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

Lecture - 27
Tutorial on Biosensors Fabrication (Continued)

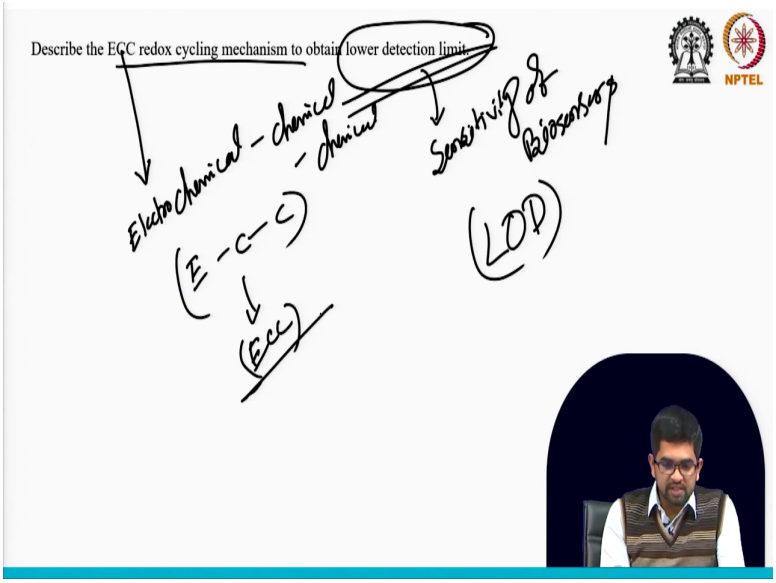
Students today I will teach you again some new questions some problems on nano biosensors. So, that you can plan accordingly the kind of questions may come during your exams, also at the same time this questions help you to think some more course related questions. So, this is kind of improvement your independent thinking.

(Refer Slide Time: 00:53)

So, let us start one by one; so, today that is why I will cover the mainly the tutorials the kind of questions may come in the exams. Also, you can think independently a kind of questions

may arises during the discussions of the nano biosensors are during the when you think something new project that time you also may think this kind of problems.

(Refer Slide Time: 01:15)

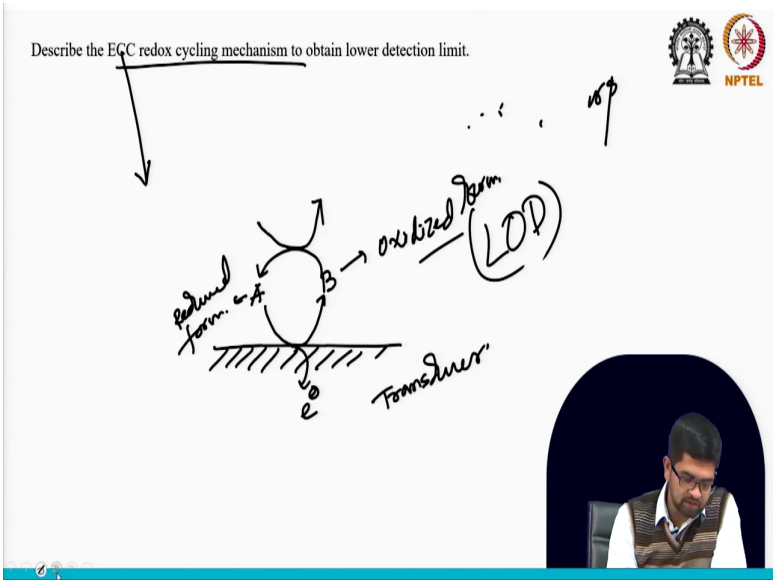


So, first of all first questions is the describe a ECC redox cycling mechanism to obtain a lower limit of detections ok. So, let show you the this question sensor, means if this kind of questions come how to solve it. Also, at the same time you can think like if you want to get the lower limit of detections, lower limit of detections this is also kind of your sensitivity right your sensitivity of your biosensor right.

So, if you design some biosensors how sensitive your biosensors for that you have to think about your limit of detections that is called LOD. So, first you have to design a ECC based biosensor; so, let us design like this way. So, because the question is describe ECC redox cycling mechanism to obtain the lower limit of detections.

