Nanobio Technology Enabled Point-of-Care Devices

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Lecture - 28
Tutorial on Biosensors Fabrication (Continued)

So, students, today I will take one more tutorial; here, see, you already seen, have you have seen the last classes also while I was discussing the many problems, that time I also I am bringing some more concept. Because during the class while I was teaching you, that time maybe it was not the right time to tell you everything in the same time.

Because, see in a one biosensor there is lots of thing, right there is so many optimizations, so many fabrications, so many mechanisms, so many concept, I cannot tell you at a time. But now, it is the right time to tell you everything on a single sensor right.

So, that is why this tutorial can help you to bring all the concept whatever I taught on a single slide. And during this tutorial also that is a one, I can bring some more concept, more optimizations; so, new concept also, that will be very much useful that is; so, this tutorial, let us follow.

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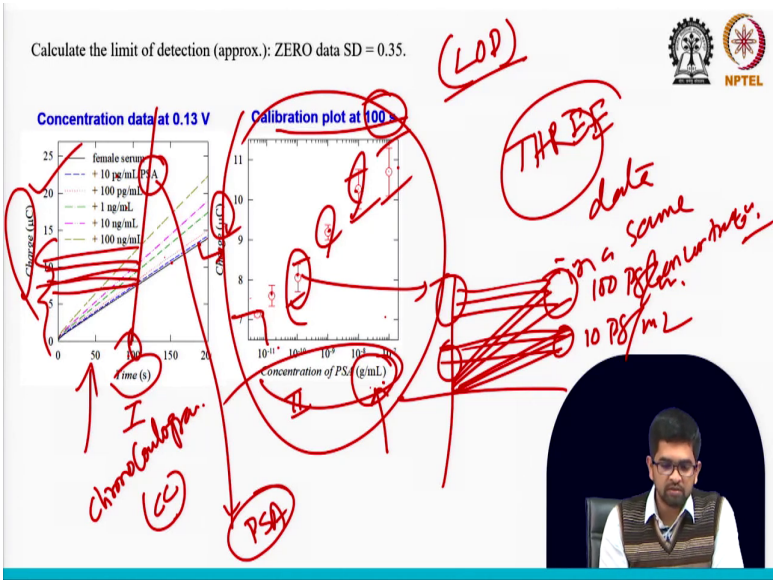

So, in this slide you can see that limit of detection calculation that I will taught you; so, let us show you first the questions. So, these kind of questions may come during the exam, this is just for your preparations of your exam.

Then I will discuss many factors altogether; so, then you can think a new design for biosensor ok. So, let us come first for your exam, calculate the limit of detection, this kind of questions may come, how you can calculate the limit of detections? Means, LOD Limit of Detections.

See, I will provide these two images, 1 is the concentration and 2nd is the calibrations or sometime maybe I can ask. There is no calibration curve; you know this curve will not be provided, only concentrations curve. So, you have to prepare a calibration curve; so, you know right how to prepare calibration curve, let us summarize.

So, if I provide you a concentration data and if I ask you make a calibration curve, then; so, these concentration data because you can see this is a charge versus time. So, this is what, this is ChronoCoulogram right, ChronoCoulogram and the technique is called ChronoCoulometry, right CC ChronoCoulometry. So, at a fixed time you have to measure all the currents; so, here you can see the calibration curve I decided 100 second; so, see this is the 100 second.

So, in the 100 second you have to measure all the currents; so, if you have like this one, just a this is the 0 data. Serum means just 0, there is no target 10 picogram per ml 100 picogram, then 1 nanogram, 10 nanogram, 100 nanogram per ml, different different concentration, then we increase hm.

So, and then you have to like in the 100 how much current you are getting; so, like this way you have to calculate, this is the current. So, from here you have to calculate the all the current and that all the current you have to plot in a graph with respect to your concentrations of the target.

So, in this case your target name is PSA, Prostate Specific Antigen right, PSA is your target. So, in the x axis you can plot concentration of PSA, unit is gram per ml; so, without unit diagram is meaningless; so, always at the unit right. Here also charge micro coulomb right; so, in this concentration this much charge we got from the diagram 1, then this then this is 1, 2, 3, 4, 5, 6, six concentration there right.

So, the six concentrations we actually plot all the current and you can see this is the error bar, right this much error bar we are getting. So, error bar means at least you have to collect three, three data in a same concentration to get this error bar at least three. So, like if you have like 10 picogram.

