Nanobio Technology Enabled Point-of-Care Devices

Prof. Gorachand Dutta
School of Medical Science and Technology
Indian Institute of Technology, Kharagpur

Lecture - 25
Tutorial on Biosensors Fabrication

Dear all, today I will start some tutorials. So, these tutorials will help you to understands all the problems like I taught you many designs, how to develop biosensors, how to develop different nanobio devices and then use them for point of care diagnosis. But if you when you see for exam kind of questions may come how to solve this it should be trained properly right.

So, this class that is why how to train you for exam, like kind of questions may come and these tutorials also you may get lots of problems and you may get some novel concept to think for your future like if you go for your higher study, if you go for internship or other institute you may work on this kind of small small projects also. So, this kind all the problems I will show you and I will give you some training. So, that you can think independently let us come those all the problems.

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So, today mainly I will cover the basic tutorials for this whole course. So, let us come one by one all the question and during the discussions of the answer of the question you also may find out and the new design and I will also solve again the new design. So, it is not only the discussions of the questions and answer also same time I will show you the new problems that you can solve based on the current problems current situations and like the COVID situations you are facing, the new issue like how to deal with the COVID like recently may be dengue.

So, how to develop and why to develop, where to develop like it all sometimes specific to the region to region that also you will get some concept.

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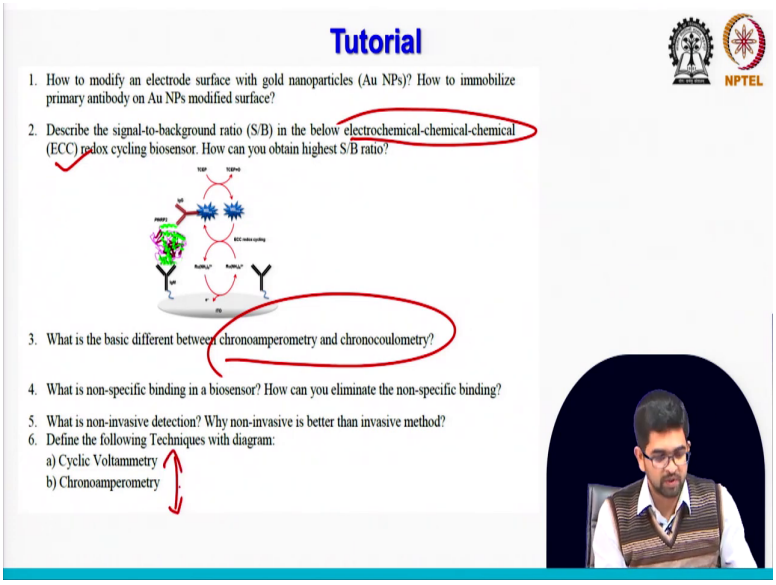

So, you can see here I already made a list of the tutorials that how to modify the electrode surface with gold nano particles. So, I already taught you how to synthesize the gold nano particle, but how to modify the gold nano particle on the surface. But now systematically you can understands like question to questions how much you have to write. Like I already gave you the clear concept.

Now, let us focus on when you write the exam how to start how to complete this problem. So, first problems I will solve like this way and the second one is the describe the signal to background ratio for the electrochemical chemical redox cycling. You already know like ECC redox cycling I taught and I think you can solve this issue.

But if I ask you the design or describe here which one signal, which one background and how we can minimize the background those things in this problem and how much you have to

write in the exam that I will cover. Then what is the basic difference between different techniques here I just mentioned chronoamperometry chronocoulometry.

Maybe I will bringing the cyclic voltammetry that I will show you. So, then what is the non-specific binding of a biosensor? So, you know all non-specific binding let us show you the answer then how we can eliminate and what is the non-invasive detections and why this non-invasive is the better than other invasive approach. Then you can define the different technique. So, how much you have to write, how to write good way to get the good marks.