So, let us first design and then describe the mechanism and how to improve the lower limit of detections ok. So, from the name itself you can start thinking to design and to think about the mechanism; so, ECC means Electrochemical Chemical right. So, that is why ECC; so, we are saying this one ECC redox cycling ok. So, let us first draw a basic ECC redox cycling is came ok, then you can explain the mechanism.

(Refer Slide Time: 03:21)

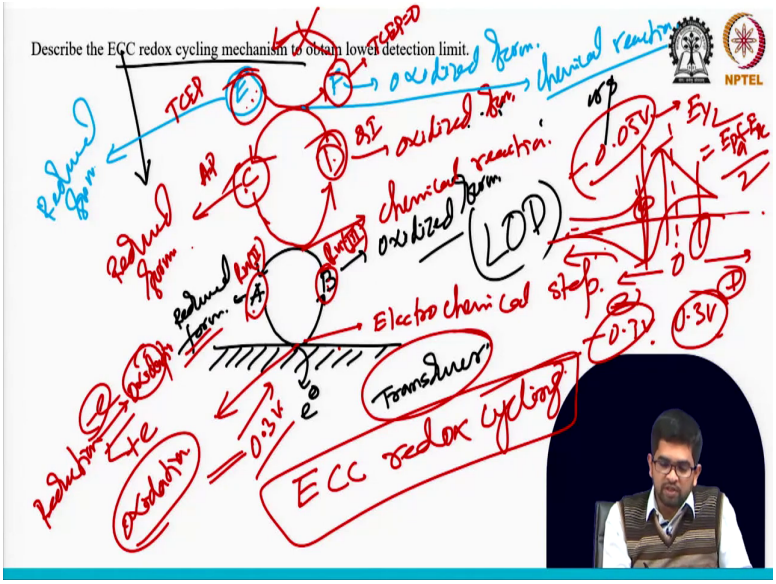


So, I will draw it first like if you have a electrode surface this is a transducer surface this is transducer surface right. ECC means here, you have to think like electrochemical right maybe your oxidations happen and then; so, this is. So, you should have a compound A and compound B mean some generic way you first think.

A; so, it is oxidizing on the surface; so, A should be oxidized or reduced form; so, it is oxidizing. So, it should be in the reduced form right; so, it is reduced form right; so, A would be in the reduced form. And B after oxidation; so, B will be in the oxidized form right.

So, now you want to convert B to A; so, you need some again here like this; so, again you need some something; so, that B can be again sorry it should be like this means the arrow you have to start thinking how you should put the arrow say. Say, A already B already oxidized to the means it is oxidized form; so, you have to convert B to A.

(Refer Slide Time: 04:55)



So, you need; so, B to A conversions you need a reducing species right; so, that B can be again reduced, suppose you have a compound C right. So, your C is the reduced form right; C

is the reduced form that is why C will reduce B to A and itself will be oxidized; so, D it will be the oxidized form right.

Now, you have to convert this D to C again right; so, this is the; so, this step is the electrochemical step right, electrochemical step. And this step means B to A formation, this is the chemical reactions B to A formation is the chemical reactions; so, this is the chemical step chemical reaction step right.

Now, one more chemical reactions will come that is D to C; so, you need here D to C means you need one reducing agent right. So, let us put one reducing agent any reducing agent; so, E to F. So, this E to F; so, A is the again you know E is the reduced form E reduced form and F is the oxidized form clear and this reactions E to F this reactions again the chemical reaction clear.

So, you have to explain the mechanism like this way fast; so, let summarize this mechanism, how? So, first you have to think that some chemical reaction this is the very basic step; so, see I just represented like A, B, C, D, E, F like this way mean. So, it is a ECC redox cycling E C C redox cycling ok. In this redox cycling; so, it is the oxidization based in the electro electrochemical oxidizations happen on the electrode surface.

So, on the electrode or on the transducers you are oxidizing a substance that is A right. So, this A should be reduced form this is in the reduced form and it form a B that is oxidized form. So, E have to take A again reduced form any C that should be in the reduced form; so, that here B will be converted to A again naturally you know the redox reactions reduction another is oxidation.

So, reductions to oxidations may it will release the electron, oxidation to reductions mean it will accept the electron right. So, when your C, your B will convert to reduced form; so, naturally your C will convert to the oxidized form because it already released the electron to the B. That is why it already released the electron release the electron itself oxidized; so, it is oxidized form.

