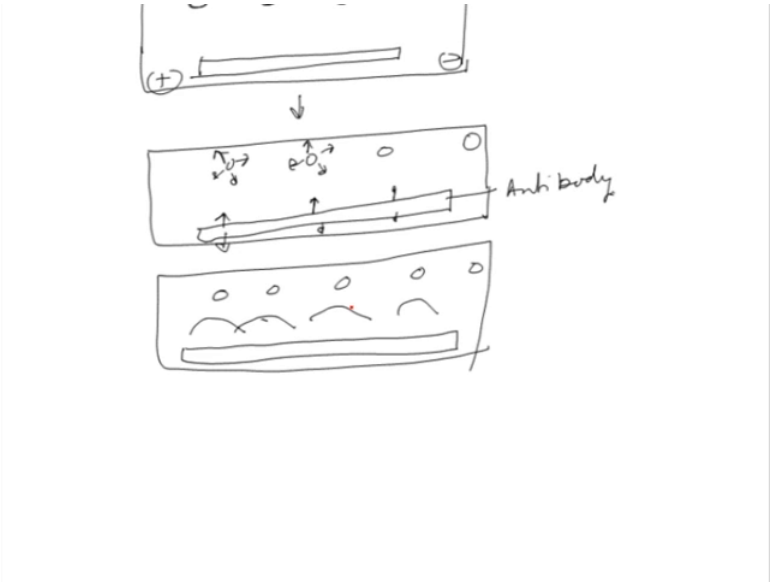

So, what resultant would be, you will find sometime like say after incubating overnight you will find them. So, what you have is, you have these kinds of patterns or you have antisera here, what you will find is you will find the precipitin lines for these samples actually, after incubating overnight. So, you will be able to, if it is to identification then you will be able to identify because you will find only precipitin line for that particular antigen sample, you are looking for. This is a very simple qualitative immunoelectrophoresis. So, that is how you can perform immunoelectrophoresis and could identify or you could analyze the particular antigen.

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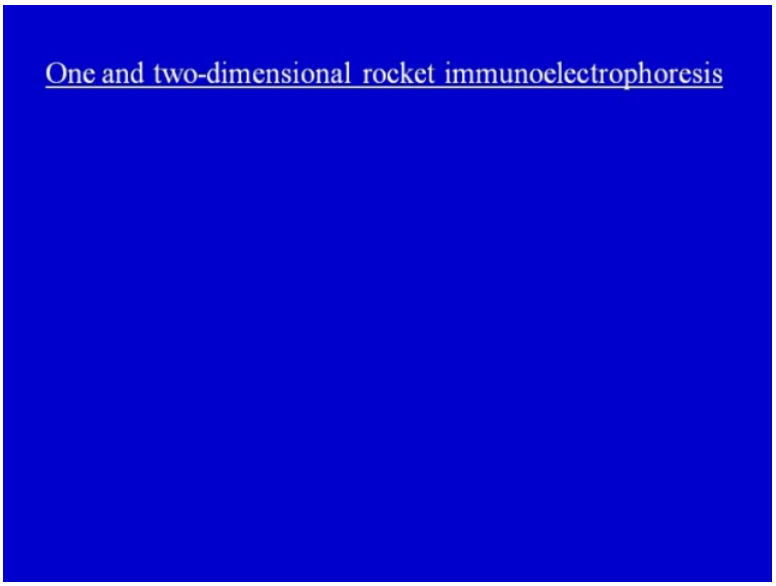

There could be quantitative immunoelectrophoresis also 1 and 2 dimensional rocket immunoelectrophoresis. Now, the two major variations of this is, one and two dimensional rocket electrophoresis or immunoelectrophoresis. And the name rocket is due to the shape precipitin patterns formed, following antigen antibody reaction. Now, height of the precipitin peaks are roughly proportional to the concentration of antigen in the sample. And therefore, these methods can be triggered as quantitative immunoelectrophoresis, in rocket immunoelectrophoresis antigens are subjected to electrophoresis, as we have shown you in agarose gel containing, suitable antibodies.

And the ph of the electrophoresis buffer is chosen so that, it is near to the pi of the antibody molecules so that, antibody remains immobilized in the agar or during the electrophoresis. Now, for one dimension rocket immunoelectrophoresis, antigen samples are loaded in the well, punched in the anti-body containing gels, before starting the electrophoresis. And what you will get is, you will get rocket shaped precipitin peaks where, antigen antibody meet at equivalence. Now, the concentration of specific antigens can be determined by comparing, rocket height of test samples with those formed by known standards.