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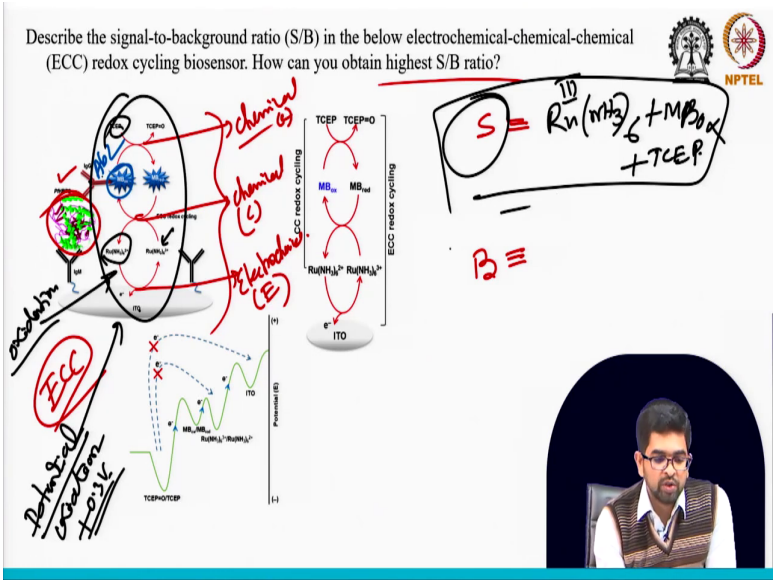

So, let us come then one by one all the questions. Let us describe these questions first. So, here I just ask describe the signal to background ratio in the below electrochemical chemical redox cycling biosensor. How you can obtain highest signal to background ratio? So, this is the question this kind of questions may come during the exam.

Let us solve it and I do not want like a one phase writing just very coin size I mean very simple way you can express your all the answers like point by point. So, first questions here describe the signal to background ratio in this below electrochemical chemical redox cycling. So, if I gave you this kind of diagram. So, you already understands right. So, here is the electrochemical reactions right electrochemical see and here chemical reaction right and here chemical right.

So, this is the E that is why this is E C and C right that is why it is ECC redox cycling it is very much clear. But signal to background ratio. So, you have to find out which is signal and which is the background.

See I already taught you in the class signal means in the presence of the target. So, here target is your P F H R P 2 your malaria target this antigen. So, if this antigen present then only this secondary antibody will bind right. So, this secondary antibody two this will bind once this target come.

So, this secondary antibody already conjugate with methylene blue. So, your signal is responsible if your methylene blue present. So, let us then combine everything like your signals is then the full this full reaction is because of the signal right. So, you have to mention see in the first things is you just have to remember in this surface you are releasing the electron releasing means oxidations right can you remember? This is oxidation because electron is released on the surface.

So, on your surface ruthenium 2 actually generated because of this redox cycling ruthenium 2 actually regenerated that is why during the cycling if you use one more two more three more cycling more ruthenium 2 will be generated right on the surface that is why we will get the higher signal. So, your starting region not there in ruthenium 2 your starting region is a ruthenium 3 and its actually reduced to ruthenium 2 that you will oxidize on the surface because of some applications of potential.

So, you need to apply some potential. How much potential you have to apply? That is the positive some oxidations potential right. So, suppose you have to apply some oxidations potential with plus 0.3 volt you are applying. So, you are going to measure some chronocoulometry or chronoamperometry for the (Refer Time: 07:07) potential.

So, your you know starting reagent that is why ruthenium hexamine R u n H 3 6 III right this one this one your starting reagent and your methylene blue that is conjugated with the secondary antibody right that plus M B methylene blue O x this form right because this one actually will react with the T-CEP. So, your T-CEP also should be there.

So, your signal is responsible for this reaction ruthenium hexamine 3 methylene blue T CEP that you have to write which one is responsible for your signal and background. Which one is responsible for the background? That is pretty clear just remove the target you have to remove the target.

