Nanobio Technology Enabled Point-of-Care Devices
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Lecture - 08
Signal Amplification for Ultrasensitive Biosensors (Continued)

Dear students, today again I will start the Signal Amplifications, but these lectures mainly I will show the applications, different ultrasensitive biosensing applications using redox cyclings, let us come.

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So, today's main concept for this lecture is the special examples using redox cycling and their applications. So, there is lots of applications. So, I will recommend I will I will generally we

can you can see in my slides I put many references. You please go through all the references, then you will get the more details.

But I will cover the all the basic part in the lectures, but if you want more examples please visit the link also different different links, also different references I put in each slide.

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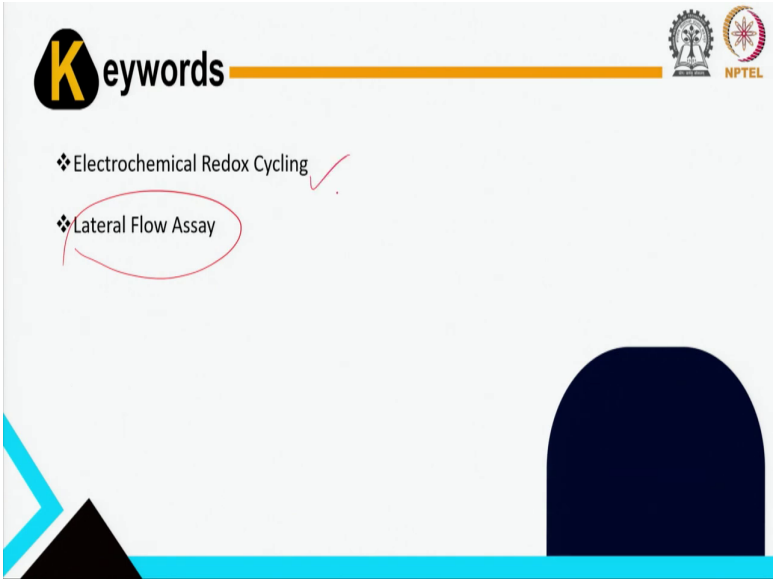

So, main keywords in the lectures is the electrochemical redox cycling and a lateral flow assay. I will give you one very special example of the lateral flow assay. So, you can search the lateral flow assay based on the redox cycling, you will get the many report also electrochemical redox cycling using the devised like a different kind of electrochemical device development that also you can search ok.

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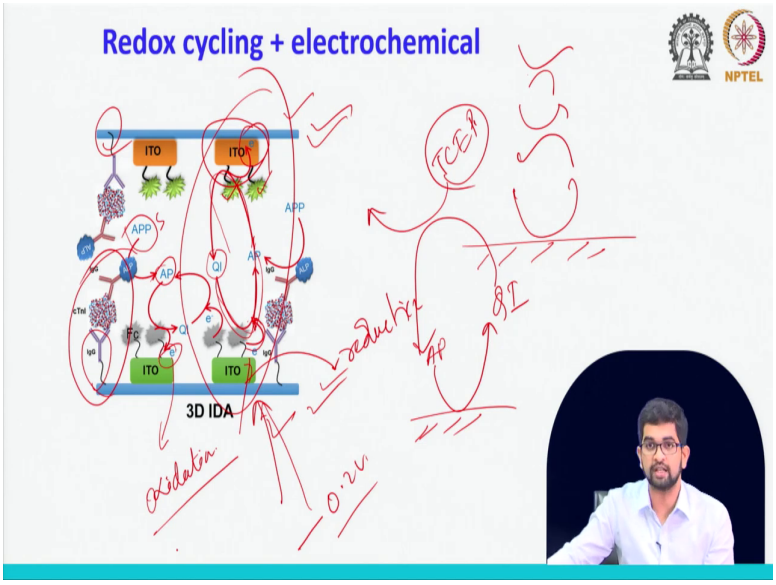

Let us come the example here. So, here a special example like you can say inter-digited array electrode we developed here. You can see so here one electrode and here another electrode.

