Nanobio Technology Enabled Point-of-Care Devices
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Lecture - 29
Tutorial on Biosensors Fabrication (Continued)

Ok students, today I will cover some new more questions and from there let us try to understands the all the new design for the biosensors. So, again the tutorials for the biosensors.

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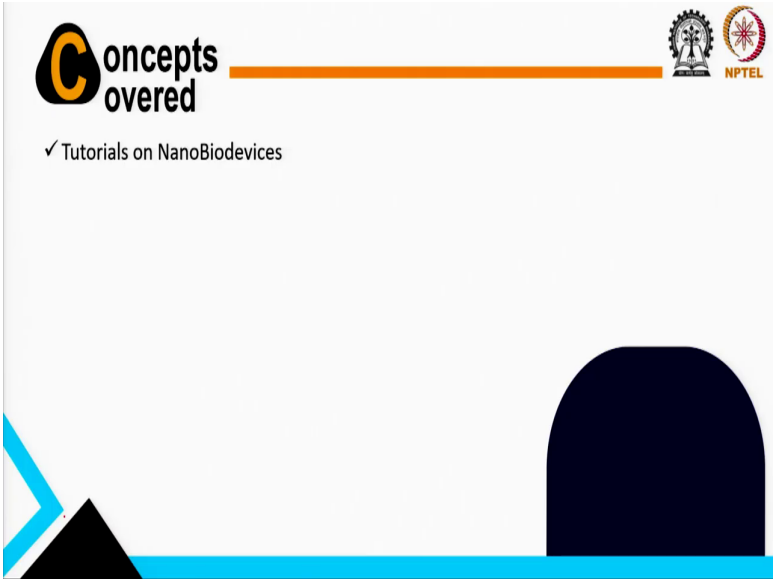

So, let us try to understand solve the questions, ok.

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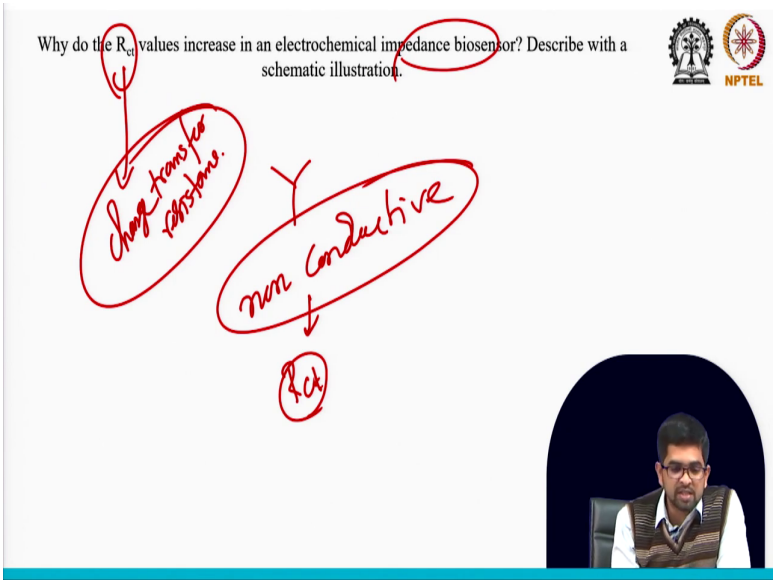

So, first questions for today's tutorial is R ct. I think you can remember what is R ct and based on this R ct we can design the many biosensors and we can characterize the biosensors and we can develop some new technology. So, let us come what is R ct? It is a R (Refer Time: 01:03) is the charge transfer resistance right charge transfer resistance.

So, these R ct value will guide you give you lots of informations for the biosensor development. So, generally in the biosensor development while you are adding the antibody, then target, then secondary antibody like the step by step when you are forming the sandwich like the sandwich elisa.

Generally, the electrochemical impedance value actually increasing. Why its increasing? Because, this all the put in stocks they are non-conductive, right non-conductive and if they are non-conductive, definitely your resistance value will increase R ct value will increase,

right. This is the basic concept. So, let us use them for the biosensor development and let us try to think this try to solve these questions and just try to understands first the problem.

See, the problem is why R ct value increase in the electrochemical impedance biosensors. So, let us first draw you can answer like this way in the exam. Let us draw the biosensor very basic surface.