Now, you have to convert again d to C; so, then you have to use a reduced form of on another reagent that is E. So, E is the reduced form and then you convert to this D to C again and its E itself converted to F; so, I am not going more cycling. So, just one electrochemical, one chemical and another is chemical, two chemical, one electrochemical.

This is the one mechanism for the ECC redox cycling; just try to remember here I am always saying in the surface is the oxidation right oxidation happen on the surface. But why not you are thinking opposite way? Maybe you can think on the surface reductions can happen right.

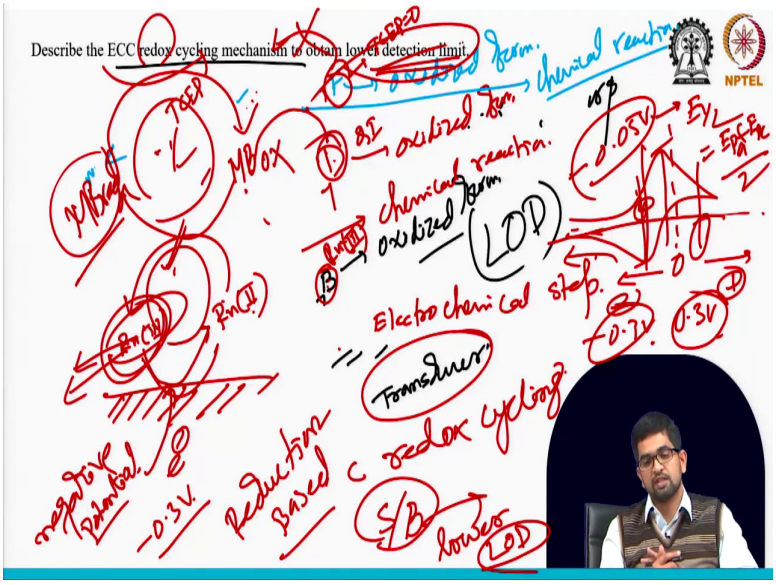
This means here actually we are applying some oxidations potential suppose 0.3 volt right. If you take like ruthenium II, if A is the ruthenium II; suppose for example, ruthenium II then B is the ruthenium III. And if C is the aminophenol and this one D is the quinoneimine and E suppose for example, TCEP and F; for example, this one TCEP O, but we can think the opposite way right.

Let us use ruthenium means somehow on a electrode surface a ruthenium III can be produced; so, it means your ruthenium C V something like this. So, here we can see like generally ruthenium cases this one 0 and in this side is the negative and this side is the positive. So, mainly we are getting the oxidation potential and reductions potential very close to the 0, it is minus around 0.05 volt this is the E half value right.

E half value you can remember like E half equals to E of the peak potential of the anodic peak potential plus E of the peak potential for the cathodic divided by 2, means this is close to 0. And if you apply like around here 0.3 volt, then necessarily oxidations will happen on the electrode surface.

Now, let us think if you want to apply the negative side potential like minus 0.3 volt; so, in this case the reductions will be predominant. So, opposite way like if you have on the surface ruthenium 3 right. So, let us show you like opposite phenomena this is oxidation base just I will show you very briefly the reductions based, let show you.

(Refer Slide Time: 11:46)



Reduction base will be; so, suppose ruthenium III; so, it will reduce right; so, it will get the electron it will get the electron. So, you have to apply some negative some negative potential ok some minus 0.3 volt you are applying. So, ruthenium III will form ruthenium II; so, you have to oxidize now ruthenium II to ruthenium 3 right. So, you can remember I taught you already that can be oxidized maybe in the presence of methylene blue.

So, ruthenium II is forming ruthenium III; so, that ruthenium II can be converted to ruthenium III. Again, if you can use methylene blue oxidized form; so, ruthenium II can be oxidized with the ruthenium III and itself methylene blue will form the reduced form clear. So, it is the reduction based this is reduction based and first one I have shown you that is oxidations based.

