Nanobio Technology Enabled Point-of-Care Devices
Prof. Gorachand Dutta
School of Medical Science and Technology
Indian Institute of Technology, Kharagpur

Lecture - 30
Tutorial on Biosensors Fabrication (Continued)

Ok, students today let us continue some new questions based on these questions you can understand some more nanobio devices and you can use your existing knowledge.

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Let us come the new try new questions and their problem. So, mainly again I will cover today some tutorials. Today I will cover some new some new problems that will very much helpful to design some new device.

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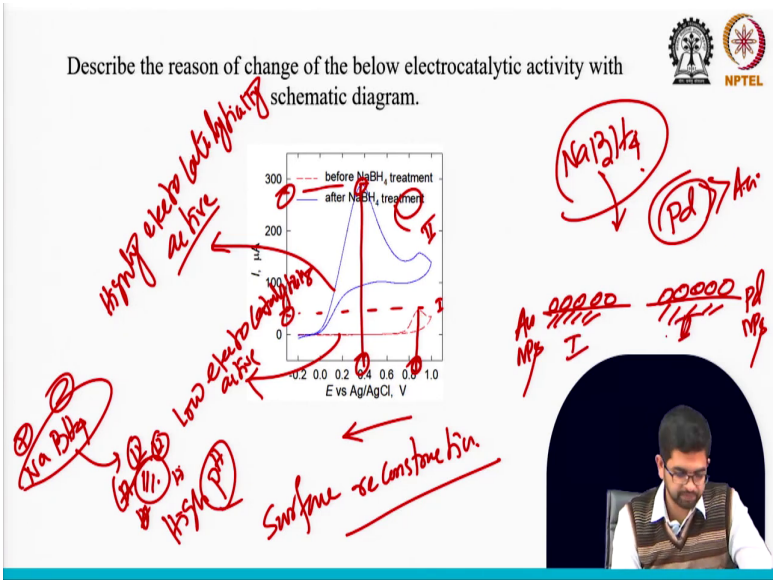

For example, I think this you can remember this figure right, when I thought you this one for sodium borohydride treatment. Today's questions I just showing you two cyclic voltammogramo, right. One just before treatment and second is the after treatment these 2 cyclic voltammogram. Let us explain this systematically, means when you observe this kind of cyclic voltammogram how you can explain and what is the characteristics of the sensor surface.

And then second when you observe this cyclic voltammogram, what is the characteristic of the sensor surface and how you can explain it. And this is very very fundamental questions. If you can understand this you can explain maximum the sensor surface modifications. See here mainly we discussed the sodium borohydride treatment, but this is this cyclic voltammetric change is very generic.

See sodium borohydride change that I told you that it can increase the surface electro catalytic activity right, that you can remember. Now, see if you have a catalyst like gold or may be palladium, you can remember that if you have a gold surface or maybe you have palladium nanoparticle coated surface suppose, gold nanoparticles or here may be you have palladium nanoparticles. You can remember when we react this surface with sodium borohydride.

So, gold nanoparticle then palladium actually this shows the enhancement much faster right, and also it is not only faster also it can enhance the activity much better than gold nanoparticles. That is reasons I taught you already. I can summarize again because the sodium borohydride NaBH 4 you can remember Na plus BH 4 minus in the solution. Then from here the hydrogen atom they are adsorbed on the nanoparticle surface.

In the case of palladium, its very quick and also self hydrolysis in the case of palladium nanoparticle is very slow, because we need high pH right, you can remember these things. So, now let us explain that why we are getting means this is the reason for change of the below electrocatalytic activity with schematic diagram.

This kind of questions would come. So, schematic diagram is you have to explain this enhancement by the sodium borohydride. And because of this hydrogen atom adsorptions. And this is because again you can explain that because of surface reconstruction or some surface strain spacing that is why electrocatalytic activity will change. Now, you can see there is two cyclic voltammogram.

So, see in this case first if we check the first one here, we see the oxidations you can see here. And the second cyclic voltammogram you can see the oxidations you can see at this positions. So, let us explain this, means when you observe this oxidations in the left side, it means you need lower oxidation potential. And your sensor surface actually is fewer for oxidation it means it is highly catalytically active.