In two dimensional rocket immunoelectrophoresis, what is done is first step is to perform regular agarose gel electrophoresis to separate the antigen in the sample. And in the next step, the lane containing the separated antigens will be cut like we have discussed in two dimensional gel electrophoresis. And will be fused to agarose gel containing, embedded antibody. Second electrophoresis will be run at 90 degrees to the first one and then again you will get the same patterns as the antigen migrates that is, rocket shaped pattern. So, the precipitin arcs are formed along the equivalence regions of the antibody antigen and number or multi simplicity of precipitin arcs formed will depend on the complexity of the antigen sample and the anti serum in the second gel. Let me show you this on your screen.

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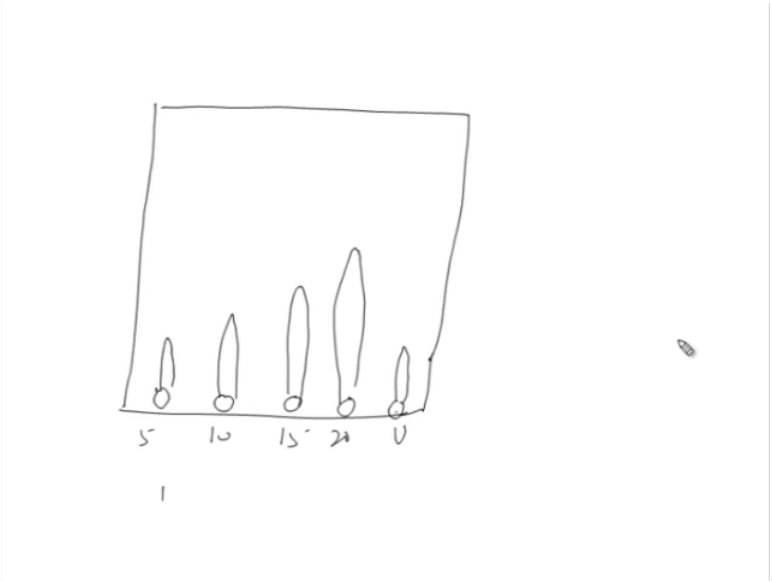

So, two ways this could be done is, one is you have agar containing gel, antibody containing agar or antibody in agar and this is, antibodies are all over they are, when you are casting the gel, you put the antibody. And what you do is you punch holes in here or slots for loading your sample and unknown. And as you run the electrophoresis in one dimension then you will, what you will get is say, we have loaded the unknown in a particular concentration say, 5, 10, 15, 20 or so.

What you will get accordingly, the antigen concentrations will get rocket shaped pattern and like in chromatography, this particular heights of the peak will be the concentration idea. And so unknown could be found out, if unknown is this height then you can through calibration curve, you can know the unknown. So, this is called rocket electrophoresis because of this pattern.

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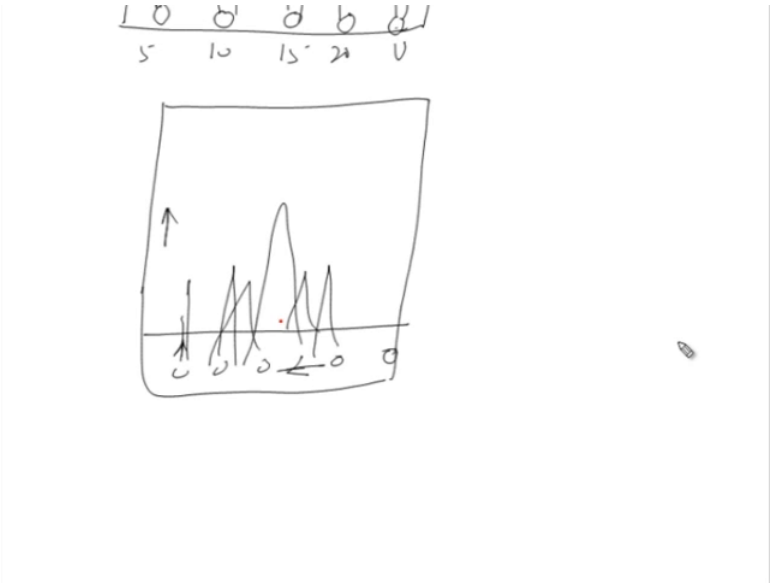

Now, in two dimensional rocket electrophoresis what is done is, that first it has been done in this direction electrophoresis, that is you separated these components here, rather than punching hole. And this whole thing has been fused into this next gel and which contains the antibody. Now, as they run in this direction now, direction of one will be this one, that is at 90 degrees and you will get the patterns which will be like I said, it could be different patterns.