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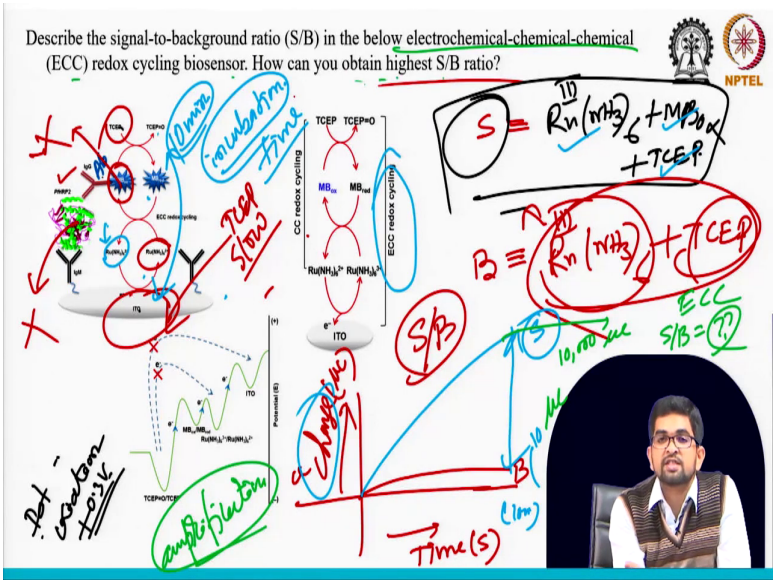

So, your target if you remove this one then you will not get the methylene blue. So, then remove this one. So, you will have ruthenium hexamine 3 and T-CEP. So, R u N H 3 whole 6 III plus T-CEP. So, this is your background and that you have to mention just very precisely no need to write like the one page sentence you can write what is the signal, what is the background and then signal to background S by B you can easily tell that and you can you have to get the like if it is chrono-amperometry or chrono-coulometry.

Suppose you are measuring chrono-coulometry CC. So, in the y axis you are measuring current or charge. So, I assist charge like micro coulomb and x axis is the time right like suppose second in the unit. So, signal and background. So, background you may get this much this is the background why you are getting too much low current because ruthenium hexamine 3 already oxidized form T-CEP is not reacting very fast on the surface.

So, on surface T-CEP reacting very slow that is why you will get very low background current clear and your signal will be ruthenium hexamine 3 methylene methylene blue X and T-CEP and always you have to keep in mind while you are using this kind of redox cycling you have to incubate incubation you need you need some incubation time.

What incubation time? May how long you are after mixing all the chemical how long you are waiting before the signal measurement how long you are waiting suppose after this adding all the reagent you are waiting 10 minute. So, 10 minute will be your incubation time to measure the signal and background and this time you have to keep always constant.

So, background case if you take 10 minute incubation and signal case also you take the 10 minute incubation then only you can compare the both data otherwise see if you incubate more than 10 minute, but slowly this also reaction can be little fastest because ruthenium hexamine 2 plus can be generated little more.

So, you cannot compare. So, always you have to incubate background and signal same time ok. So, your background signal is ruthenium 3 methylene blue x T-CEP and maybe you are getting this much signal. So, this much different current means in this case charge this much charge you are getting. So, suppose in background you are getting 10 micro amps. Sorry this one is the coulomb like it is the charge right. So, in this case. So, just you have to remember this one will be you know the unit right.

So, in this case the unit will be micro coulomb and signal because of ECC redox cycling only you are enhancing the signal to background ratio you are getting you are developing a ultra sensitive biosensors. So, it will be definitely around maybe 10000 or maybe 1000 something like this micro coulomb. So, something like this. So, then you can divide like signal to background ratio then how much you are getting that is your I mean amplifications right and that amplification will guide you how sensitive your biosensor right.

So, based on these questions now see I am just already combining all your concept here. So, discuss the signal background ratio in the below electrochemical chemical ECC redox cycling

biosensor how you can obtain the highest signal to background ratio. So, I already discussed now you have to find out these things like this answer now you have to write how you can obtain the highest signal to background ratio.