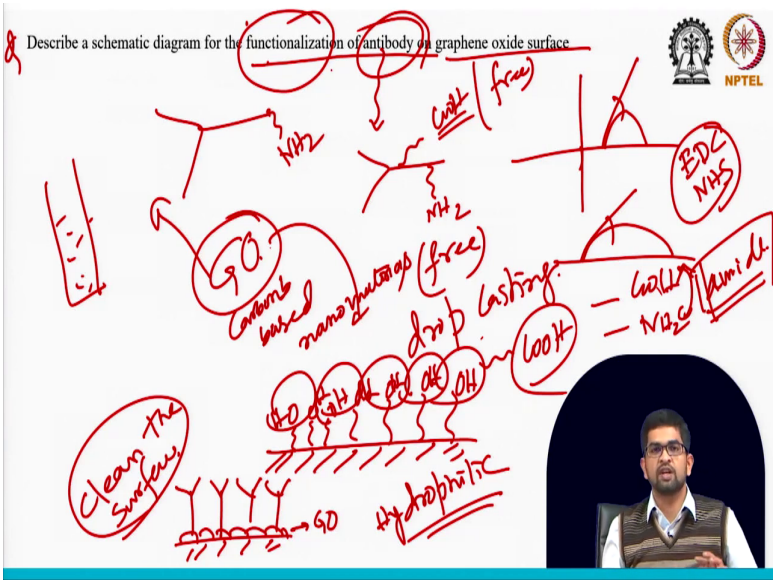
Now, again like now in your surface you have the methylene blue oxidized form that already oxidized ruthenium III, you have methylene; so, methylene blue actually converted methyl red. Now, you can convert again methyl red to methylene ox by using some oxidizing form, some oxidized form of something you can try that can be that can converted the red to ox second; so, like this way you can design some new scheme.

As I told you at the very first class to get the limit of reduction ECC redox cycling case; see, there is one cycling, another cycling, then there is a oxidation; so, reductions happen. Because of the cycling reactions on your surface electrode surface, your like the starting one that you are going to oxidize or reduce on the surface that will be repeatedly to be generate because of the cycling.

And it will generate very high concentration because of many cycling, very high concentration of these things and we will generate the high current. And very high current means, you will get the I mean you can amplify the signal your signal to background ratio; you can easily amplify, you can get lower limit of detections.

So, how ECC redox cycling is helping to get the lower limit of the detections? Because this many cycle will generate on the electrode surface move this oxidized species or reduce species whatever you want to oxidize or reduce on the electrode surface. It is concentration with time will increase and you will get high signal. Naturally, your limit of detections will be; so, very very low very sensitive you will get highly sensitive biosensor.

(Refer Slide Time: 14:53)



So, next questions now; so, in this tutorial I will teach you again some very important step that is the bio functionalization that I already taught you. So, let us summarize the bio functionalizations, how you can use this bio functionalizations process for biosensor development. Here I ask you questions; so, this questions is describe a schematic diagram for the functionalizations of antibody.

so, when we will ask this kind of questions immediately just think antibody means say, it has many functional group, amine group free that all this functional many functional amine group or carboxylic group. Free cooh; so, cooh I needs to be (Refer Time: 15:43) that you have to functionalize on the graphene oxide surface.

So, graphene oxide you know graphene oxide, it is the nanomaterial for carbon based nanomaterial, this carbon based nanomaterials. So, this kind of nanomaterials will be very much useful to functionalize different biomolecular on the surface.

See, you have the transducer surface; so, before the fabrications of graphene oxide graphene oxide, you have to clean the surface that is very common that you have to follow. So, first you have to clean the surface, the cleaning method I already taught you. Then you will have lots of hydroxyl group on the surface, because this surface will be very much hydrophilic, this surface is very much hydrophilic after the cleaning hydrophilic.

So, what is the hydrophilic? Hydrophilic it can attract the water easily. So, if you measure the contact angle, contact angle may hydrophilicity, hydrophobicity you are measuring. So, if you measure the contact angle; so, your water droplet will be like this means you will get lower contact handle. But if your surface is not clean properly, then you will get means in this case you will get the water droplet like this means your contact angle will be very very high.

Now, you just drop cast your graphene oxide on this electrode surface, you have to follow the drop casting drop castings means some graphene oxide. You can prepare in a test tube you have to disperse it in a water DI water in a solution you disperse it. Then you can drop cast I mean just drop it on the sensor surface then you have to wait for drying; once it is dry, then you can use for antibody immobilization.