Because of, there will be lot of mixture, as complex is the mixture, the pattern will be that complex. So, two dimensional rocket electro immunoelectrophoresis could be performed or it could be, one dimensional immunoelectrophoresis. Both ways it could be performed, so once precipitin lines formed in immunoelectrophoresis, they should be visualized.

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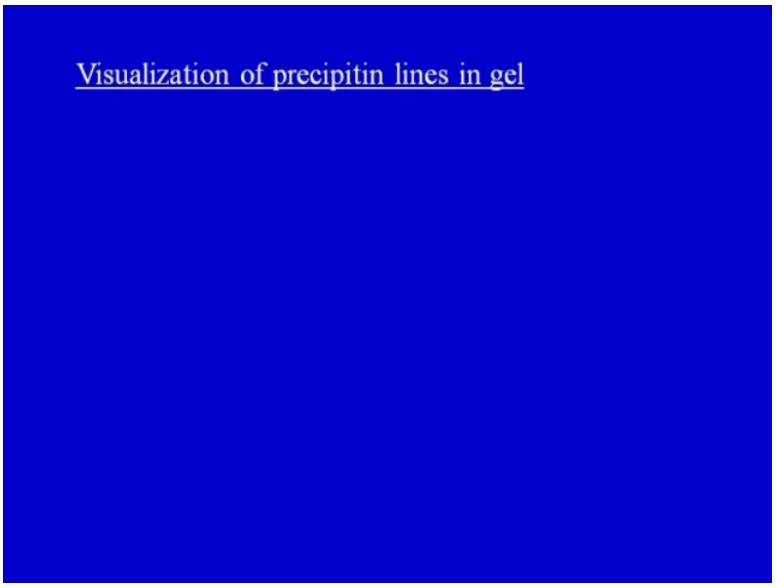

They could be like I said, there should be precipitin lines should be developed in humid chamber and they could be directly seen by the naked eyes. And if not, you can illuminate from the site and on the dark background they can be seen clearly. Many times, staining might be required, they could be stained with regular proteins and then they could be like, excess thing could be washed off. And they could be seen they, could also be permanent records can be made by photography and other means. So, this is all about immunoelectrophoresis.

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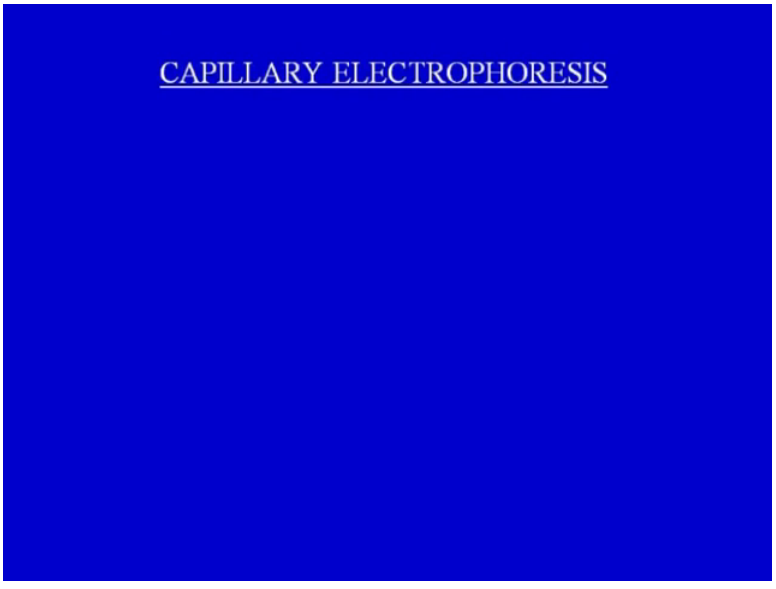

Let us move on to the next technique, that is capillary electrophoresis, if you could recall we have talked about a phenomenon called electro endosmosis or this particular, in electro isoelectric flow actually. In electro endosmosis, there is a net cathodic migration so capillary electrophoresis works on or takes advantage of that particular phenomenon. Capillary electrophoresis could also like, it is also known as capillary zone electrophoresis and there are other names, it separates the ionic species by their charge. And then other factors could be frictional force and hydro dynamic radius, but mostly on charge.

Capillary electrophoresis involves, electrophoresis of samples in a very narrow bore tubes, fused silica or other and these are like say, internal diameter could be 50 micro meter. And external diameter could be 300 micro meters so these are very narrow bore fused silica tubes. Now, one advantage of using these capillaries is that, they reduce the problems resulting from heating effects. So, because this particular surface to volume ratio is large surface to volume ratio and heat could be dissipated easily.