So, ITO indium tin oxide coated electrode where actually we modified some electroactive species like the ferrocene; FC means the ferrocenes we modified here. And we modified the primary antibody on the electrode surface, but these two electrode is a two inter-digited array electrodes.

So, one will behave as a, where one behave as a anode 1 behave as a cathode. When where one will apply positive potential and another electrode will apply the negative potentials when something different design that we can make. You know, that is just for example, I am saying not just like the like one sensor surface and then redox cycling, redox cycling like this. Means

In this case again we can reduce the number of the chemical by using just two different electrode.

Just kind of like a sandwich here like two electrodes we can use just one for where we can apply one positive potential and another electrode where we can apply the negative potential. So, where we are applying the negative potentials that will behave just like a like we do not want the reducing activity they you can supply you know something like this.

See, this is the sandwich assay that already form on the surface and APP that is the substrate, react and form the AP, right. So, this AP can be oxidized, this AP can be oxidized on the sensor surface, and it will release the electron. So, this electron release right, so here oxidation happened, right. So, in this electrode surface oxidation happened. Now, see the and here so in this case the electron release and in this case here you can see here we are giving some electron, right

So, here reduction happened right; reduction means electrochemically we are reducing. So, you can see in this these electrode in this electrode AP actually oxidizing see it is oxidizing, right. So, here oxidizing and here reducing means in this case means we do not need any extra reducing agent. So, AP actually forming AP forming based on this ALP reactions, then these AP can be oxidized and form the quinoneimine and this quinoneimine can be reduced not by T shape right.

Last time I told can you remember like aminophenol can be oxidized on the sensor surface from the quinoneimine and that quinoneimine can be that quinoneimine can be reduced by T shape right. This is a reducing agent, but here we are not using any extra reducing agent, here we are actually this quinoneimine actually reducing by here applying some negative potential suppose minus 0.2 volt we are applying for example, if you have a negative potential it getting the electron and it will be reduced.

So, this is one special examples where you can design something different different redox cycling plus biosensors. So, you should know like how we can design different different redox cycling method.

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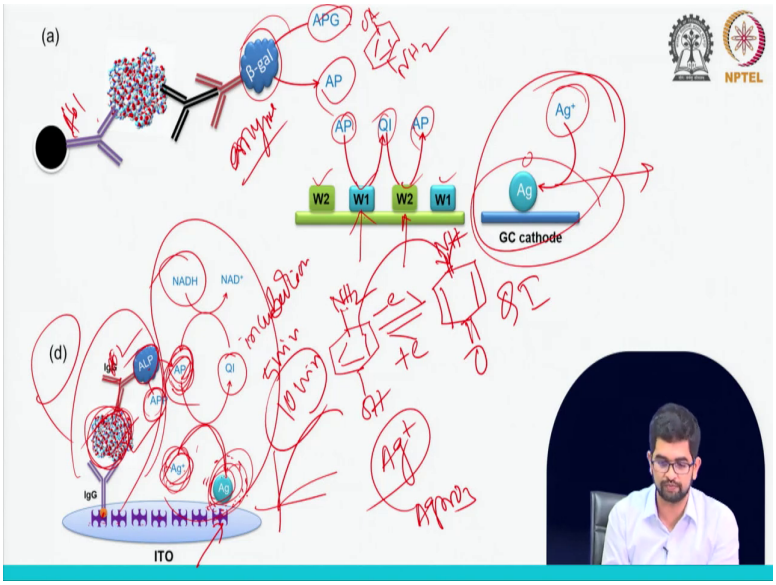

See another example I will show you here. So, in this case say again this is the primary antibody 1 and here we use the like two antibody they are the secondary antibody and they have the secondary antibody actually conjugate to the beta galactose this is the enzyme right this is the enzyme based biosensors. So, this is enzyme ok.

And APG this is just a beta galactose enzyme substrate and it will form the aminophenol this aminophenol is forming, right. So, here we can design something biosensors like see here like different working this is the all the our sensor surface working 2, working 1, working 2, working 1 where working 1 where we are actually oxidizing the species. So, aminophenol is

producing that actually we are oxidizing and quinoneimine can be reduced in this working electrode 2 it is reduced and form the aminophenol again.