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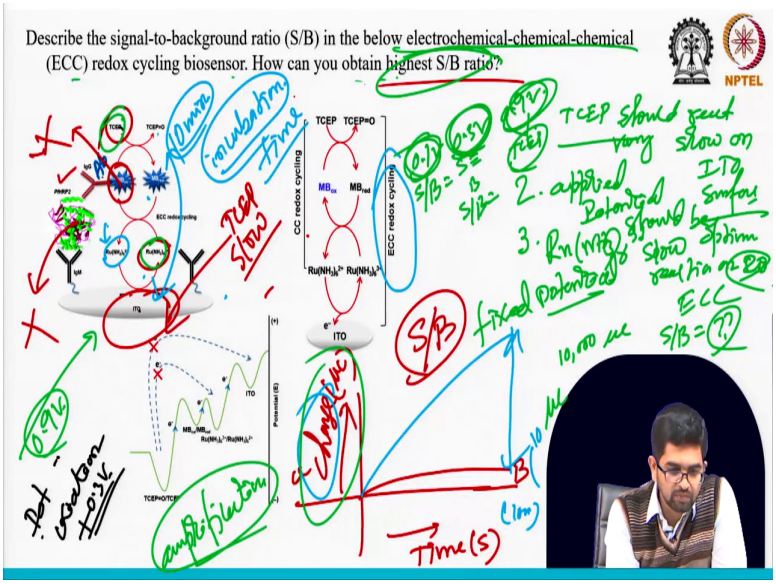

So, I will prefer let us try to answer it point by point like first to get the highest signal to background ratio your background should be very low. So, your T CEP should react very slow on ITO surface right. This is one point because if T-CEP react very low on the surface then you will get low background current. So, you have to select a potential because you are applying some potential right see this is chrono-coulometry here you are applying a fixed potential.

So, on that potential this T-CEP are oxidizing, but it is swings very low, but you maybe you may apply like not point may be 0.9 volt at that high potential T-CEP can be oxidized it may

cause high background current. So, it should react slow that is why your applied potential should be optimum; should be optimum.

Why I am saying optimum? Because some of the cases maybe you are applying suppose 0.1 volt or 0.3 volt or 0.9 volt. So, 0.9-volt case you are getting maybe T-CEP is reacting very very slow, but your signal also very slow then your signal to background ratio will not be that much high, but at the 0.3-volt case your background is little high, but signal is very high.

So, then signal to background ratio will be very high right then we will choose the optimum potential 0.3 volt not 0.1 volt, but 0.9 volt why we will not choose because in that high potential your T-CEP can be readily oxidized its reaction will be very very fast that is why very high potential I should not choose. So, we should choose a optimum potential ok and another things just I have remembered that is ruthenium hexamine 3 R u N H 3 whole 6 3 plus this is also your starting reagent.

So, this also should react very slow right. So, this is also slow reaction this also should react slow reaction on ITO then your answer will be completed right and you will get the full marks. That is the things you have to remember.

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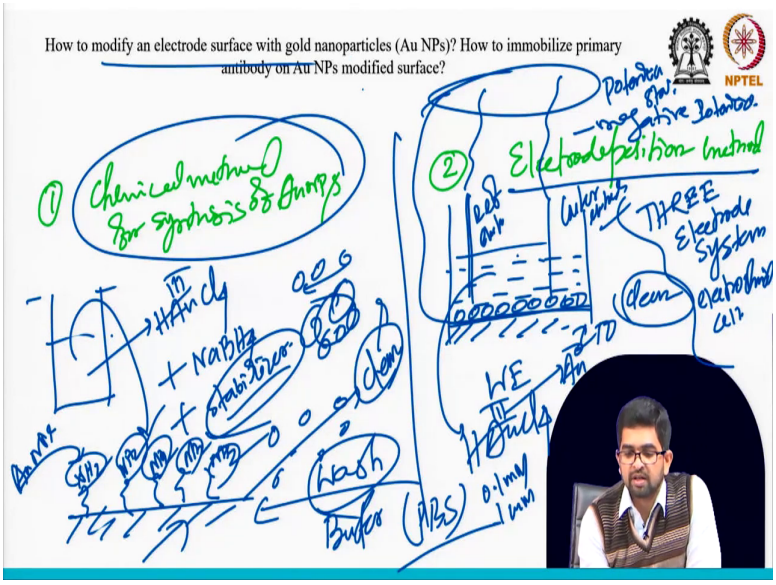

Let us come the second questions how to modify an electrode surface with gold nanoparticles. So, how to modify the electrode gold nanoparticles? I taught you two method one is chemical method for synthesis of gold nanoparticles right and another method is the electro depositions method; electro deposition method.