Now, but it does not mean you can keep any length to do that, there is certain balance between the length and the voltage. And these are run at high voltage so high voltage will generally cause lot of heating effect, but since they are long and thin bore or narrow bore capillaries. Therefore, the heat dissipation can take place, but I said like increasing the capillary length will not improve, as such the separation here.

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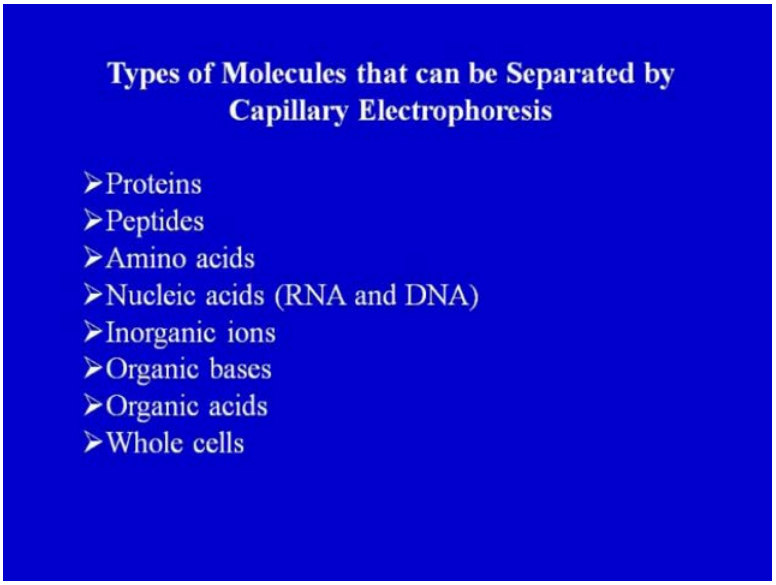

Now, types of molecules that can be separated, there can be different kinds of molecules can be separated by capillary electrophoresis. They can range from say, protein peptides amino acid, nucleic acid, inorganic or organic molecules, organic acids and lot of other different kinds of molecules, could be separated on capillary electrophoresis. As we have talked about this particular phenomenon of electro endosmosis.

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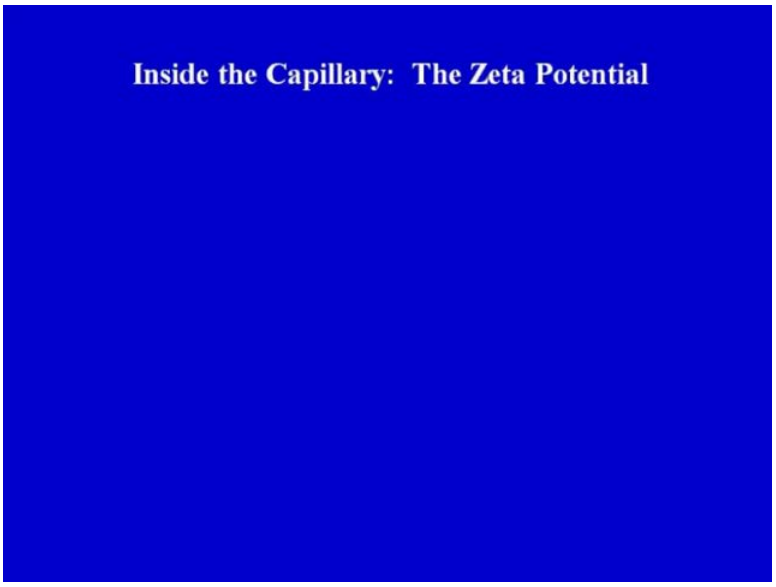

The inside wall of the capillary is covered by, if you could remember it is, we have discussed about this that is ((Refer Time: 39:18)) group, which are charged above ph three or starts charging after ph two. And this negatively charged groups attract cations to the, inside wall of the capillary. Now, what will happen the distribution of charge at the surface is like, there is a stern double layer, which is like kind of fixed. And there is diffused layer and this result in zeta potential. Now, zeta potential is the potential at any given point in double layer and decreases with the increasing distance from the capillary walls. It will be more at closer to the wall and the stern model is also referred to as electric double layer model.