So, like this way means we can design something different sensor surface. You see here in this case like if you have any silver plus in your solution like silver you have silver nitrate Ag plus. So, you can form on the sensor surface by using some reactions you can reduce this silver to silver just silver Ag 0 and then it can be oxidized. So, this can also help to form the biosensors.

This example I will show you I will show you one design the how silver can form and it can generate the signal again that redox cycling I will show you here. So, this redox cycling example is the this d you can see here. Suppose you have a biosensor like this. So, here so, you know this one I have told you like this is the avidin coated like this is the avidin and your antibody already conjugated with the biotin alright. So, this biotin, biotinylated antibody you immobilize on the sensor surface and this is your target.

Now, your antibody 2 that is the your secondary antibody that conjugated with alkaline phosphate right ALP. So, that will react with the APP and form the AP aminophenol means that you know already. Now, as I told so, this one AP aminophenol NH 2 OH. So, it you know this can form easily quinoneimine alright, this can form easily this one. So, it can just release the electron it can form this and if you give the electron then it will form this right. So, like this way it will actually participate in the redox cycling reaction.

Suppose you have Ag plus in the solution like silver nitrate whatever Ag plus means some salt like silver nitrate something like that, so Ag plus is coming here. So, silver nitrate then it will oxidize the aminophenol right. So, it can oxidize to quinoneimine QI and itself will be reduced to Ag 0.

This g plus will form Ag 0 then by the help of aminophenol. So, the so this is the another redox cycling reaction right something else, but the concept is same like EC redox cycling; EC redox cycling something like this, but here something different way we are trying.

So, here we are just use a salt silver salt this silver salt will react this aminophenol and form the silver 0, but this silver formation means if you make so many silver particle on the surface, then you can easily oxidize those that I will come means you can apply some potential or maybe you can run some electrochemical other signal and you will get the signal means if you have the less number of silver maybe you will get one signal if you get very high number or many numbers of a silver then you will get the higher signal something like this.

So, if you have more AP, then more silver plus will form the more silver right on the surface like if you so, that corresponds to that so, a target concentrations. If you have more target in the sensor surface then more ALP on the sensor surface right and more ALP means more APP will react and form the more AP. So, number of AP will be very high and more silver plus will form the silver 0 clear.

And this we need like AP should be regenerated otherwise AP concentrations will be slowly to decrease. So, how we can form the AP just we can use the reducing agents here nad is actually acts as a reducing agent nothing else.

So, this is used reducing agent so, this again aminophenol will form and this aminophenol again will react with the silver 0 and form this. So, this reaction that is why we need some incubations when this kind of EC or ECC redox cycling will form we need some incubation time incubation. So, how long? Like 5 minute or 10 minute incubation we need. So, during this time this reaction will happen and you will get the silver on the surface. Now, let us turn on your electrochemical technique you will get the signal ok.

So, like this way you can apply this kind of redox cycling reactions for your biosensor development why like if you like just I now, I told you. So, based on the concentrations the silver will be different so, you can use ok.

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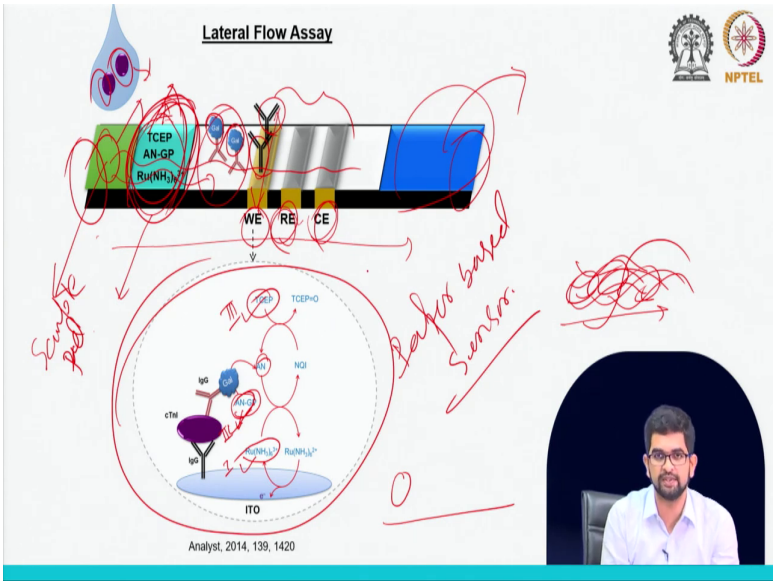

Now, I will come a very very important and very fancy examples of the lateral flow assay. So, what is the lateral flow? Laterally flowing the solutions right, it is just a paper based; paper based sensor. This is a paper based sensor we are using different kind of cellulose membrane.