So, you can solve both the method for your gold nanoparticle modifications on electrode surface. So, chemical method what do you will say you will synthesize on a bigger on a test tube a gold nanoparticles. So, how you will synthesize? You have to use H A u C l 4 a gold salt right while and you have to use a reducing agent. Suppose, sodium borohydride you are using plus a stabilizer why you need a stabilizer?

Because it will not means make the all the nanoparticles together like a not make a coagulation when coagulate coagulation means. So, that all the nanoparticle should not mean

come together make a big particles right no not a (Refer Time: 16:16) like this. So, this should be well separated or mono disperse for that you have to use a stabilizer.

For example, sodium citrate that you can say right by using this one I will synthesize the gold nanoparticles then I will draw to this gold nanoparticle on a amine functionalized surface right as I told amine functionalized surface. So, why we need the amine functionalized surface? Because amine group and gold group gold nanoparticle they have the strong interaction like an easily bind in the surface.

So, because after the modifications of the surface you have to wash the surface thoroughly with some buffer sample buffer means something like PBS phosphate buffer saline you do wash it. So, the non-bound one will wash out from the surface and we will get a reproducible surface every time. Otherwise, if you not wash properly number of nanoparticles may be not same always.

So, you have to be careful about the washings also and this amine modification of the surface I think that I will I already taught you maybe I will show you some more questions how to modify the amine surface how to functionalizations that I will just give you more example, but that I have already taught in the class.

So, like this way you can answer one part you have to first chemically synthesize the gold nanoparticles then you can draw from a amine functionalized surface. Just keep in mind that you already know that during the class I taught, but no need to write the answer everything on a questions (Refer Time: 17:57) I mean in the answer sheet while I give you the questions this kind of question.

Before the amine modifications you have to clean the surface properly right you can remember show that your surface become more hydrophilic not hydrophobic that process you have to follow, but that no need to write during the your exam like this way. So, chemically mean chemical method you can complete.

Now, come to the electrodepositions method. Electrodepositions means, you have to take a very clean electrode surface suppose ITO clean surface right always you have to clean. Otherwise, if there is lots of impurity and if you, I mean deposit your gold nanoparticle on the surface. So, they will actually deposit on the impurities. So, they the surface will not be that much reproducible and that is not good for the biosensor development ok.

All I is you have to clean properly the surface before depositions can any kind of nanomaterial. So, this surface you have to use as a working electrode on a electrode three I mean three (Refer Time: 19:01) three electrode system right you know three electrode system three electrode system electro chemical cell electro chemical cell.

In the three electrode system electro chemical cell your ITO will behave as a working electrode and you have to use the electrolyte right the electrolyte will be your chloroauric acid right HAuCl4 and we will not use very high concentrations of the chloroauric acids we will use like 0.1 millimolar or maybe 1 millimolar chloroauric acid. Then you can dip your reference electrode and your counter electrode ok.

So, reference electrode, counter electrode, your working electrode then add this all three in a potentiostat right potentiostat and in the potentiostat now we apply generally we like here to apply negative potential. So, that your HAuCl4 that is three oxidation state it will go to the gold nanoparticle that is zero oxidation state. So, you will apply a negative potential negative potential ok.

So, once you apply negative potential then gold nanoparticle will be deposited here and you will get gold nanoparticle deposited ITO surface clear like this way so, you can modify electrode with gold nanoparticles ok. So, this part done, now is the second part of the questions. So, in the first part of the question there was two part right how to synthesize one is the chemical process and another is the electrodepositions process.

Now, let us come here like how to immobilize the antibody see your surface you have lots of goal nanoparticles.