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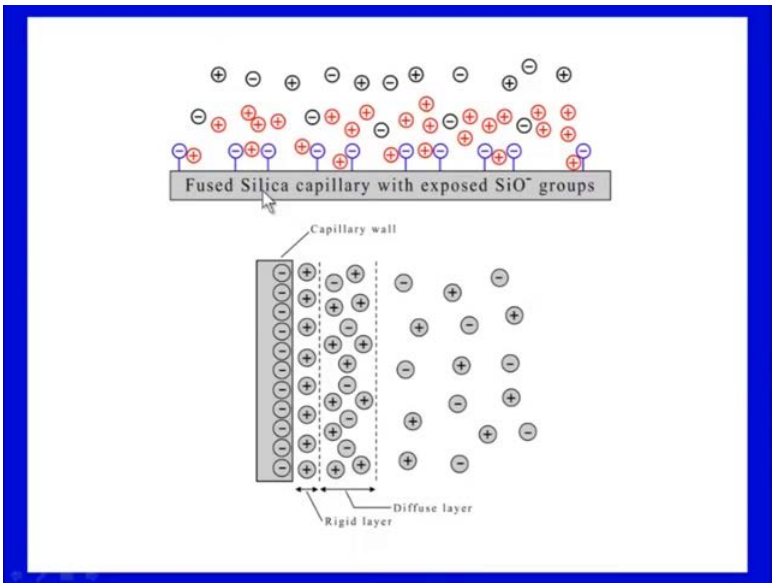

So, what is happening here is you can see here this is fused silica capillary tube, it carries negative charge and negative charge will increase as the, ph increases. Now, what you have seen here, there is fixed or you can say rigid layer and there is a diffused layer, stern layer actually. So, rigid layer is very close to this and diffused layer has some leeway to move around.

So, the electro osmosis or electro endosmosis flow, is utilized in capillary electrophoresis and the separations occur due to this phenomenon. So, what happens is that in electro endosmosis, the charges or the positive charges which is in diffused layer, moves towards the cathode. And carries the whole solution with it so there is a net flow towards cathode so net flow occurs as solvated cations drag along the solutions.

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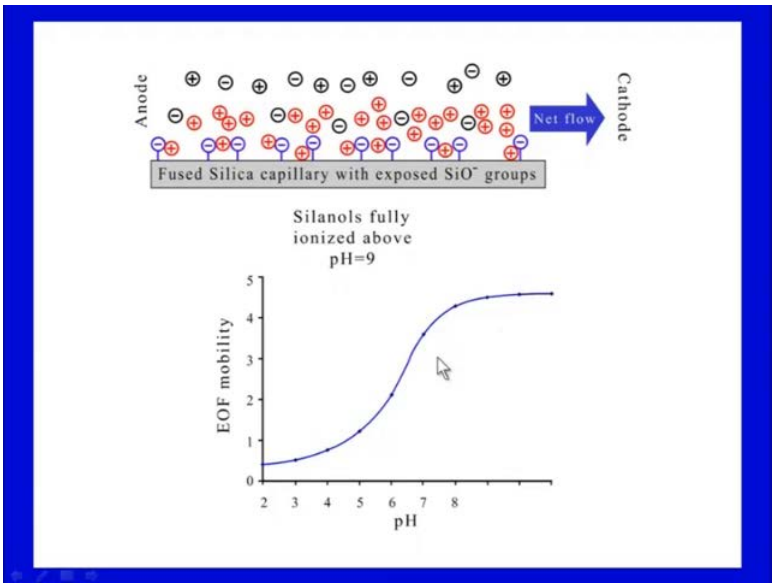

Now, in this figure as the ph increases the mobility also increases here and that is because the charge is increased over here. So, net flow is towards the cathode and it carries everything towards the cathode, that is even negative charge is, entities towards cathode. Now, if you consider like say there is 50 mille molar ph 8 buffer, that will flow through a 50 centimeter capillary at say, 5 centimeter per minute speed, with 25 kilo volt applied potential. So, that is how it will flow and key factors that affect electro osmotic mobility will be directly constant and viscosity of the buffer.

Now, this is like so if you see here, since they are all moving towards one side, many times it is hard to really separate neutral or negatively charged species, as you want to. Many times electro osmotic flow could be quenched by say, protection of ((Refer Time: 42:16)) or low ph. You can quote the inner wall of the fused silica and so to quench or to press the negative charge, but then problem will be that only one kind of ions will move towards the cathode, because the net flow or your detector is towards the cathode.