And one good example of this lateral flow assay that is already available in the market that is pregnancy test kit, but that is calorimetric that is not electrochemical. So, there we just where you can just drop the sample one part and because its made by paper you know is there if you see the papers if you see the microscopic structures there is lots of like different different like this kind of random like kind of threads is there. So, and because of this why you are facing lateral flow in the paper?

Because there is a kind of it is called like microfluidic kind of. So, it is called you can say this all the channels they will help to flow like from one part of the paper to the another part. So,

this is lateral flowing. This is the kind of microfluidic or you can say it is kind of flow that is helping the papers. So, I will refer please check the microscopic image of the paper that will basically help you why actually liquid flowing from one end to another end.

So, if you draw off your sample on the one end of the paper. So, basically because of the lateral flow it will start. So, you need different kind of chemicals as I told. So, we can design a electrochemical lateral flow assay. So, pregnancy test kit kind of things that is also lateral flow assay, but they are calorimetric they are showing the change of the color that I will come. But first I want to show you the electrochemical lateral flow.

What the electrochemical case I always saying we need some electrode one is working electrode; one is reference electrode; one is a counter electrode right. So, here on the working electrodes there we actually immobilize our capture our antibody right our receptor, capture means they are receptor.

So, as for example, an antibody. So, they immobilized on the working electrode on the paper and we can immobilize we can makes a paste like reference electrodes that made by like silver or silver chloride paste and if countered electrode also you can make another something by using something like carbon base. So, this kind of three electrode system we can make on the paper.

So, this is the sample pad; this is the sample pad on the lateral flow where we just drop the sample. It can be blood sample or it can be urine sample, it can be plasma sample anything. So, this sample will contain the target. So, if its target present, then it will also flow through the your paper. See here some compartment you can see.

So, here one compartment this is called a sample pad, this is another compartment this is called a reagent pad because as you see this kind of redox cycling will start on this paper based lateral flow assay, alright. So, what is the starting reagent in this redox cycling? You can see that we use the galactose one enzyme that is conjugated with a secondary antibody.

So, AN-GP is the enzyme substrate for the galactose that will form the AN. So, basically this is as it is substrate it should be your starting reagent and it should be on the reagent pad there. So, this is the reagent pad in this reagent pad we will use all the reagent. So, we have to find out first which redox cycling you want to use, I already taught you different different redox cycling let us try to find out which redox cycling is the best for your work ok.

So, you optimize that all the optimization possible I told I already taught based on this you select some chemical. So, here I suppose we selected AN-GP one chemical. So, this is the starting reagent and ruthenium hexamine 3 plus is the starting reagent and here TCEP is the starting reagent right that I mentioned many times. So, that is why I see I used here in the reagent pad ruthenium hexamine AN-GP and TCEP.

So, I, II, III, so this 3 mixture I use here and always I told you also that they should not react each other. So, although I keep them as a mixture, but because they are not reacting that is why I can keep them easily and it will be stable if they react, they cannot keep.

But it is the best way if you can keep them like a lyophilized conditions like we can back home drive and put the we can make a this mixture and like a solution and in the back home drive it means like a lyophilized condition if you keep and we can go for a good packaging right.

In this lateral flow if you buy a pregnancy test kit if you see they are the packaging system we are keeping this all that kit inside a packet. This is the very good methods you are following there is not much water not much oxygen. Why we are following these things? Because they can destabilize the reagent. They can destabilize the this reactions or this destabilize the sensor that is why we have to be very much careful packaging also very much important and how we are storing the reagent ok.