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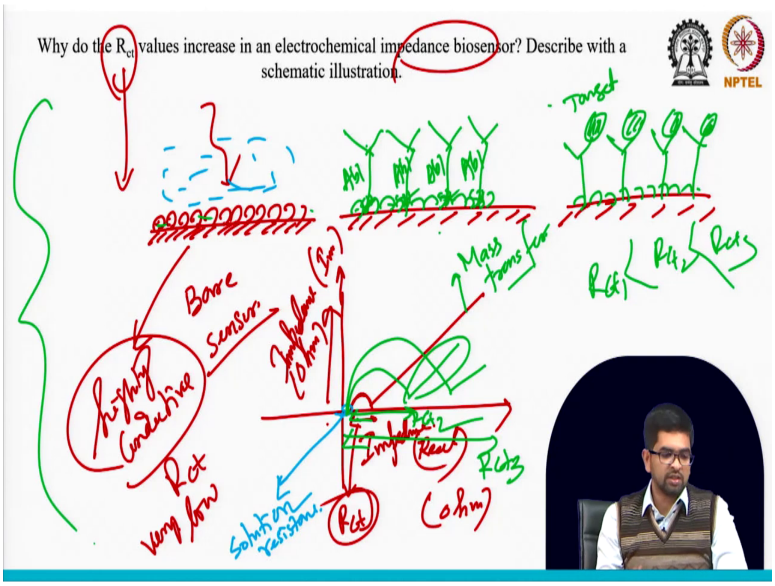

So, first you have may be a one nanomaterial coated bare surface ha bare your surface is bare. So, this is bare biosensor sensor surface, right. There is no protein molecules. So, as it is nanoparticles there and your surface become it is highly conductive, right highly conductive. So, as your surface highly conductive definitely R ct value will be very low.right It will be very low.

So, you just think then your impedance spectra you can remember the x axis y axis x axis we are measuring the impedance, right impedance of x axis is the impedance of this is the real value right. So, here you can see the real impedance, right. So, impedance of the real and y axis you are measuring impedance of imaginary value, right and generally the unit is ohm, ok. So, in the bare sensor case as it is the highly conductive, I think you can remember you may get very small R ct, right.

This semi circle region is the R ct. This is the R ct over the semicircle region and there will be there will be a straight line, right. This straight line is because of the mass transfer, right. So, here mass transfer also very fast and you very highly conductive that is why you are getting very low impedance value. And you can just you can remember there is starting point. See the starting point this is because of solution resistance, right solution resistance.

So, it will not start from exactly from this origin 0. So, it will start from there should be some resistance. This is because of because see your sensor mean there is some solutions right some electrolyte (Refer Time: 04:47) using some previous buffer or some real sample you are using. So, that sample resistance will be this one, clear.

Now, let us come the second modifications. So, your sensor surface has nanoparticle and maybe suppose it is the gold nanoparticles. And your antibody is thiolated antibody, gold and thiolate are strong interaction. Your thiolated antibody you already conjugated on the surface. So, as this antibody come antibody and it has some non conducting behavior with their protein. So, definitely your surface resistance your surface impedance value will increase, right.

So, what will happen then? Then you will get higher R ct right. So, this this R ct 2 will be for your because of this antibody already on your surface it will increase and your straight line this portions also will decrease. This one will increase will decrease in the straight line because this straight line actually gives you the information how mass transfer. This one give the information of mass transfer.

So, so how fast your mass is transferring towards the electrode surface. Now, the third step. So, third step case your antibody you can now can it can means you when you add your real sample or your that contain your target. This is your target. It will bind because of antigen antibody interactions. So, definitely there will be again some hindrance of electron transfer rate.

So, as they are protein. So, electron transfer rate will again decrease and again impedance value will increase, right. That is why electrochemical means when you are developing any this kind of immunosensor your R ct R ct value will increase slowly. So, again this is the R ct 3 R ct 3 will be higher. So, R ct 1 R ct 2 and R ct 3 see the trend will be like this and like this way. So, based on the impedance that is why you can characterize your surface, right.

Like if your sensor surface is highly conductive with this nanomaterial then you will get the lower R ct then slowly the R ct increases then you this is the confirmation that yes your surface is slowly binding with this material. That is why impedance value is very good. It is a very informative and this all the information you can put together that your sensors actually developing.

This is a good method for the characterization of your sensor, ok. So, these all the things you should keep in mind.