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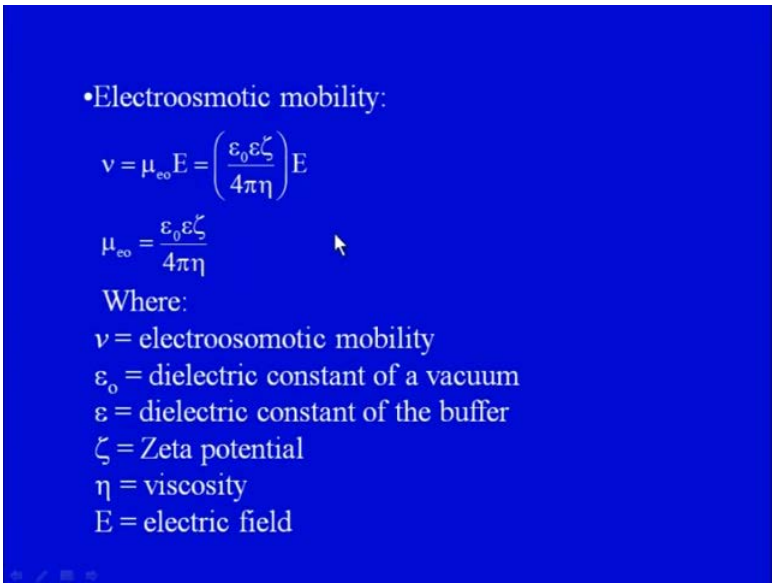

These equations here give you an idea about the electro osmotic mobility and which are dependent on like, dielectric constant of the buffer zeta potential viscosity. All these factors affect the mobility.

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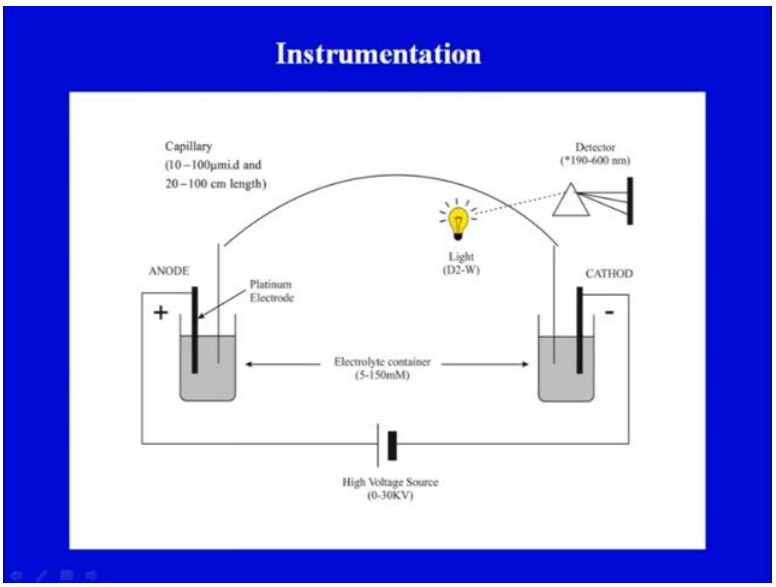

Now, a typical capillary electrophoresis, a very simple schematic is shown in here, in this figure. If you see here, there are two reservoirs and this is anode and this is cathode, this is anodic reservoirs, this is cathodic reservoirs here. The capillary tube is dipped in here, anodic and cathodic and this is, it could be 110 to 100 micro meter inner diameter plus,

20 to 100 centimeter length could be taken. This is high voltage supply, which could go up to say 50 kilovolt, but around 25 kilo volt utilized. This is a detector at cathode outside, which can detect or which will be monitoring the whole run and in a recorder, data recorder these things could be recorded and analyzed.

So, this is a typical capillary electrophoresis set up and the sample is loaded towards the anodic side, we will see how. So, what is happen is systems like we have seen, main components are sample wire, source and destination wires, capillary electrodes, a high voltage power supply, a detector and data output and handling devices. The source wire and the destination wire, which I have shown you these are source wire, anodic one and the destination wire. They will like here, these are filled and capillary also is filled, with an electrolyte which is aqueous buffer solution.

Now, to introduce the sample here, the capillary inlet so what will be done is the anodic buffer chamber could be removed and the sample chamber or sample containing say, it could be simple syringe type or a beaker could be put in here. And sample could be loaded or introduced into the capillary by, different ways like capillary action, it could be a pressure injection where you can through a syringe, it could be by pressure you can put in sample, small amount of sample or it could be high voltage injection, where you replace the anodic chamber by sample. And very instantly for a very small time, high voltage is supplied which leads to the introduction of the sample into the anodic side of the capillary.