That is why you can say that after sodium borohydride treatment your surface, this sensor surface highly electrocatalytically active alright, that you can see. And this surface this one

you can see your oxidations here this is low, electrocatalytically active. So, not only potential also you have to check the current you are getting see your current your current how much enhancement you are getting.

So, generally which this enhancement we are comparing in a fixed potential. So, you have to fix any potential and you can compare the amount of current we are getting. That is also another important point to compare the electrocatalytic activity of two different surfaces. So, you have two different surface you can compare.

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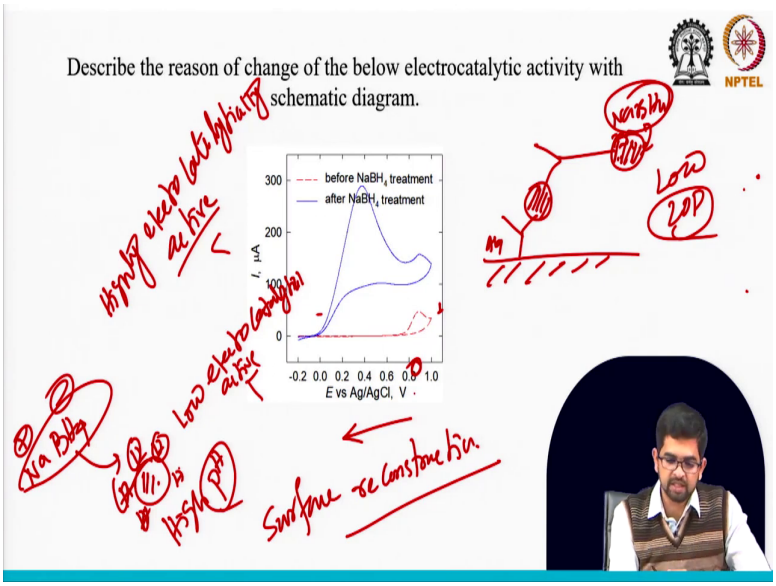

So, that you can explain now let us show you like a very basic diagram of the sensor that you can design. So, based on this enhancement you can design a biosensor like I think I taught you like just you can modify the sensor surface with antibody one that is the primary

antibody. Suppose this is just for example, you want to detect just some cancer biomarker. So, this is your cancer biomarker.

And secondary antibody that you can conjugate with some very nanoparticle like gold or palladium which one you like. Then you can this nanoparticle, now you can add the sodium borohydride, right. And these activations can help you to get very low limit of detections. So, that is simple now process you have to explain. The basic things that you have to understands this cyclic voltammogram shift, ok.

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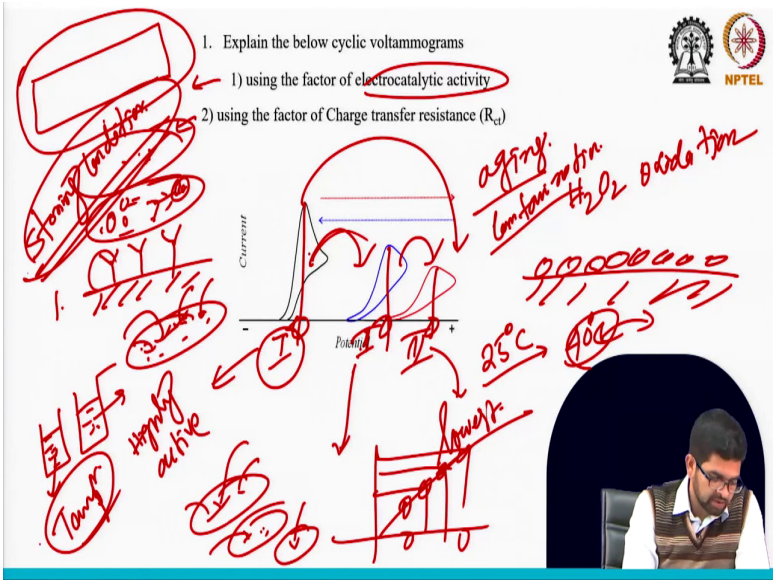

Now, let us come to the second questions that again we will discuss in the in these problem. Let us discuss the is this two here you can see that two problems I want to discuss, here you can see three cyclic voltammogram. What is the factor for this change of the cyclic voltammogram.