Let us collect three data, but in this diagram there is no three, there is three data not available. If I put all the three data it will too much hazards we cannot find out the data that is why I just showed you I have showed just only 1, 1, 1, 1 like chronocoulogram. So, if I ask the

calibration make the calibration probably this diagram, then you will not have the scope to push use this error bar.

But if I show you, but if I provide you like different different concentration like this is the like 10 picogram data and with three concentration this is 10 picogram per ml, then this is the 100 picogram per ml. So, you will have three data then you have to collect the standard deviation. So, in each concentration actually you are getting this much variation that standard deviation you have to put it here clear.

Now, let us come to the limit of detection calculations. So, before the limit of detection calculations I think I already taught you that you have to collect the standard deviations of your 0 data, 0 data means without any target data that is the 0 data. So, your 0 data is like just the female serum like just serum there is no prostate specific antigen ok.

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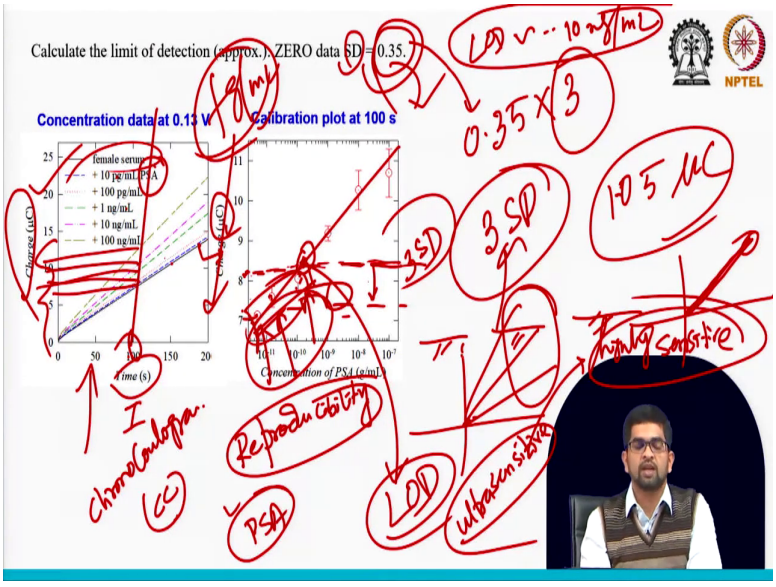

So, in this case suppose your zero data SD means Standard Deviation is 0.35, then you have to multiply 0.35 into 3 right, 3 time standard deviation you can remember 3 SD value that value means right 1.05. So, 1.05 micro coulomb is your 0 data standard deviation 3 time standard deviation; so, that much current that much current you have to consider just like a 3 time standard deviation value.

And offer this three time standard deviation value will be your considerable detections for the biosensors. Otherwise, if see suppose your this; so, suppose this one your 3 time standard deviations may come here, if your 3 time standard deviation may come here although you did. So, this is a 3 SD for example, although you got 1, 2, 3 this concentration data, you can detect, but at their below of the 3 time standard deviation.

So, this concentration this data is the meaningless with your biosensors, you cannot show them, you cannot tell them this is your limit of detections, your limit of detections you have to so, with this concentration you have to draw a straight line. With this all the concentrations and this intercept this one will be your limit of detections, this intercept.

Although your sensor can detect these 3 concentrations also these 3 this 3 can detect, but the below of this 3 time standard deviation show they will be useless they this data will not be considered for your sense with sensitivity of your sensor; so, your sensor is not that much sensitive.

So, if you want to make your sensor very sensitive, then your standard deviation should be very very low. If like once you are developing 3 sensor if their data like chronocoulogram data if they are very close to each other, then your sensor is really reliable because their deviations very low.

But if you are getting like one same concentration one is here, one is here, one is here, means large deviation then your sensor is not that much reliable because, they are not reproducible. So, reproduce reproducibility can decide your and if your standard deviation is very low, then

your very much reproducible very high means not reproducible your biosensors. So, like this way you can calculate a limit of detections and you can predict your sensor sensitivity.

See in this case if your 3 time standard deviation come here all the concentration here, then your detection limit around nanogram forever right, LOD close to something nanogram like 10 nanogram per ml; so, your sensor is will not be considered as ultra sensitive.