And then again the capillary could be returned to the anodic chamber then the migration of the analytes is then initiated by the electric field, that is applied between the source and the destination, while that is anodic and cathodic chambers. And is sup, this is like supplied to the electrodes by a very high voltage power suppliers I have shown you in this case. So, it is important to note that all ions, that is positive we have discussed earlier negative, positive, negative or neutral ions will be pulled through the capillary in the same direction by electro osmotic flow. Now, the analytes separate as they migrate due to their electrophoretic mobility and are detected near the output end of the capillary, that is cathodic side.

So, output of the detector is sent to a data output and a handling device, such as an integrator or computer where it would be analyzed. Data is displayed as an

electrophoretogram, which reports, which reports detector response as a function of time. Now, separated chemical compounds appear as peaks with different migration times, in an electrophoretogram. So, in electrophoretograms if you see, like we said detectors are placed at the cathode side under common conditions. And these detectors should have high sensitivity because like the speed at which these are gone are very fast.

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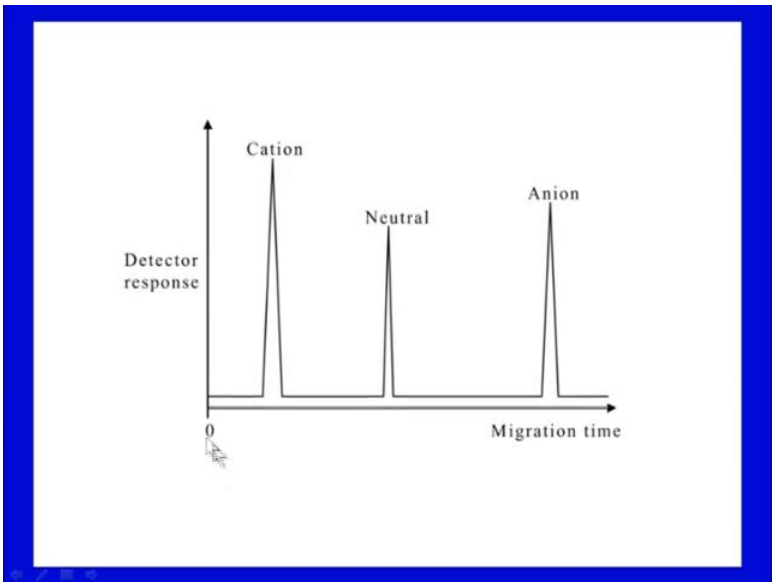

Now, if you see that electrophoretograms what you will see is, the cations will come first because they are obviously moving towards cathode, they move faster. And depending upon how much charge is present like say, if some analyte contains more positive charge it will move faster then neutral will go, will be pushed in. And then anionic, because anion will also have electrophoretic mobility, that will push them or move them towards the anode. But since net flow is towards the cathode, they will be the last to appear. So, that is how the different analytes will be separated.

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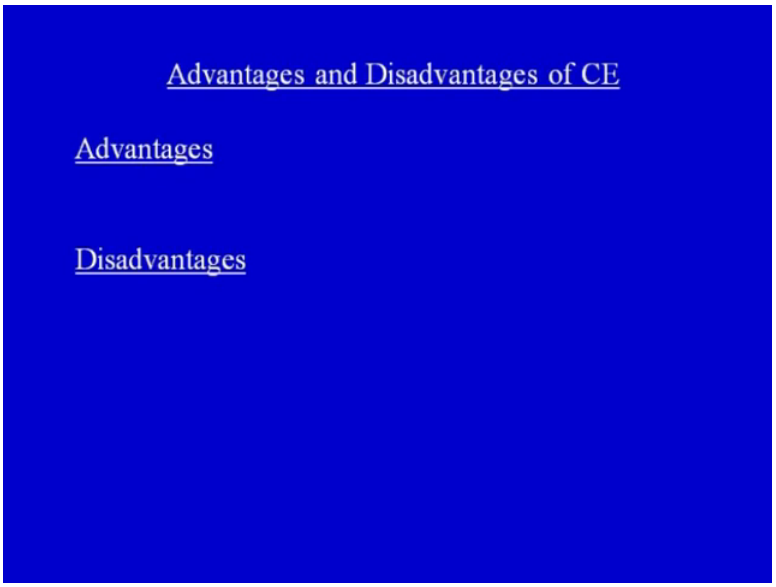

There are lot of advantages of capillary electrophoresis, in advantages it offers new selectivity and an alternative to HPLC. Though HPLC is more widely used, it is a very easy and predictable kind of selectivity it has high, separation efficiency that is you have high theoretical plate number. This is a very small sample size is put in, separations are very fast like say 30 to 40 minutes may be less. It is automated, it could be quantitative also, it could be easily coupled to mass spectrometry or others. And lot of different kinds of modes are available like, as we will see.