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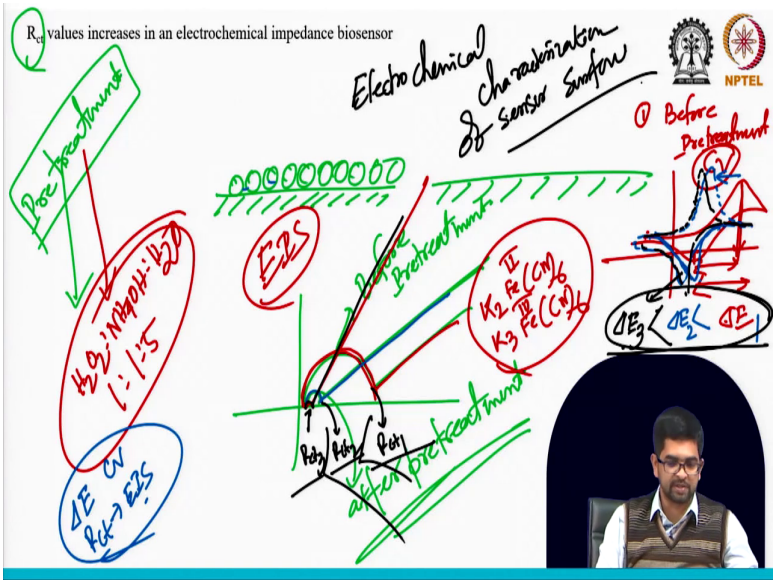

So, these are simply increases in the electrochemical sensors. Let us try to think more like some details way so, that you can design some biosensors. Based on this RCT let us design some biosensors. Suppose you have a sensor surface just I think you can remember last time I taught you different pre-treatment right pre-treatment method.

So, you can characterize your pre-treatment method by the help of R ct also. Why? Before the pre-treatment your surface may be not that much conductive your surface was hydrophobic not there may hydrophilic. So, your after the treatment your surface become very conductive. So, definitely if you measure the RCT value definitely you will get the small R ct right small R ct and higher this straight line.

So, you can predict the as your surface become clean. If your surface we cannot clean properly your if your pre-treatment method not effective that much then you will get the

higher R ct. So, before and after so, this one should be before pre-treatment right and this one would be after pre-treatment clear. So, like this way you can characterize that your pre-treatment method how effective and this is the electrochemical characterizations. Now, see after the pre-treatment again you modified with some gold nanoparticle.

So, what will happen if you modified the gold nanoparticle? So, this R ct value again will decrease or increase again it will decrease. So, you will get again the very low very low R ct value and this very long straight line you might get. This is because your surface become again conductive that is why you are getting again the very low R ct value ok.

So, this characterization technique that is why definitely you should follow side by side your cyclic voltammogram. So, what effect on the cyclic voltammogram? When side by side cyclic voltammogram means. So, first before pre-treatment right before pre-treatment of your sensor surface. So, kind of cyclic voltammogram you may get. Suppose for example, you are taking the ferro ferricyanide like K 2 Fe CN 6 or K 3 Fe CN 6.

So, here 2 oxidation stage and here 3 oxidation stage right. So, if you take this couple and if you take the cyclic voltammogram. So, for the before pre-treatment case when surface is not that much conductive maybe you may get something like this this kind of cyclic voltammogram you may get and if you see this oxidations peak potential reductions peak potential they are very far to each other and you may get before pre-treatment is this much R ct.

So, you can correlate cyclic voltammogram and EIS ok these two you can correlate. So, when your oxidation potential reduction potential very far from each other it means the surface is not that much catalytically active not conductive and you are you are getting higher R ct like this way higher R ct and delta E value very high right.

Now, you are using some pre-treatment like hydrogen peroxide, ammonia hydroxide and water mixture right 1 is to 1 is to 5. After this pre-treatment what you can see may be your you can see your cyclic voltammogram actually shifted right. So, see your oxidation peak potential shifted left and reductions and your reductions peak potentials also maybe it should

come like this it should show near here not toward very very left it should be near the right side.

So, reduction peak potential it should be actually like this and delta E value will be less than I mean it is delta 2 and it will be delta E 1. So, delta E 2 value will be lower than delta E 1 and after pre-treatment generally see this is your this value right this is your EIS value. So, delta E value also decreasing your R ct value also decreasing.