So, this reagent all the things we kept this is the reagent pad and here is the detection antibody. So, the here we can drop this detection antibody that is conjugated with enzyme that also we can drop and we can lyophilized ok. So, that is done. So, your fabrications part of this

lateral flow done and this is the last part this is the waste chamber I mean this is another paper just here like excess reagent, excess fluid everything can be adsorbed absorbent pad.

See this is the absorbent pad where excess chemical, excess reagent when they will restart flowing here then it will come, they will absorb. So, this is the whole design of the lateral flow assay. Now, let us start the reactions when I drop the sample what happened and how we can measure the signal right let us start this part now. So, let us remove the basic things that I taught now let us come the mechanism part of the redox cycling on the paper ok.

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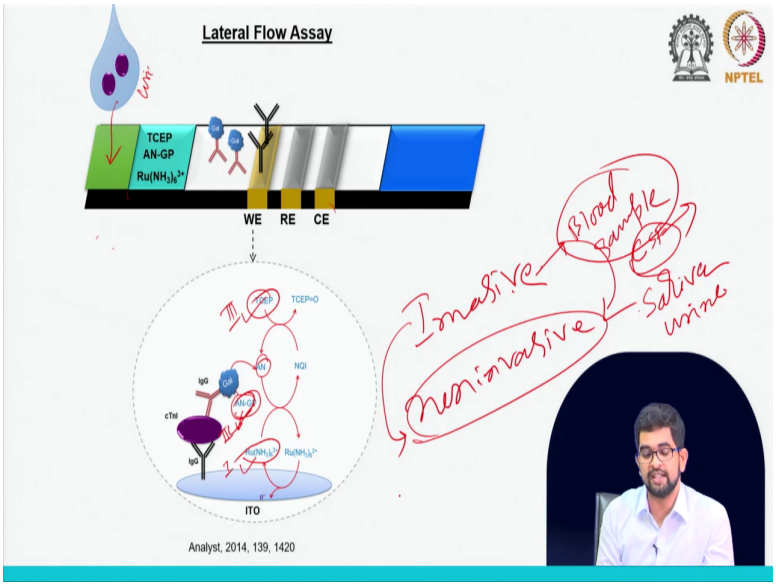

See here once you drop the sample on the sample pad then suppose it is a urine sample; urine sample because sometime you know the invasive method non-invasive method. So, that I can show you first that in invasive and non-invasive. So, invasive and non-invasive; invasive

means it is like a painful method like we need to prick your finger or collect the blood sample from like using needles that is the invasive method.

So, basically for point of care and for the user friendly method if you really want to develop we that is why we try to go to a non-invasive. So, in this case no need any prick of the fingers and no need to collect the blood sample using the needles it is the less painful that is why, but almost no pain. That is why invasive means generally we need the blood and also invasive sometimes you can CSF Cerebral Spinal Fluid. So, this kind of sample we are getting there cerebral like we need very trained persons also.

So, collections of the cerebral spinal fluid also not easy from the spinal cord will be collect the cerebral spinal fluid right. So, like common person like if I make some point of care device if I keep home and if you want to go for like test based on cerebral spinal fluid like its really really difficult for me also right. I mean common people laymen they cannot use the cerebral spinal fluid they should have some basic expertise some basic training and same thing for the blood sample collections.

But at least the pricking of fingers are still good, but it is not all I some people they cannot prick their fingers. Suppose the glucose test case who are the diabetic persons they have to prick their finger every day you know how many times they have to prick their finger maybe for their meal they have to prick their fingers right. So, every time say the per hour if they want to check their glucose because without checking glucose they cannot take the food its kind of something painful for them every day cannot prick.

And mainly if you think about like sometime this kind of diabetic things now you can see even in the very very younger age also. So, baby so they can we cannot prick their little they are always their finger they daily many times. That is why we are thinking now let us approach to the non-invasive method. Non-invasive means we can go for the different kind of sample like saliva; saliva, urine that test you can go for the testing.

Also, sometime people think like interstitial fluid just below of your skin if you can collect these samples also non-invasive way, but that is also not pretty easy you have to use some

microneedle. Sometime people thinking like using microneedle and getting the interstitial fluid just under your skin that is the interstitial fluid that is also sometime become invasive, but sometimes you can be minimally invasives.