There are lot of disadvantages also it could not be, cannot be preparative like you cannot do very large scale preparations or separations. Low concentration and large volumes are very difficult to do then there are problems of sticky compounds like for example, certain proteins might stick to the negative charges, positively charge. And they could be streaking of the protein and then you will get peak. There might be certain species which are hard to dissolve, reproducibility is another problem, it is very hard to get many times reproducible results these are certain disadvantages, but there are many advantages to use capillary electrophoresis.

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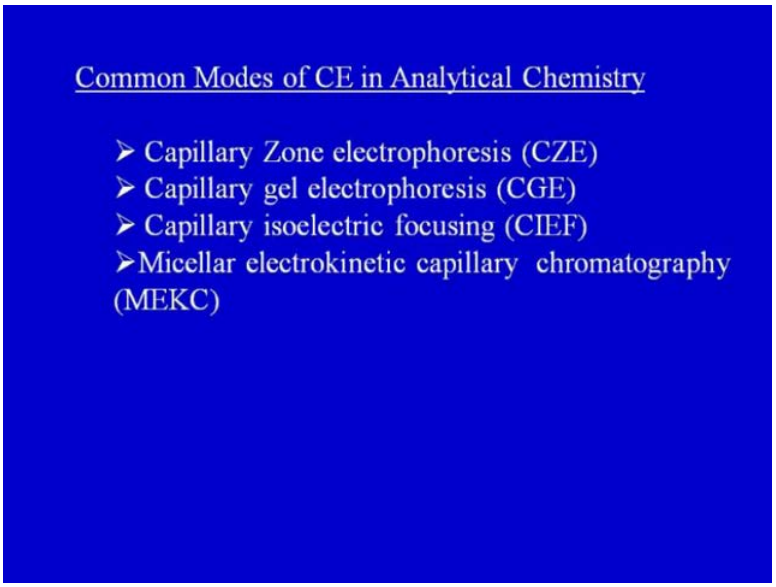

There could be many modes of capillary electrophoresis like say, there could be capillary zone electrophoresis, where in free solutions you are doing or free buffer you are, the experiment is being done. There could be capillary gel electrophoresis, where a particular matrix material is loaded or matrix material is packed in these columns. Again it is very hard to do by one self, it is like commercially available columns are available. These are commercially available columns and they can, it is like by different strategy the gel is put in there. Iso-electric focusing mode could be available and for neutral molecule there is Micellar electro-kinetic capillary electrophoresis or chromatography we call it.

Where, a detergent could be used and a neutral molecule could partition, when these missile formation of detergent at certain concentration takes place. So, these are different modes where, could be utilized in for capillary electrophoresis for different applications. There could be many, many applications of capillary electrophoresis, it is a versatile technique for lot of different kind of compounds to be separated and analyzed.

Though certainly instrumentation and it is an expensive instrument, but it is very, very handy. Lot of different kinds of like pharmaceuticals, in pharmaceutical industry it is utilized for reaction intermediates, purity validation, stability, final product testing, ion analysis and lot of other different applications.

In bio sciences peptides, proteins, DNA carbohydrates can be analyzed. In food industry inorganic cations, anions or organic acids, amino acids etcetera could be analyzed. In environmental science lot of pollution studies or pesticides or lot of other different kinds of metals, surfactants, dyes environmental pollutants could be analyzed on capillary electrophoresis.

Then, forensic science like say drug of abuse, explosives, gun powders and other things could be utilized in, could be analyzed in capillary electrophoresis. So, lot of different areas utilize capillary electrophoresis for different applications. So, this was about capillary electrophoresis, another very important technique which could be utilized for particular applications, while various kinds of, various kinds of fields actually, various fields of bio technology.

So, this completes our section on electrophoresis, in this lecture we have discussed about immunoelectrophoresis and the capillary electrophoresis. Both are very important techniques, immunoelectrophoresis has been now a day's replaced by immunoblotting techniques. Where like in investing blotting and others. And the particular molecules say, protein molecule can be transferred on nitro cellular membranes.

And then can be probed by antibody linked to an enzyme and then it could be, the bands could be or the patterns could be seen with enzyme reactions. So these, this has, but still immunoelectrophoresis is utilized for various applications likewise, capillary electrophoresis is very good, very simple to use automated technique for various applications. In the next lecture, we will start a new topic, which is also very important, that is centrifugation techniques.

Thank you.