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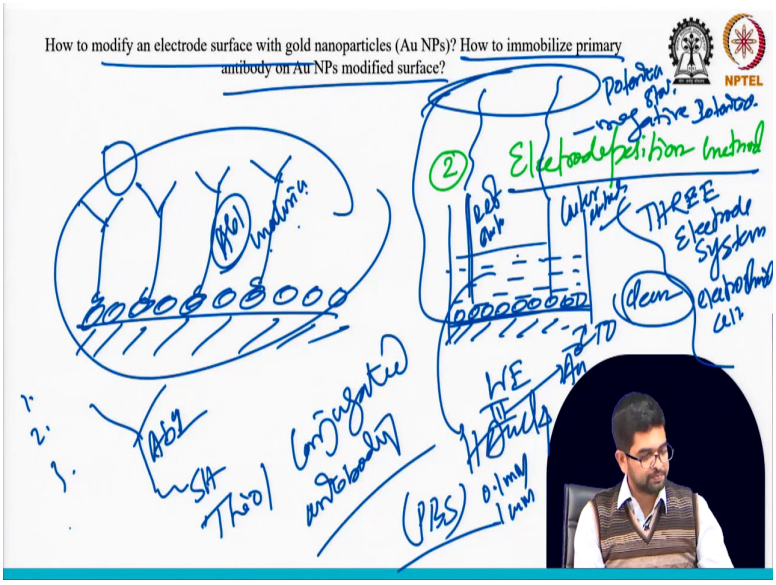

Now, let us immobilize your antibody. So, you can choose a antibody primary and Ab1 with thiolated means thiol conjugated thiol conjugated antibody. Why I choose thiol conjugated antibody? Because gold and thiol they has very strong in to the they are very strong interactions immediately they can bind maybe few minutes is enough then thiol and because of thiol and gold, you will get a very good sensor surface antibody modified gold surface and now this surface is ready for detections.

So, this surface now ready. So, now specific suppose your immobilization malaria antibody then it can detect malaria if it is dengue and dengue means you can detect dengue if it is any kind of cancer it can detect the cancer. So, this is the basic concept you can write during the exam not everything, but like point by point 1, 2, 3 like this way if you can answer that is really good.

But generally, in this NPTEL I will prefer to give you some MCQ type so, that you can try to you have to remember this kind of the concept and you have to solve the question, but as this is the tutorial, I am just giving all the concept and like this way you can think some new idea also.

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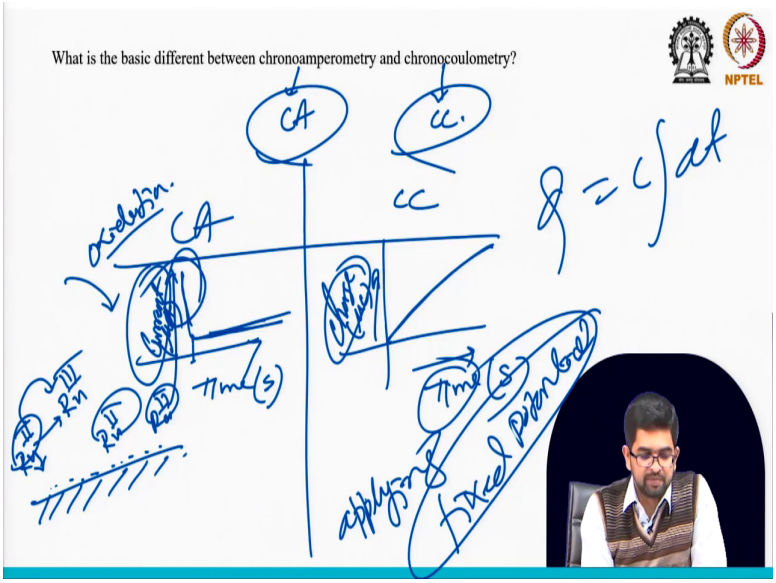

Now, here you can see what is the basic difference between the chrono-amperometry and coulometry. I have already told you I have taught many times what is the chrono-amperometry what is the chrono-coulometry. Amperometry CA, coulometry CC. So, let us make a basic difference CA and CC. See both cases you just try to remember CA like this right CC like this.

What is the y axis? y axis is the current, x axis is the time as I said without unit it is meaningless. So, always write time second current is micro amps and coulometry y axis is a charge lomb micro-coulomb and x axis is the time second.

So, that is the unique difference that here we are measuring current with respect to time and CC case we are measuring charge with respect to time ok and C here chrono-amperometry case like it is at the very beginning see the current is very high then its decrease decrease decrease and it will be it will slowly it will be equilibrium.