But if you can detect your sensor with some femtogram per ml very low and with three time standard deviations like this SD value will be its position is like this like your femtogram per ml is just above of this line. You can say that your biosensor can detect very very low and a light concentration. So, your sensor will be considered as a ultra sensitive then or it is called highly sensitive; so, your limit of detection can guide you ok.

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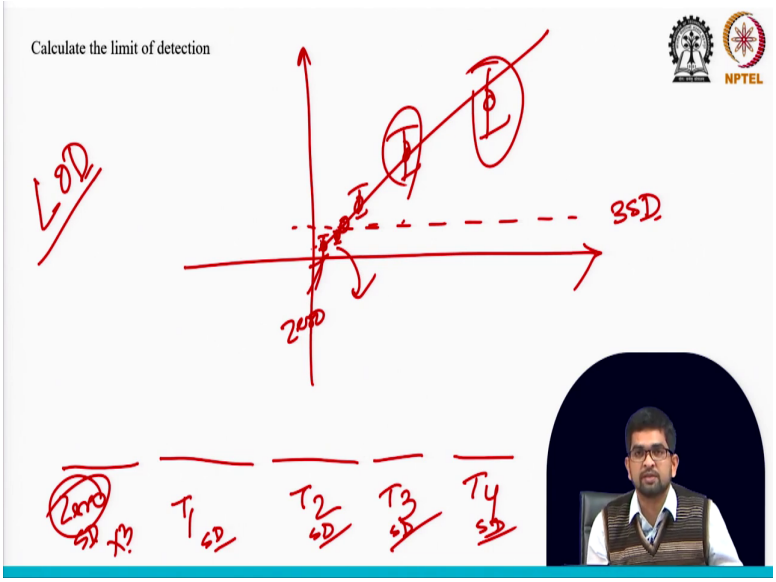

So, like this way you can calculate the limit of detections that I showed you and another thing just try to remember just I showed just unless. Because limit of detections calculation is very very important parameter for the biosensor device just, I am summarizing here. So, first you have to you have to like collect different different concentration data like, 0 like here target concentration 1, target concentration 2, target concentration 3, target concentration 4.

And each concentration at least 3 data you have to collect each concentrations, then get their standard deviation value each concentration get the standard deviation value. And for calculations of LOD limit of detections, you have to multiply with 3 or with the standard deviations of 3 then that 3 SD you have to draw.

Now, T 1, T 2, T 3 all the concentration; suppose, your 0 data right your 0 data here you are getting your 0 data here. And your target concentration, but 3 time standard deviation here your target concentration T 1 suppose here, T 2 you are getting here, T 3 you are getting here T 4 here.

So, they are the standard deviation SD value like all the standard deviation you have collected. See although your 0 data here and second this one 0 data and second concentration data also more than 0 data.

But this one will not be considered with your biosensor means your biosensor is not that much sensitive, because your 3 SD value just above of this concentrations. Then you have to just draw the straight line, then because this one your limit of detections clear; so, like this way you can determine the sensitivity of the biosensor.

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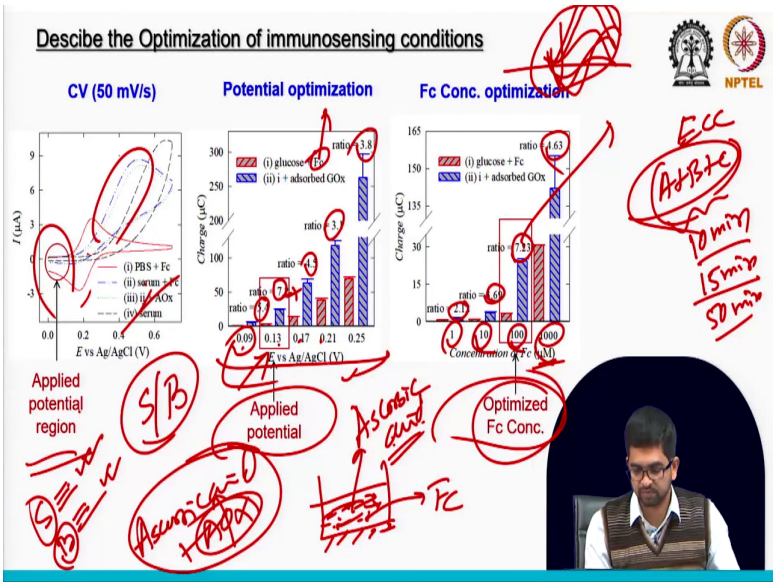

Now, the next questions in this tutorial I will show you like describe the optimizations of immunosensing conditionsm, I think many times I taught you the optimization technique for the biosensors. Like all always I am saying that you have to optimize the applied potential right because; so, if you apply very high potentials maybe in that case some interference species they can oxidize right. So, you have to apply a in such positions; so, the interference is should not be oxidized.