Suppose this is the hydrogen peroxide oxidations we are measuring hydrogen peroxide oxidations we measure on a sensor surface. Suppose you have a gold nanoparticle modified sensor surface. And you got number 1, 2, 3 this different different kind of cyclic voltammogram.

Let us explain this one in the factor of electro catalytic activity. So, now it is very much clear like which surface is the highest electro catalytically active which surface is the lowest electro catalytically active. So, number 1, I think now you can easily predict is the highly active. And number three is the lowest, is the lowest electro catalytically active. And this one medium moderate electro catalytically active because of the potential positions. This potential position you can predict.

So, if I ask you that electro catalytic activity why is changing? You have to explain that this activity it can come here because of aging it can be possible right, if you have one nanoparticle you are stored for the longer time in room temperature. There is different different factors the one factors can be like that just aging, another factor can be contamination, you can remember that I taught you with the help the help of XPS study also the AFM study also we have measured.

So, like this way you can explain the why you are getting number 1, number 2, number 3, you may get of the same surface. So, that is why I told the packaging is very much important. So, how that is how you have to store the electrode. So, storing condition, storing condition is really a important factor for biosensor development it is this factor always you need to be careful. Otherwise, you cannot make a very reliable sensor; you cannot commercialize your product. So, this is very important factor.

So, first things you have to remember your sensor that you are modifying it should not react with. Just suppose you develop a sensor surface, you have primary antibody and you are making some suppose you have some paper based membrane, right. On that membrane you just use some other reagent. So, they should not react each other that is I am going to tell you.

Suppose you have your secondary antibody some conjugate it is already conjugated you have some other reagent. So, they should be very much stable, it should not react. So, one thing you can do. So, you can just make the solution like secondary antibody or other reagent solution and drop on your like paper based membrane if you want to use then drop on that paper.

This how much you have to drop that you have to optimize that optimization conditions everything I thought you that how much you need that you can drop it here. Then you can lyophilize it. You may need like different different membrane like for each case you may need separate membrane, but you can make a lyophilized conditions the all the reagent you can keep. This is one thing. Another thing may be in area oxygen and other humidity right, they also slowly can interact on your sensor other material.

So, its activity also can slowly decay or they may start reactions because humidity. So, what you will do? If you see that if you buy like very commercially available sensor like glucometer, Princeton test kit they are very they are actually storing in a very good inside a packet. So, may be that is inside you can put it like in your case may be in nitrogen environment you can or may be in vacuum condition right, that you have a store, ok.

Otherwise, you can see this kind of change I cyclic voltammetry the change, right. It can decay slowly, then whatever may be suppose you already made the calibration curve, right. So, that calibration curve some current you like some current you got because of some concentration and from the unknown sample you want to predict the unknown concentration.

But if somehow you are not getting your surface table, then you will be misguided that how much actual concentration present your target concentration present the real sample you cannot measure. So, your sensors should not change the catalytic property. So, that is a vacuum conditions or nitrogen environment you have to store or it should not react with other like other interfines species like oxygen or humidity also should not affect, that you should keep in mind.

And other things you have just all the reagents you can keep in the lyophilized conditions. Like this way you can improve the storage like a maybe long time you can keep for your sensor surface. And another things is a temperature, you have to keep in mind like can you store your sensor in the high temperature or not that you have to properly investigate, you have to properly optimize.

And which temperature your sensor is really active, if they are very much sensitive. Suppose you make your sensor at 25 degree Celsius and at 40 degree Celsius your sensor actually behave something different. Then when you use this one in some rural area may be you do not have much like refrigerated conditions then your sensor may not work, right. So, it should not be very much sensitive with temperature.

Also, your protein other protein that you are using that should not be denatured high temperature, that you should keep in mind. So, that all the things that I told you that is the stability of your sensor surface, that you should keep in mind, ok.