So, this parameter from the CV and R ct value from the EIS you will get lots of information on the surface. Now, after pre-treatment you are modifying with some gold nanoparticle. So, what will happen on the cyclic voltammogram? So, cyclic voltammogram will be again shifted right.

So, it will be; it will be like this. So, it will be right very very reversible means delta if think in this case it is the delta E 3. So, it will be very low delta E 3. So, delta E 3 delta E 2 delta E 1. So, this will be the sequence of that you will get from the cyclic voltammetry. See I taught you at the very beginning at the very first few classes that time it was difficult to give you all the information all together at the same slide.

But now in giving the tutorial I am showing you everything all together because I already taught you everything all the like what is delta E value what is cyclic voltammetry what is the electrochemical activity see everything, I am put together in one single slide.

Now, you can think like how you can correlate all the concept for one single problem because it is very so many information there in a single diagram. Now, you can correlate. So, this is the sequence of the delta E and you see the del R ct value this is the R ct right this is the R ct. So, if you think this is the R ct 3 this is the RCT 2 and this one is the R ct 1. So, in this case also sequence will be like this. So, R ct 1 will be higher than R ct 2 and R ct 3 will be very very low.

Like this way you have to characterize the sensor surface very properly and this is the very good electrochemical characterization electrochemical characterization of your of sensor surface you should follow this and then you will have the whole idea about your surface, ok.

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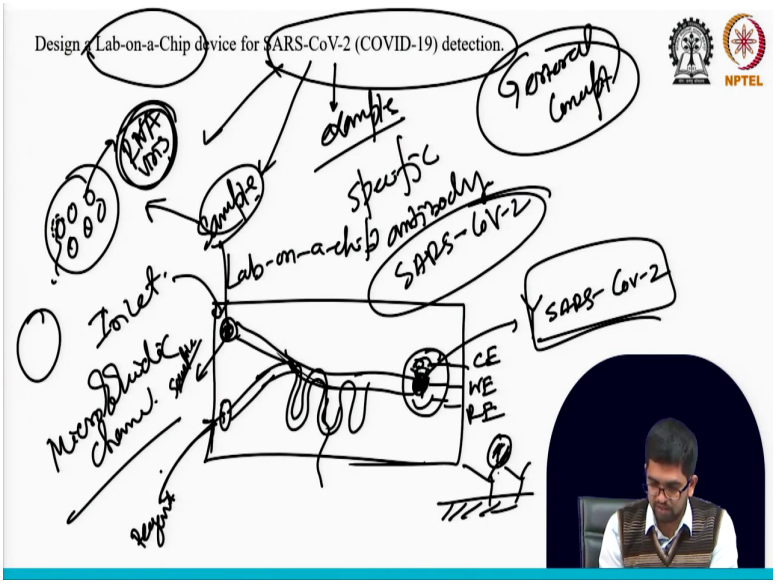

Now, put together your all information again to design a SARS-CoV-2 detection that is very timely when this times you wanted to develop a SARS-CoV-2 detection say biosensor. Now, you all have you know already like how to design a surface how to clean the surface how to make the very good sensor surface and how to immobilize the antibodies.

Now, let us put together all the your ideas for detection SARS-CoV-2 this is a very generic concept this is a very general concept you can use this concept not only SARS-CoV-2 for any target detections because here for example, I put this is just the example for SARS-CoV-2.

So, because here I may be wanted to use a specific anti body specific anti body for this SARS-CoV-2 right specific antibody for SARS-CoV-2. Now, if I say this one for dengue or for malaria maybe I can replace SARS-CoV-2 for dengue or malaria then just replace the antibody with dengue or malaria then problem solved right that is why let us put together all of your ideas here see your biosensor can be like this.

So, I told let us develop a lab on a chip lab on a chip means all your lab all the development concept like sample processing sample purifications sample amplification everything on a single chip. So, let us design a chip like this. So, this is called lab on a chip. So, where first things you here your sensor surface right here one working electrode here counter electrode your reference electrode.

So, this is working this is counter and this is reference electrode. Now, here actually you are modifying your antibody that is very specific for SARS-CoV-2. This antibody is very much this was SARS-CoV-2 that you immobilized here, ok. Now, you have to you should have one inlet here from there you can inlet means here actually I wanted to integrate on microfluidic channel.

Why I need to integrate the microfluidic channel because easily you can handle the very low volume of the sample no need any sample pre-processing and you can try easily with the microfluidics, I just show you. So, here may be one inlet here you are using your sample.