Why it happened like this? Because on the electrode surface suppose you are because here suppose you are oxidizing here right this is oxidation with chrono-amperometry right you are oxidizing. So, at the very beginning oxidizing means you have the ruthenium 2 right. So, at the beginning you have many ruthenium 2 on the surface.

So, when you apply some potential immediately you will get the high current because you have very high concentration ruthenium 2 and then ruthenium 2 will form ruthenium 3 and there will be I mean after that. So, at the very near surface at the beginning there is lots of ruthenium 2 that is why you are getting a very high current.

Now, ruthenium 2 concentration decrease because ruthenium two converted to ruthenium 3 and that is the current will decrease decrease and after some time and maybe it is 6 second or 10 second there will be equilibrium of the 2 and 3 then we will get like almost stable. And these things, but in the chrono-coulometry case. So, you are actually integrating right in the chrono-coulometry case you are integrating all the current with respect to the time.

So, with respect to time as you are integrating it. So, always you will get with time with increase increase increase and another another point you just have to mention the similarity of this two topic that always here you are applying a fixed potential right fixed potential that you have to keep in your mind both cases you are applying fixed potential right.

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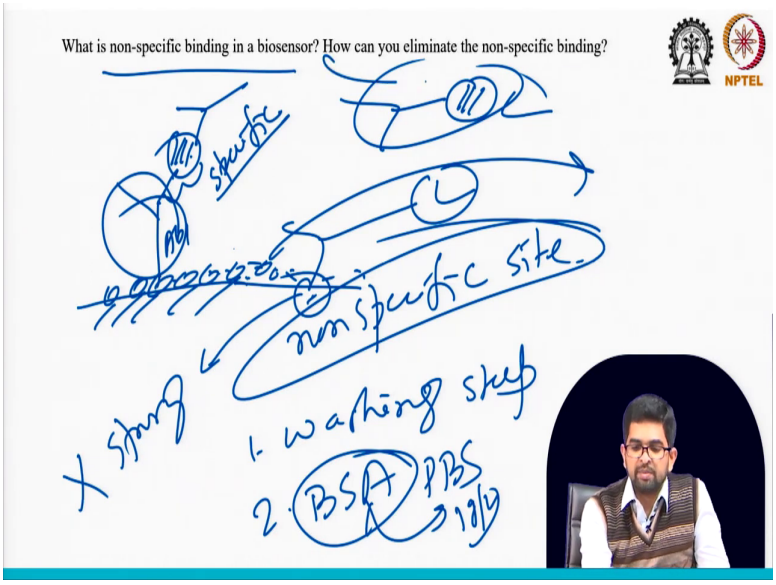

Now, the next questions what is the non-specific binding for biosensor how you can eliminate the non-specific binding right. I already taught you the non-specific binding non-specific binding what before that you have you should know the what a specific binding.

So, if you have biosensor and if your target bind with the antibody then this is this antigen antibody binding is the specific binding. But non-specific means suppose this target is not bind here suppose maybe other side of the electrode surface your secondary antibody that is already level. So, it should bind with the antigen with target, but if it bind here on the electrode surface somewhere then it is a not specific site right it is non-specific site non-specific site. So, this is the non-specific binding.

So, how you can eliminate? The best things is the you can wash first thing is the wash washing step if you wash properly because this binding is not very strong binding not strong

not strong. So, if you wash it properly it will be wash out this is the one best way and second method BSA Bovine Serum Albumin small small protein you can use. So, that if you after the primary antibody binding if you use the BSA on the surface.

So, that the secondary antibody may not be may not bind on the surface very strongly during the washing they can easily wash out. So, you can add PBS buffer and BSA BSA around maybe you can try suppose few like 1 gram per litre something like this you can make a solutions and then you can drop the surface on the surface like this way you can eliminate the non-specific binding.