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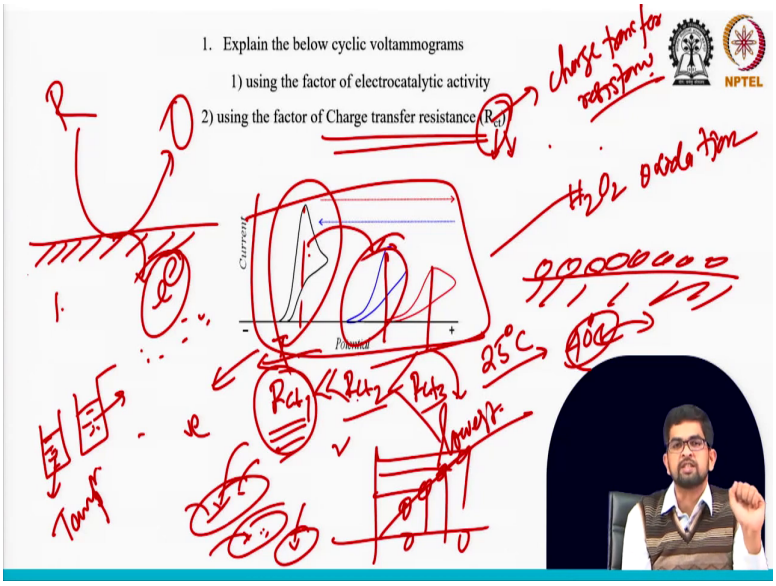

So, these things actually I just discussed because of to understands the catalytic property of the sensor surface. Now, just Let us come to the second part. Second part of this I just wanted to discuss that use this cyclic or voltammogram, I mean this cyclic voltammogram change you have to explain in the factor of charge transfer resistance, R ct. R ct R mean resistance c means charge, t means transfer. So, this is charge transfer resistance, right.

So, you know that charge transfer resistance this factor is depend on how fast this is sensor surface, how fast your electron actually transfer through the electrode surface. Suppose you have the reductant it is oxidizing right, it is in the electron so, how fast? So, you can see the number 1 the cyclic voltammogram, here actually electron is transferring very fast, right. That is why you are getting your oxidization speed in the negative side.

So, suppose this R ct 1 and the second case this is R ct 2, third case this is R ct 3, you just made you absorb it, right. So, you now you just think which one is the highest which one is the lowest. So, you can remember like when your sensor surface is highly active, if the electron can transfer easily, it means your resistance or impedance value is very low, because the electron can transfer easily, right.

So, it means R ct 1 should be very very low. So, it is very very low then the R ct 2. So, because its shift towards right in this cyclic voltammogram 2 in 2, it means its catalytic activity slow electron transfer slow R ct will be higher then R ct 3 it will be higher than R ct 2. So, that you can remember, but you have to explain the (Refer Time: 16:16) like this why. So, why this happen? So, also you can explain like little bit like this like.

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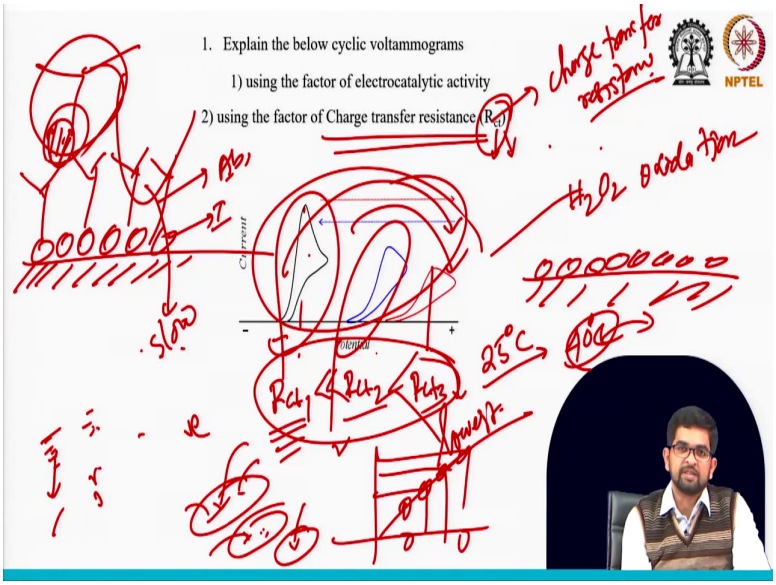

You can remember like; suppose you have this a sensor surface and you have some nanoparticle coated sensor and you have the antibody coated and then you have the antigen and then maybe secondary antibody. So, those all the steps you can characterize by the means of cyclic voltammogram and R ct, right. So, first steps only gold nanoparticle.

So, in this case you may get like this series, second step antibody coated. So, you may get like this series number two. And third case suppose after the additions of antigen or the antigen antibody. So, it will form of the sandwich then definitely what happened the electro catalytic activity slowly while electron transfer rate will be slow. Because they are the protein, they are this protein stock actually they are not that much highly conductive.