So, optimization potential is important, chemical optimization their concentration is important, time how long incubations you are using. As I told you suppose ECC redox cycling right, ECC redox cycling case A plus B plus C 3 chemicals you are using.

So, maybe you have to incubate it for 10 minute or maybe 15 minute or maybe 50 minute. So, which time you want to use for this reaction incubations that also you have to optimize right.

So, this kind of questions may come that from the optimizations of immunosensing, which conditions you have to optimize and how to optimize.

See here this kind of diagram I can give you the exam 1, 2 and 3 and you have to mentions see I already put the answer, like this is the applied potential region where I got the very good data that is the applied potential optimized data. So, an optimized concentration data that everything we decided, let us show you why we decided this one is the optimized conditions. So, first see here number 1 curve is the previous plus Fc means ferrocene; so, only ferrocene this cv is the only ferrocene.

Now, number 2 this one in the ferrocene in the serum sample, when if you want to use the real serum real sample like serum and if your ferrocenes means your electro meter inside this serum they may behave differently right. So, because there is some other species can interfere.

So, that is why you have to collect the actual cyclic voltammogram in the real serum sample. Because, you are using the means you are not diluting just real serum sample you are drop casting inside a biosensor that is the biosensor like this is the real serum sample.

Then you have to check your ferrocene how it behaves; you see the behavior actually change. That is why, during the optimizations process you have to think like the other mediator, other like chemicals you are using, how they are behaving in the real sample not only the previous in the real samples also that is also kind of optimization process you have to check. Then see to an AOX, AOX means Ascorbate Oxidase, generally you can remember in the real sample it may contain ascorbic acid right ascorbic acid.

So, ascorbic acids really interference species and then it can be oxidized easily in the lower applied potential; so, you have to remove this. How you can remove? You can add some ascorbate oxidase and you can remove the ascorbic acid and let us see the cyclic voltammogram after adding the ascorbic acid. It can help you like which concentration of

ascorbic ascorbate oxidase you want to use, how long you want to react with ascorbic acid as ascorbic acid plus ascorbate oxidase right.

So, you have to check the time how long they can react each other and maybe all the ascorbic acid can be removed by the ascorbate oxidase that also you have to check. Then only serum sample you can collect a cyclic voltammogram only the serum sample how it looks like.

So, it is showing see it is almost like it start the oxidations from here, you mean ascorbic acids may contain many species that start the oxidations from this region. So, you should not use the applied potential more than this, because from here only you can see the interference species oxidations can start right.

These things only you can decides if you can measure the all the cyclic voltammogram, before optimize when before final decisions. Like, once your biosensor ready well like fabrication sub antibody, your chemicals already selected everything done. So, before that you should have this all the informations, then only you can decide like which potential you want to apply.

So, applied potential region it is the best area if you can use which position you should use for the application of potential from the cyclic voltammogram I think it is already very clear see this position is the best positions. Otherwise see the when the serum presence see the oxidation actually starting from here with serum.

So, it is better in this region almost there is no contributions from the interference species that is why let us try the potential optimizations. So, we you can try the potential optimization 0.09 volt in this region 0.09, 0.13, 0.17, 0.21 0. 25 like as we thought that this region is the best region. So, let us check few potential and let us check the signal to background ratio, if you suppose you want to use a glucose, ferrocene and glucose oxidase.

So, this reactions you want to use; so, you know like I already taught you like what is the signal corresponds to what is the background corresponds to. So, if signal the chemicals you have to collect that implies the signal and that is and you have to select the chemicals that

implies the background those chemicals are selected. And then collect the signal to background ratio and from there you have to decide from there you have to decide which potential is showing highest signal to background ratio.

So, here you can see 5.4, 7.2, 4.5, 3.1 and 3.8; so, 7.2 is the best; so, 0.13 is the best; so, 0.13 potential you can select for your biosensors. So, from now onwards then when we will design these reactions biosensors these biosensor, then always you have to apply 0.13 volt, because this is the best potential you are getting best signal to background ratio. Now, one thing you can consider like the concentrations of the ferrocene; so, concentration of ferrocenes may play a very crucial role; so, this is the mediator.