So, once your surface dried; so, it will be covered by graphene oxide and in graphene oxide also has many functional growth. So, one important functional growth is the carboxylic acid ok; so, it also has some carboxylic acid. So, now let us come the functionalization; so, your antibody contain amine group NH2 and your graphene oxide that content carboxylic acid.

So, now C double OH NH2 group you can couple these two, they can form the amide bond right. But this amide bond the thermodynamically you can ok, but kinetically very slow; so, that you have to use EDC and NHS. They can catalytically they can react each other and very fast they will form the amide bond on the surface.

And your surface will be ready like the nano nanomaterial coated that is the graphene oxide and your antibody will be immobilized on the surface. Now, this sensor surface is ready for the detections clear; so, like this way you can draw a schematic diagram for the functionalization of the antibody on the graphene oxide surface ok.

(Refer Slide Time: 19:44)

Ok, now, next questions this is the very important class I took last time like sodium borohydride treatment on the nanoparticles and use them for biosensing applications right, you just remember the NABH 4 right. So, if you want to treat some nanoparticles suppose you wanted treat your gold nanoparticles right, your sensor surface. Maybe you have some you know primary antibody coated your sensor surface and if you drop your target like antigen it will wind.

Now, your secondary antibody that is conjugated with gold nanoparticles right, that you can use this gold nanoparticle for the sodium borohydride treatment. So, what happen the sodium borohydride treatment? So, if you sodium borohydride means NA plus BH 4 minus.

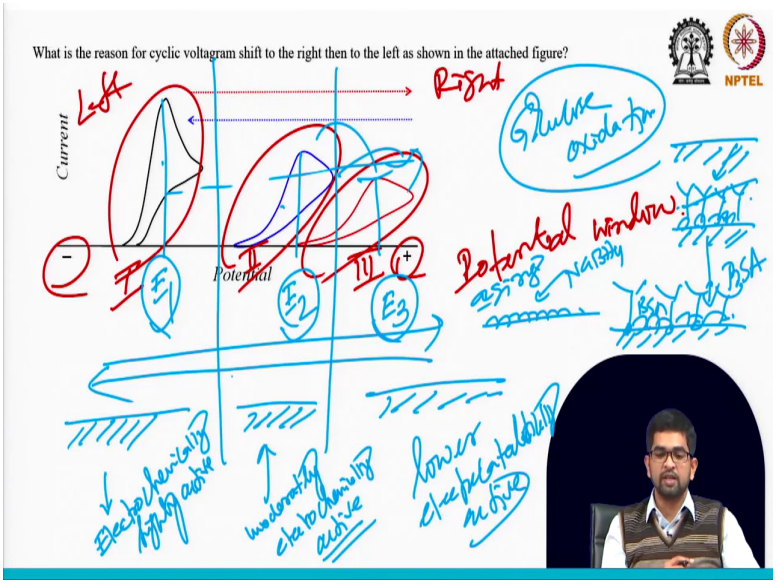
So, this borohsydride it will form atomic hydrogen atomic hydrogen, this atomic hydrogen will adsorb on the gold nanoparticle surface these all and they will adsorb. And because of this hydrogen adsorption it will form a very this surface become highly active right you can remember highly active surface; so, this mechanism you can mention.

So, once this secondary antibody 2, this is antibody 1, antibody 1 when this antibody will come secondary antibody with gold nanoparticles as it is highly active that will be very useful for very good signal amplification. So, you get very high signal because, it is very much active; even last time I told you., instead of gold you can try palladium nanoparticles also.

Because, the activations of palladium (Refer Time: 21:24) passed also you will get very high signal with sodium borohydride. So, the reason is this that hydrogen adsorptions can happen on the outer surface and it will activate the surface it will form, because of surface reconstructions. So, there is a chance of to get the high signal and naturally your limit of the detection will be very low ok.

So, if your limit of detection is very low, then your bio biosensor will be called the ultra sensitive biosensor ultra sensitive biosensor ok. So, like this way you can think how to design a ultra sensitive biosensor, how to get a lower limit of detections very low limit of detection you may get. And where you want to apply this sodium borohydride treatment, how this treatment happen this is the mechanism, clear this questions ok.

(Refer Slide Time: 22:25)



Now, let us come another questions I think that also I taught you now let us show you systematically how to answer this questions. First questions is what is the reason for cyclic voltammogram shift to the right and then left as shown in the attached figure.