So, electron flow will be slow that is why it can be like this then R ct 3 will be very much high. So, your electro catalytic activity will be low and R ct value will be high. Like this way you can explain. So, there is cyclic voltammogram and charge transfer resistance here very much correlated at each other, ok.

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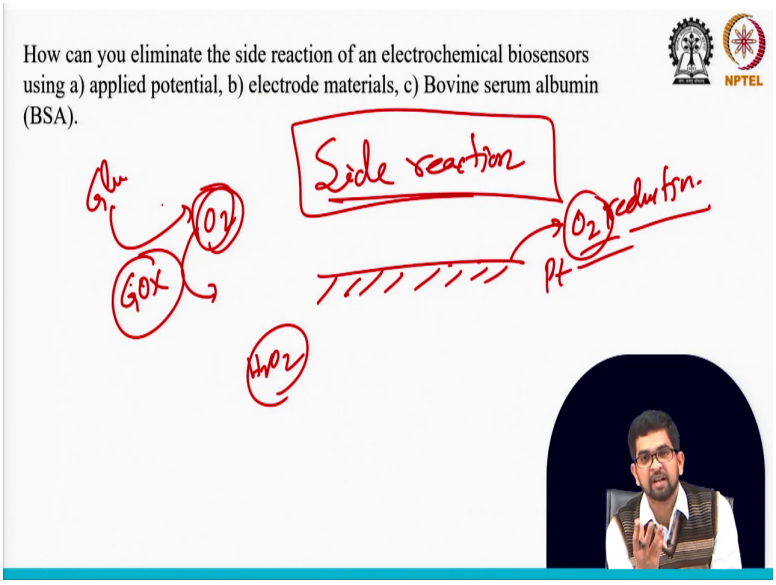

Now, next point how you can eliminate the side reactions of electrochemical biosensor? So, this is very very important factor side reaction. What is the side reactions of a biosensors? Side reactions can be like if your electrode surface is very highly catalytically active.

Suppose platinum surface is a highly catalytically electro catalytic active. So, in this case oxygen reductions will be very high. So, you are using a buffer sample, you are using some real sample, but it contain oxygen. So, it will show some like a background current. And if you may face reproducibility problem because of oxygen.

Because oxygen concentration may be different place to place, sample to sample. So, you may not get the similar background current because of oxygen. Also, because of oxygen reactions

you can see some current. So, they are the side reaction, right. Also, another factor oxygen. See you know the glucose oxidase when glucose will react.

So, in the that case oxygen also can take part, it will produce the hydrogen peroxide. So, these oxygen side reactions also can hamper. So, that is why you have to be very much careful about this oxygen dissolve oxygen effect in your biosensor development. But if you not get like it very much reproducible data then you have to think how you can reduce those effects.

There definitely there is some solution how to reduce those effect. So, as I told like if your sensor surface is highly electro catalytically active. So, in that case very definitely oxygen will show some effect.

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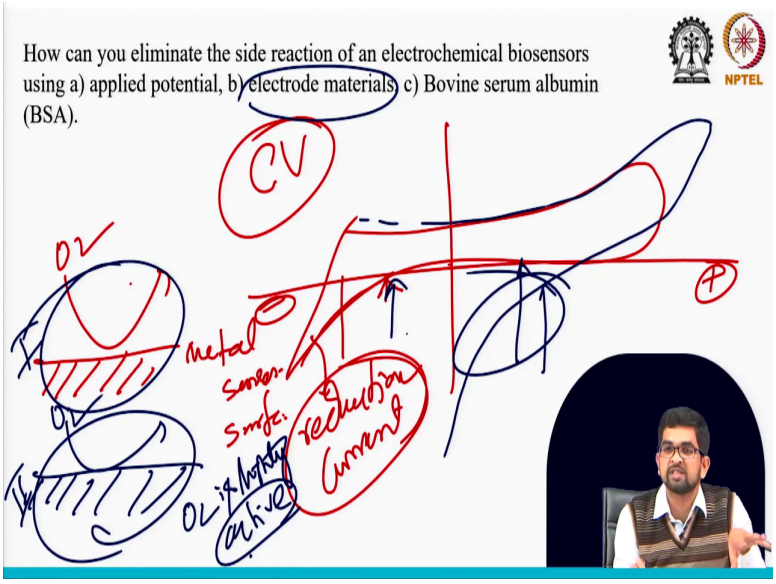

If you measure so, what you will do? You can measure a cyclic voltammogram on your sensor surface. Suppose you have a metal base metal base sensor right, surface. And you wanted to check the oxygen side effect oxygen, how oxygen is reacting on this sensor. What you will do? On the sensor in a buffer sample let us measure a cyclic voltammogram.