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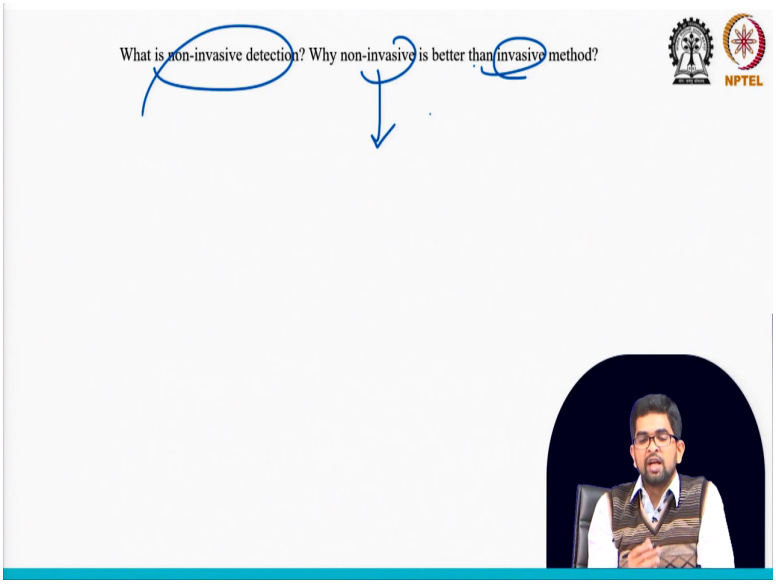

Now, what is a non-invasive detection and why non-invasive is better than invasive method? Non-invasive means, first you know the invasive method right. Invasive means you have to prick the finger collect the blood by needle very painful method right invasive method.

Non-invasive not invasive means, like when you collect the sample like saliva sample urine sample sweat sample, they are non-invasive sample.

So, non-invasive non-invasive detection means, when you are using like those non-invasive sample like saliva, urine or sweat sample they are the non-invasive sample right and this method because it is not painful and also immediately you can collect even in the (Refer Time: 28:40) I mean they can collect the sample by themselves and they can try this detection easily.

So, this is very important technique non-invasive technique is very important technique and you can easily develop a biosensor. So, for point of side applications means even the people they can use by themselves.

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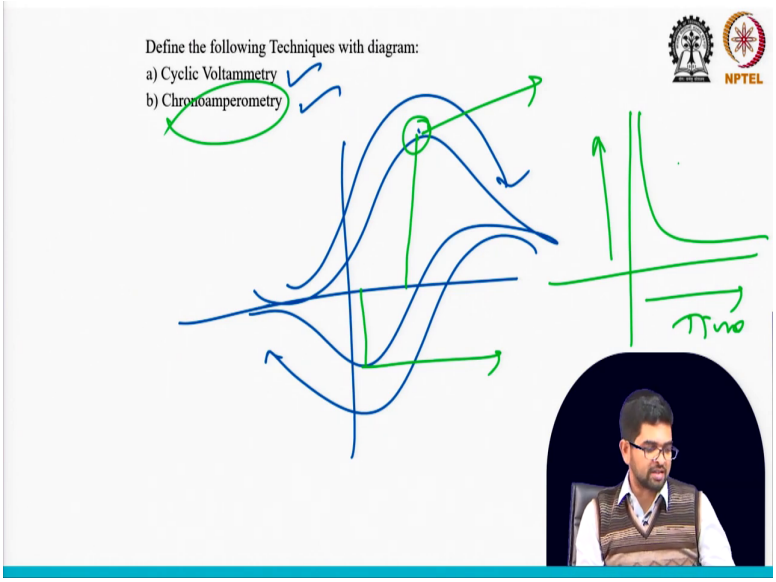

Last questions define the following techniques with diagram? Cyclic voltammetry chronoamperometry this questions may come. So, what is the technique cyclic voltammetry means just you have to draw your diagram, then you have to say this is the oxidation scan right this is the reduction scan and then you can mention this is oxidation peak potential, this is reduction peak potential.

This is oxidation peak current this one is reduction peak current right those everything you have to describe with the cyclic voltammetry that I already taught you with the proper diagram you have to mention and amperometry that is just I have told you right amperometry means, it is something like this.

So, in the x axis it is time and y axis is the current and you have to describe all the parameter that I have already taught you like this way you can properly answer the questions ok. So, that is all for today's tutorial. So, next tutorial I will bring the more innovative questions so, that you can think something new idea and you can plan accordingly for your future study also it will be very very helpful for you.

Thank you, that is all for today's class.