So, we can develop some microneedle like a banded you can put on your screen and this needle actually we can will not hurt your nerve systems. So, that is why it is not much painful just keep like a banded on your skin and it will absorb the interstitial fluid from the skin and in the interstitial fluid there is a glucose there is other biomarker that we can go for the detections also.

So, this is the different different sample we can use for the lateral flow assay or for the sensor kit development, ok. Now, let us come back to the this mechanisms of the biosensors this lateral flow biosensors, ok.

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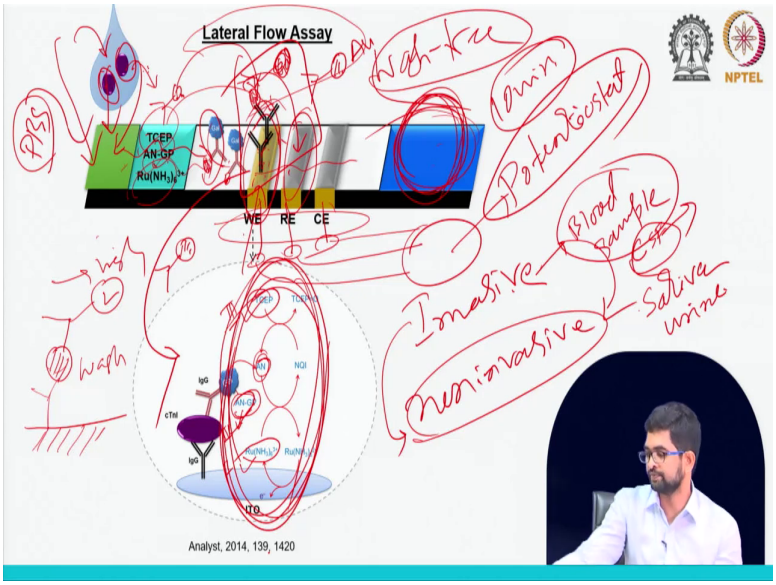

So, I drop the urines sample that contain the some target, ok. So, naturally this chemical they will dissolve right and they will flow.

Now, if you have the secondary antibody and this it is already content target so, target will bind right, if your sample content target then it will bind with your secondary antibody right ok its. Then it will come to your detection region like this is your region in the detection region where you have the working electrode that where already primary antibody coated right.

So, then your target will bind secondary antibody will bind that is galactose the bind and this chemical also will come here. So, naturally with this galactose, then I will focus this then these reactions. So, this reaction will start here. So, galactose will react with the NGP formed by AN and it will react this then this redox cycling will start.

Now, here you can see the working electrode, reference electrode counter electrode these three we have to make a adapter and we can add this one with the potentiostat; potentiostat. This is the electrochemical analyzer or potentiostat that can help to measure the signal. So, if the target is very high concentration, necessarily then you will get this redox cycling very fast and you will get the high current, you will get the high output and you can measure this one with potentiostat.

Similar, like the glucometer like if you drop the sample and if you have the high good number of I mean number of I mean if you have the high concentration of glucose in your blood samples you will get the you can see the value.

Actually, we are getting the all the current that we are measuring you by flowing the electron that will show the in your display the some numerical value showing. Similar way we can use the IOT that I will teach you slowly at the end of this course that IOT- enable biosensor we can develop. So, how much current you are getting that you can see that digital a digital value on the skin.

Like you can use some similar something like just your mobile phone you can use there you can use your app and just run the app and then you can see on your mobile skin that how much current is generating corresponds to that much current it will show a digital value ok. So, this is the IOT- enable biosensor that we are saying. Now, see maybe you have so, after binary there have lots of secondary antibody, but your target is very low concentration. Suppose you have only one target.

So, other excess one other excess one what will happen then it will go through this and it will absorb on the absorbent pad. So, excess one will be absorbent by absorbent pad excess reagent will be absorbent by absorbent pad. So, excess part will be here and generally we need the you know when we are forming the sandwich alloy assay as I mentioned after this is the primary antibody then your target, then your secondary antibody that conjugate the level.