The sample means in the for the SARS-CoV-2 generally we are using nasopharyngeal sample or saliva sample whatever you want and that sample will contain your whole virus and you are going to detect that virus this is the SARS-CoV-2 is the RNA virus right. So, RNA virus.

So, in this case maybe you want to detect the RNA that you may think or maybe you can detect the whole virus. So, if you want detect to the whole virus a specific antibody already immobilized here. So, whole virus that may come here with the microfluidic channel and it can bind with this antibody. So, you are working area here your antibody here. So, whole

virus can bind here then you can take the impedance spectroscopy like if the virus bind on the surface you will get the higher impedance value right.

But if you want to measure like you want to develop a genosensor, genosensors means you want to detect a DN RNA kind of genetic material. So, you know the SARS-CoV-2 is the RNA based virus. So, you want to detect the RNA not the whole virus. So, in this case so, you have to; you have to I mean this upper part of the virus will dissolve. So, that RNA you may get. For that you may have to use some other chemical for this dissolutions of this virus has upper part will dissolve.

So, some chemical you may need that chemical so, you may need some kind of serpentine chemical that will dissolve this virus. So, that chemical you can inject here another channel. So, it will then connect with this main microfluidic channel. So, this generally microfluidic channels are here you can make the serpentine and last time I showed you already right. So, that it will get some time to mix the reagent with the sample, ok.

So, from here your sample coming right then from here your reagent coming. So, this reagent will react with the sample and you will get the RNA and in this case your surface will be like this.

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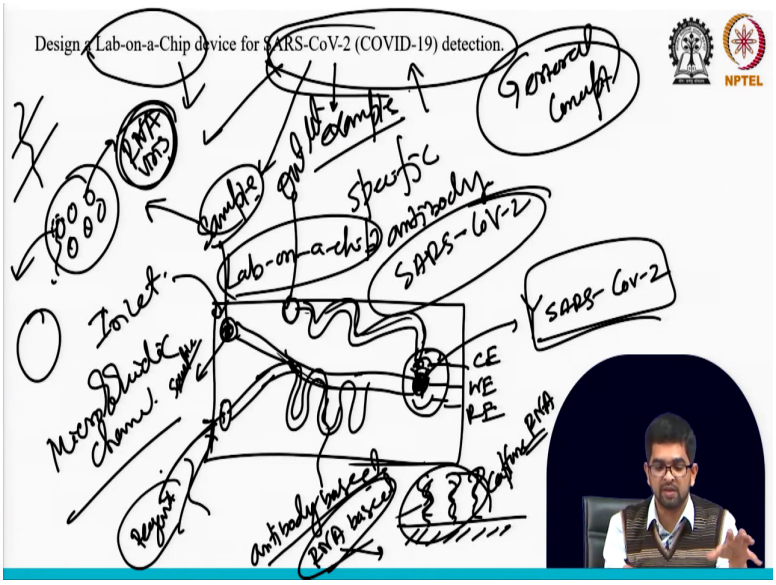

So, you will have some capture. So, because this is the RNA virus you want to detect the RNA. So, on the surface you will have some capture RNA, ok. Also, you have to use some more reagent. So, that because this is the very big RNA you have to break this RNA. So, all the chemicals you can try here, ok.

Then very PCB RNA maybe suppose it is 100 nucleotides or maybe 18 nucleotides you are using the capture. So, this RNA will come and bind here. So, PCB RNA. So, your target RNA will come and bind your target RNA mans that come from here what is in the virus, ok. Then you will get the electrochemical signal change. In the impedance you may get the change in the chronoamperometry you can show the change in the cyclic voltammetry you can show the change that all the concepts I have already taught to you.

See in the lab on a chip it is this is the whole systems on a single platform. So, you should have a here one (Refer Time: 22:11) chamber that is the outlet one outlet you should have this is the inlet and there is outlet. Means the excess solution they should go through this channel and we store here. This is the basic concept I taught you just I am putting together all the concept in a single chip.

So, we can use the lab on a chip this concept for the detections of the SARS-CoV-2 and I taught you here two concept at a time all together in a single slide. One is the antibody based antibody based biosensor you can develop or maybe RNA based. So, why I use the I want sometime we wanted to use the RNA because with sometime we want we have to think about the very much specificity, right geno sensor they are very very specific if there is a single nucleotide change maybe they will not bind here.