So, you see maybe cyclic voltammogram can be like this or it can be like this because it is negative it is positive. So, what you will see at certain potential slowly you will you can get some reduction current. You can see some reduction current at the negative side. Just in a buffer sample measure is cyclic voltammogram, you can see like this. So, you can see a cycle thus reduction can start away from these positions. So, from here is reduction starting. This is because of dissolve oxygen.

Now, you have the another sensor surface, you are measuring again cyclic voltammogram. In that case you may get the oxygen can reduce from here very early means here, it can start the reduction. It means in that surface oxygen is highly active, because it can reduce easily.

See, you may get if you apply in this potential suppose for your biosensor the oxygen will show its contribution. So, your number 2, if this is number 1 sensor surface this is the number 2 sensor surface the number 2 sensor surface is not good for the bio sensor development because of this oxygen side effect.

So, that is why if I tell you that let us decide the electrode material that is the number 2 questions the electrode material. So, you have to choose the certain material of the electrode that will show lower side reactions of this kind of oxygen, ok. Like this way you can select a best material.

That is why you should not select a very highly electro catalytically active surface like platinum or not also very very non-conductive. You have to choose some moderate one so that you can avoid those side reaction, clear. Now, let us come applied potential another factor.

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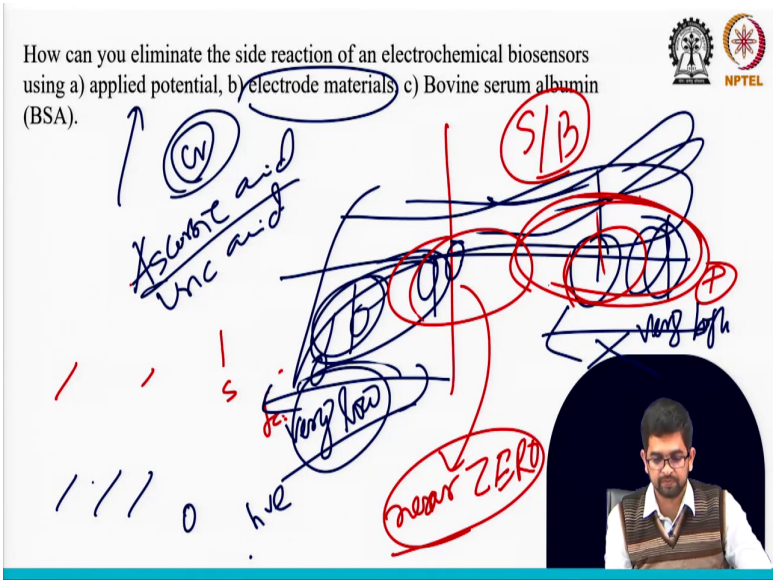

So, another factor is applied potential. See in the first cyclic voltammogram let us remove the second one, ok. Let us remove I as I told cyclic voltammogram is a very very important tools, you can get so many informations before development a biosensor. First measure a cyclic voltammogram in the your chemical solution. As I said that your reduction is started from here and you can see the here reduction is actually increasing, right.

So, what you have to do, your applied potential you can select. As you can see here oxygen reduction actually started it means side reaction is much, here do not choose this potential because there is you may get a high background current then select around this position.

Also, another factor it may come. So, do not choose very high potential again not very low, also not very high. If you apply very high then you have to think your real sample may contain some different interfering species, like as I said ascorbic acid or uric acid.

So, this kind of interference species, if you measure the cyclic voltammogram as I told this is a very important tools to get the information about your sensor. If you can oxidize you can see the ascorbic acid can oxidize here uric acid also can oxidize here.

So, it should not apply this much high potential. So, that is why we are avoiding this much high potential during the biosensor development. And we are avoiding very much low potential because there is some other background like oxygen dissolve oxygen related effect.