And every time after adding the target we need wash right. So, that excess one can go wash out from the surface after secondary antibody we have to wash they show that your secondary excess level antibody can be wash out. Otherwise they can show the extra signal background signal I can show, but in this case its kind of wash free. Why?

Because excess antibody secondary antibody that conjugate to the enzyme as the this sample flowing so, excess one can flow and it will come to absorbent pad right. So, excess one will not be here.

Even after adding this urine sample or your sample you can add some previous buffer extra previous buffer you can draw to minimize the background current. Which can cause the background current? Maybe if you have the extra this secondary antibody if you absorb if it absorb maybe some non-pacifically here it can cause the higher background current. So, if you can add some little bit previous also that you can optimize also.

So, that can help to like all the thing wash you do not need extra washing step that is why we are calling the washing frame. We do not need extra washing step just drop or add some previous and you can wait for 5 minute or 10 minute as I said you need some incubation time

then only this reaction will be completed right. So, after this you will see this signal on your portable potentiostat or maybe in the mobile phone based if IOT enable some biosensor you can develop that you can see.

So, this is the lateral flow basis a very good technology you can use, but this lateral flow also where is the pregnancy test kit is available in the market that is the calorimetric. So, what technology they are using? They are here using some antibody that conjugate the gold nano particle. So, here they are using some secondary antibody conjugated gold nano particle and if target comes.

So, this gold conjugated all the antibody will be attached on your test zone all they will be accumulated and so that is you will see some reddish color. So, then if you see the color, then you will say the positive test and they have some contour region although. Contour region means they all the secondary antibody that conjugated with gold if you do not you do not have target. So, in the contour region they all will come they will accumulate in the contour region.

So, contour region can confirm you that is this sensor at least fine is working fine its I mean this sensor strip is ok contour region showing. But that is the calorimetric strip that I can describe more after few class, but today just I am showing the electrochemical strip this is just electrochemical strip.

But you can ask me that why the calorimetric is the very pretty simple method, why I should go the electrochemical where calorimetric is pretty easy, but sometimes if you have the very very low concentration you may not see the color that is the point. But electrochemical case if you say very low concentration also that the that low concentration you can measure because of these signal amplification that is the utility of the electrochemical method. That I will show you later again very detailed so.

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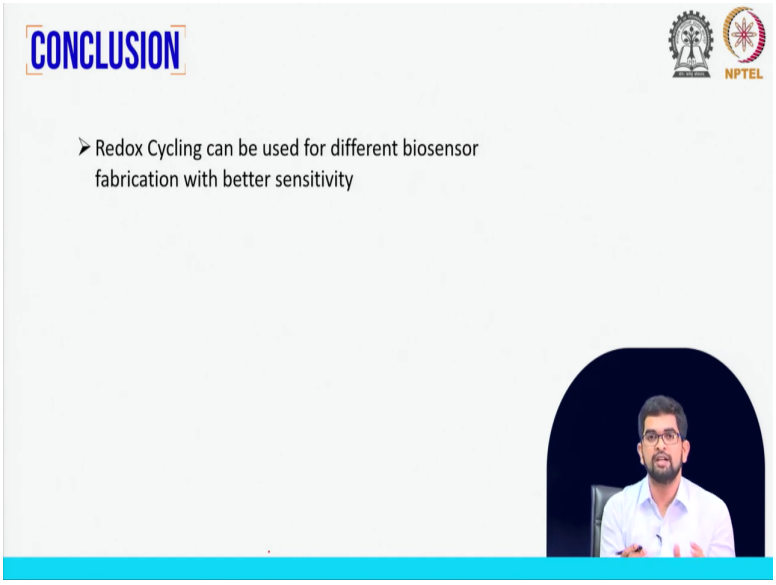

So, conclusions of this today's lecture we can use the redox cycling that can be used for different biosensor development, we can fabricate different different applications that I showed a very good examples lateral flow assay or this can be useful like different different design that I showed like silver nanoparticle also you can form from the silver nitrate different different like in you can go for different design not only the sensor design even in the chemical part also you can go for different design ok.

So, that is all for today's lectures and thank you very much. I will show you more example in the next class based on this signal amplifications.

Thank you.