They are on the electro surface they will not may not bind you will get the very specific sensor. And you will get very sensitive very sensitive biosensors very specific biosensors you can develop. So, like this way you can plan a lab on a chip. So, this is just for the example on SARS-CoV-2, but you can think some other virus detections also like dengue virus other detections that also you can develop simply like this way, Ok.

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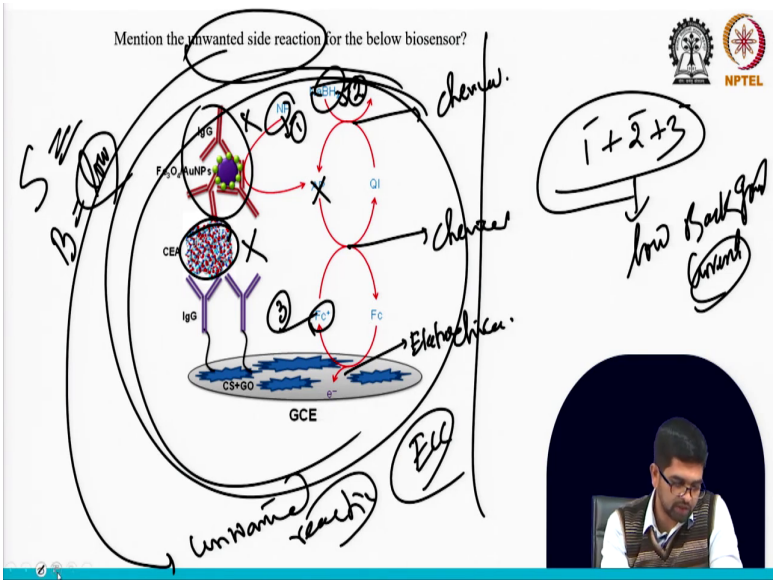

Now, let us come on next tutorial questions is the maybe in the exam I can give you something like this a schematic diagram. This kind of reactions case let us try to gather the all the information that is responsible for unwanted side reactions let us write. So, you do not need write the many sentences very basic things you can write unwanted side reactions means that can increase the background current that is not actually your signal that actually you do not want.

So, you want signal basically right. So, background you want very low background you want low signal should be very high. See you can think if I provide you this kind of a reactions you can first you just thing this is the redox cyclic reactions right here you just see here the electrochemical and this one chemical this one again chemical. So, this is the ECC redox cyclic now we can easily correlate your ECC redox cyclic concept.

So, what is the unwanted reactions unwanted reactions means without target the reactions that happen like see if there is no target no target then this nanomaterial this secondary antibody that coated nanomaterial that will not be present if this is not present this NP that is nitro phenol you not convert it to amino phenol. So, this will not be there right.

So, only your starting reagent will be your nitro phenol right this is already you are your sodium borohydride is starting reagent and you are using this one right ferrocenes plus this is your starting reagent right this ferrocene plus will form the ferrocene 0 only after the whole reactions. So, these three 1 plus 2 plus 3 these should not react very fast each other then only we will get the low background current low background current and if they react then they are the unwanted reaction they are the unwanted reaction.

So, you do not want these 1, 2, 3 they should not react that is the unwanted reactions right it should not be happened ok it should be clear number 1. Number 2 you have to mention like what is the unwanted any other unwanted reaction now first you have to think about the total reactions. Then one by one you can mentions that you are starting reagent.

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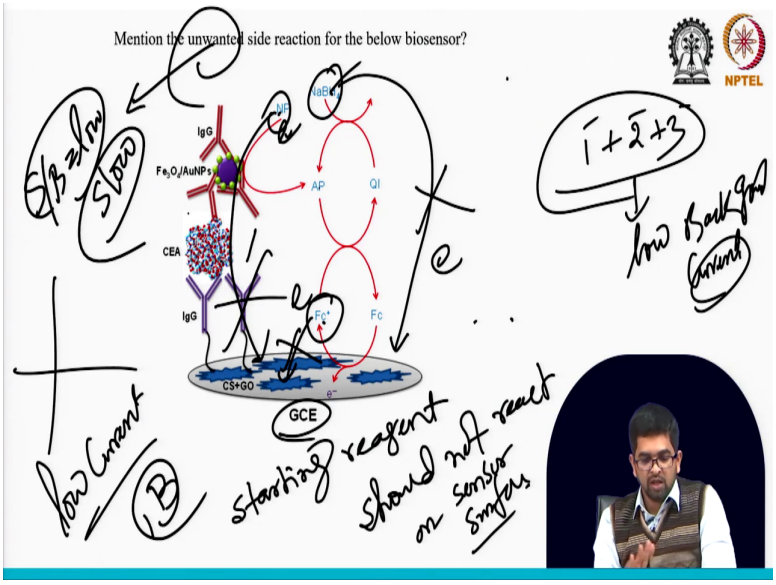