It is better the best selected potential is, which potential you can say? Is this region. So, this is called near zero near zero, this potential is the best for any biosensor development specially handling the real sample. So, here I am saying that this region is the best, but is depend on sensor to sensor, right. Some sensor case you may get in this position, but that is not maybe in that case maybe you are not handling you will see them, not maybe you are not handling the (Refer Time: 25:12) means something else you are handling.

So, in that potential may be, in that biosensor maybe in that by potential you may get higher signal to background ratio then you may choose. But in that case other background I mean other interference species that is red that can increase the background current. So, if you have to avoid those, ok.

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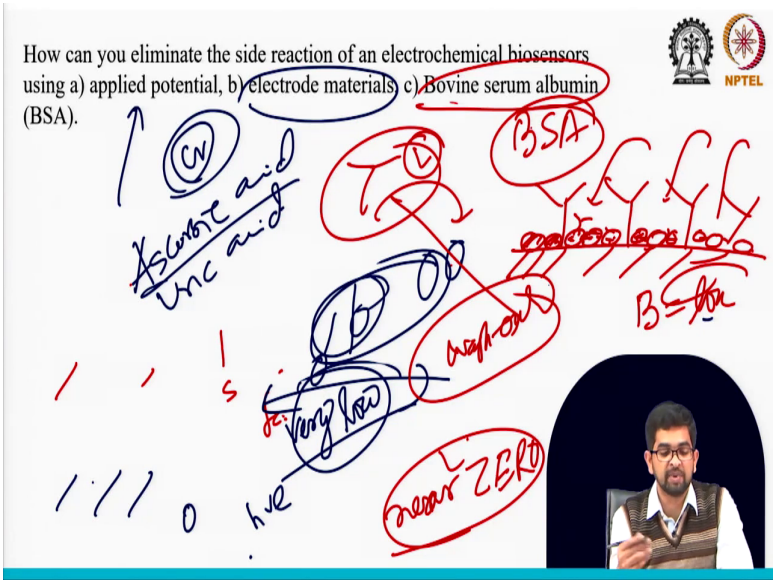

Now, let us come the another factor that is Bovine Serum Albumin. See Bovine Serum Albumin we are saying BSA that is very very important. You can use this for the development of the biosensor. Because this I think you can remember once you are immobilizing the antibody and also, we are adding some BSA. What is the role?

These you can bind the other non occupied phase the very small molecule, bimolecule and when you are going to add the secondary antibody that is conjugate some level they will not bind. Because sometimes you are getting the high background current because your secondary antibody that level that may also can bind here. All they do not have target, but they may bind here.

Although, you do not have target, but they may bind here although by you may have less target concentration, but if they bind here, they can increase the background current. But they

may. So, you may get the false positive data. That is why if you use the BSA, they will have not to bind the other like secondary antibody level they cannot bind.

Because after when you will add the secondary antibody and when you will wash the sensor surface, they can be wash out and your sensor will be your sensor current will be I mean background current will be low. So, bovine serum albumin can help like this way, ok.

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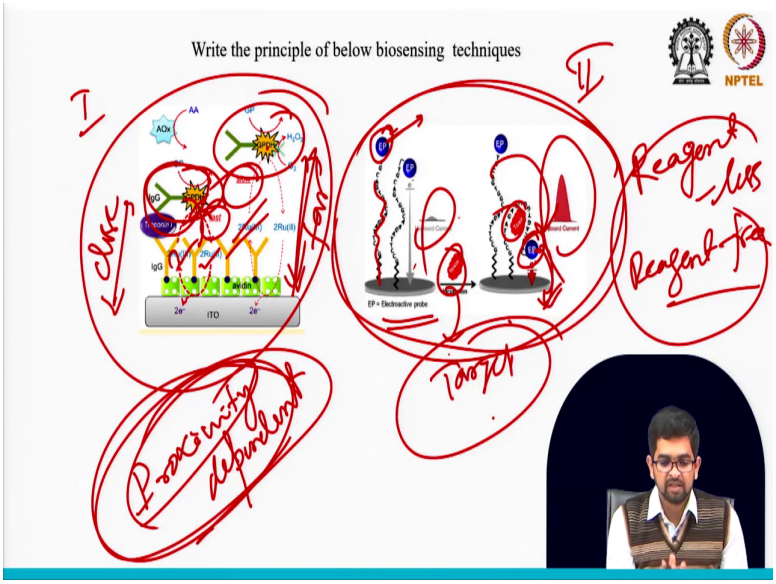

Now, the last problem for this today's tutorial is the discuss the principle of this biosensor. So, I may ask in this number 1 problem. So, what is the principle of this biosensor development? I taught the whole principle you can see your secondary antibody that conjugated with some enzyme like here GPDH some enzyme, right. And here the secondary antibody conjugated enzyme is the very far from the surface right, here far and here very close.