See, first from left to right this cyclic voltammogram is shifted from. So, this is the number I, number II, number III; so, this is the minus to plus you can remember the left side is the negative right side is the positive this is called potential window we can remember potential window.

So, we will scan a negative to positive, positive to negative; so, this whole scan window is the potential window. And based on the positions of this potential window we can decide the activity of the electrode surface. If you add some reagent on the electrode surface how it will

behave, means it will react fast or it will react slow that this cyclic voltammetry can give you the information.

So, cyclic voltammetry is really important tool really important technique that can guide you that how your surface behave. Based on the cyclic voltammetry positions, based on the cyclic voltammetry current you can think. See; so, what is the reason for cyclic voltagram shift? See, in the number I, in this number I case you are getting; so, so total 3 cyclic voltamogram.

So, here your oxidations potential this one E 1, number II case your oxidations potential E 2 and number III case your oxidation potential E 3. See these oxidation potential actually slowly shifting from left to right; so, you need more energy basically to oxidize this species on your electrode surface.

So, in this case you need less energy and this surface is highly that is why you can say electro catalytically active. So, your surface highly electro catalytic active because you can oxidize the species; suppose for example, this is the glucose oxidations glucose oxidation.

So, you are oxidizing glucose on the surface and it can be oxidized easily in the very low means a negative potential low potential. So, you can say this surface; so, you can think like 3 surface; so, number I surface is electrochemically highly active, this surface electrochemically highly active because it can be oxidized very easily.

But in the second case is shifted; so, why this shifting can happen? It can be because of the aging, because of the contaminations or because of the your biomolecule fabrication. Suppose you have the very bare gold surface; so, you may get this kind of cyclic voltammogram; now, your bare bare gold surface you already immobilize a antibody.

So, after immobilization antibody definitely your surface electrocatalytic activity will decrease. And because they all are the protein, they are kind of non-conductive that is why it means your substrate can be oxidized in the higher potential you need higher energy to

oxidize this substrate. That is why this cyclic voltammogram flows that your electrode surface is moderately electrochemically active, moderately electrochemically active ok.

Now, third step third cyclic voltammetry it is showing that again shifted towards right. So, it means if your sensor surface after the antibody maybe you are adding here BSA right, Bovine Serum Bovine Serum Albumin; so, that you want to block the other empty part. So, that you can minimize the non-pacific binding; so, your surface now we can looks like this second antibody and so, that is nano-material somehow and here BSA so, BSA.

So, that is why your surface become little bit again non-conductive; so, it may be low lower electrocatalytically electrocatalytically catalytic lytically active. So, like this way you can characterize your surface step by step that yes, your surface actually modifying by it is modifying by some and as you added the antibody yes antibody there you added the BSA that is why it is decreasing.

So, like this way you can characterize the surface; so, reason is that is why. The main reason is that the electrocatalytic activity actually decreasing when you are moving from left to right. There is another family like aging effect I told you, the aging phenomena last time I told you know that gold nanoparticle. If you have coated the gold nano on the surface.

If you kept a long time like 1 month it can decrease the surface then if you check the cyclic voltammogram it can shift here. But now if you add the sodium borohydride and if you can activate then again you can see the shifting form again right to left it means again electrocatalytic activity increasing.

So, this is also because of electrocatalytic activity shifting from lower to higher electrocatalytically active your surface that is why; so, this is the reason like this you can explain. See so, like this today I just discussed these all the questions that actually help you how to design a sensor and how to explain. Definitely if you can explain then only you can move to the next step.

Generally, when you characterize some biosensor when you design a some biosensor, every time we are characterizing a by using the cyclic voltammogram or by the impedance spectroscopy the next tutorial I will teach you some impedance spectroscopy also kind of problem. So, that you can use them for biosensor characterizations and you can conclude; yes, biosensor is already formed or not right.

(Refer Slide Time: 29:37)

So, this kind of problem actually will help you to characterize ok. So, that is the conclusion for today's talk that just think the problem and how to solve this and this problem can guide you to go to the next level like if you have one problem just think the another problem may come from this. And you may get lots of other solution and you can design some new sensors ok; so, that is all for today's tutorial.

Thank you.