So, the main concept the starting reagent that you used. So, they separately they also should not react on the transducer GCE means glassy carbon electrode. So, this is one just a transducer surface on that surface individually starting reagent individually should not react; should not react on sensor surface, right.

So, if they react then it is the unwanted reaction. So, if they react that is the unwanted reaction sodium borohydride is the starting reaction so, if they react on the surface that is unwanted these reactions should not be happened this nitrophenol is the starting reaction if they react on the sensor surface.

Then that is the unwanted this reaction should not be happened this nitrophenol is the starting reagent if they react on the surface then that is the unwanted this reaction should not be

happen and this ferrocene plus is the starting reagent. So, these reactions are also should not be happen

Only ferrocene plus means if you take the one cyclic voltammetry of this one individual this one individual nitrophenol this one should (Refer Time: 27:39) individually they should show very low current not very fast current. So, these electron transfer rate should be very very these electron transfer rate should be very very low that they are the unwanted reactions ok. So, this is a very generic concept what is the unwanted reactions and what is the role for the biosensor development.

So, unwanted reactions case if they are very high then background then background current will be very very high right if background very very high then signal to background ratio will be low right. So, unwanted reaction that is why should be slow ok.

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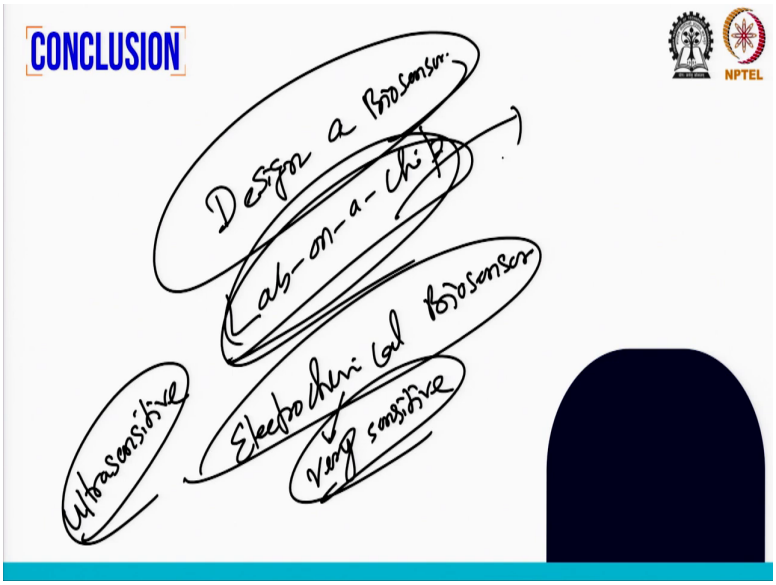

So, this is the concept for today's lecture and I taught you how to design a new biosensor based on lab on a chip I just I gave you one example like SARS-Cov 2. So, design so, design a biosensor let us put together all of your knowledge in a single platform that is lab on a chip that I taught you right.

And also, I gave you some many examples of signal amplifications their unwanted reactions that always you have to consider when you will develop especially electrochemical biosensor electrochemical biosensor I think I told you at the very beginning they are very sensitive right.

So, as they are very sensitive then you have to consider that other reactions like the chemicals you are using, they should be background currents will be low that is why you have to think very critically otherwise you may not get very ultra sensitive sensor. Electrochemical sensor is really ultra sensitive they are very ultra sensitive, but sensitive, but you have to design properly if you if not if you cannot think properly like how they are reacting each other then you may not achieve your goal.

So, that everything should behave properly on your single platform also all the concept that you are getting from this course let us put together all in a single plan and just think that you are getting higher current for the signal and lower current for the background then only you can get a very good electrochemical ultra sensitive sensor, ok.

So, that is all thank you for today's tutorial and next classes again I will show you many problems how to think independently and design some new sensor for biosensor development, ok.

Thank you.