So, it is called proximity dependent, proximity dependent, alright. So, this is the principle of the biosensing development. In this case, what is the principle? It is a proximity dependency. When GPDH your secondary antibody is very close and you are getting this redox cycling reaction very fast. And when this one far then you are getting slow reaction. So, this is the principle that by using close this is very proximity dependent factor you can develop biosensor and you can differentiate the concentration, right.

Second problem, what is the principle here? Here you do not need any extra chemical, right. Only your DNA or (Refer Time: 28:38) or whatever that is already conjugated with some electroactive probe and they are highly electroactive. See here also proximity dependent, but it is reagent less reagent less or reagent free biosensor, right. You do not have you do not need any extra reagent in this biosensor, right.

Some of this is your target toxin your target. So, once target bind in a specific side, see it bind it bend and your electro active probe will come very close to the surface. Say in say first case proximity dependent here also we are applying the proximity dependent. But in this case, we do not need any extra reagent, but in the first case we need some extra reagents. So, this is also kind of a good sensor development technique. You can use proximity dependent at the same time no reagent. What is the principle?

They can actually fold they can bind once chemical present. Chemical means some toxin means some target present, we can fold, we can come close to the surface and we will get the higher current. Then without target like this way you can differentiate the concentration. So, like this I have to explain the principle and you can think something about biosensor design, ok.

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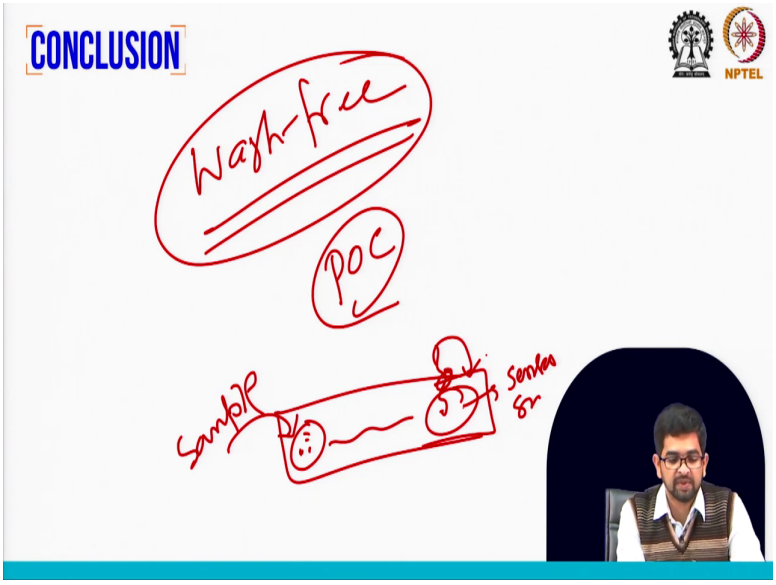

So, today's conclusion that is why I taught you some biosensor design. Although I explained many problems like some biosensors, like how to design like the like the wash free that proximity dependent, reagent free this is the very important tool. So, this kind of problem I discussed in the tutorial at the same time I just asked you how to design a new biosensors.

So, this wash free or reagent free technique is really important for point of care device development. Why? Because you do not need any sample handling, right. You just will drop the your sample here and it will go through the micro-produced to the your sensor surface, right.

And your if you have some anti like some like DNA that is very specific with this target then it will bind and it will show some signal change. Reagent free it will be very easier. That is why also wash free you do not need many washing. So, just drop and wait for the data. That is

why these two concept is really important for using development, use means point of care testing device, ok.

So, today I taught this tutorial and you learn this technique again. And I just guided you to how to apply those technique for the practical applications, ok. That is all for today's lecture.

Thank you.