So, is sometime this mediator concentrations can vary your signal to background ratio, set us check which concentration is the best. So, you can try different different concentrations of ferrocenes like 1 micromolar, this is the micromolar, 10 micromolar, 100 micromolar, 1000 micromolar, different different concentration of ferrocene you can try. And let us check the signal to background ratio; so, you got the signal to background ratio 2.12, 3.69, 7.23 and 4.63. So, 7.23 is the best.

So, this is the optimum ferrocene concentrations, like this way you can optimize each parameter in ECC redox cycling there is lots of parameter; like, you may have to optimize like all the reagent concentration. Then you have to think about the optimizations of like applied potential everything you can optimize like this way.

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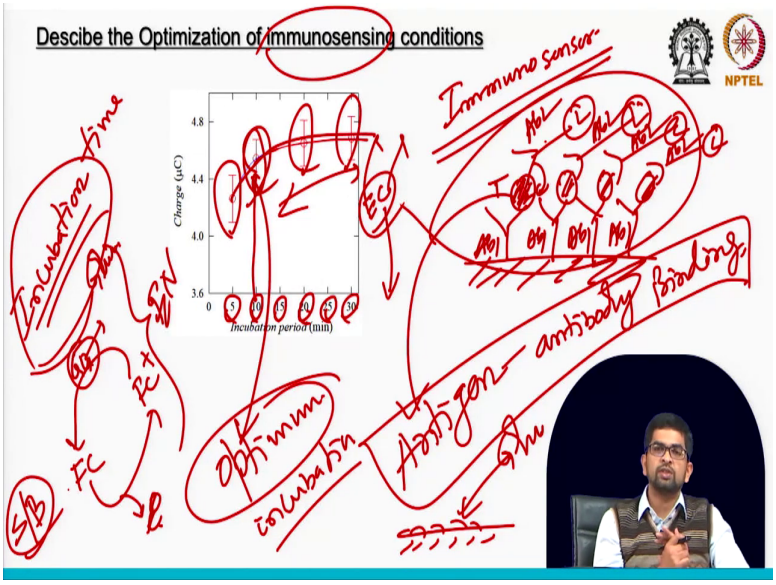

Now, let us come the optimizations of immunosensing conditions with time. See this is also very very important factor, because your biosensor is the immunosensor right, immunosensor. In this case you have to little bit careful, you have to optimize your incubation time, what is incubation?

Because your biosensor ready for the detection primary antibody coated that it is the sensor while and you add the target your target already bound. And your secondary antibody that is already labelled right, this is labelled, this is labelled, this is labelled right.

So, you add on the primary this is the primary antibody 1, antibody 1 and this one secondary antibody antibody 2. Now, on the primary antibody coated surface when you drop like say target and secondary antibody mixture of this surface my antigen antibody binding. So, this is

the antigen here sandwich; so, sandwich forming know; so, antigen antibody binding is forming; so, it is some slow reactions antigen antibody binding.

So, this antigen antibody binding may take some time, these incubations that is able to optimized. May be it may take 5 minute, 10 minute, 15, 20, 25, 30 minute in different different time interval you can check their binding effect, how you can check? You can check with the three signal to background ratio something like this way.

In this after 15 minute how much signal to background ratio you are getting, after 15 minute, then 30 minute, 50 minute, 1 hour like this you just check it. Then we can plot like with standard deviations you can plot like how your how much current variations you are getting. See you can see almost after 10 minute they are almost saturated right; so, after 10 minute, 10, 20, 30 they are almost saturated.

Then no need to consider the high means long incubations, the 10 minute can be the optimum incubation time, this is the optimum incubation for your biosensor. So, like this way you can optimize the time; so, let us summarize. Like, why you need the optimizations? Because there is a antigen antibody binding for that you need some incubations, because this is not so rapid, like just drop and check not like glucose.

So, glucose case it is one things like if you have glucose oxidase and if you add the glucose like glucometer you know within a few second it can give you the data. But for the immuno-sensor case, you have to incubate your sensor after adding your sample that may content with target and your secondary antibody.

For that this incubation time help you to bind this antigen antibody. Also, if you are using like redox cycling; suppose you are using here, some electrochemical chemical redox cycling; so, in for that when you are using some chemical right.

So, you are using some ferrocene that is oxidizing ferrocene plus oxidizing ferrocene plus. And then ferrocene plus again reduce back, for that maybe you are using with the help of some glucose oxidase; so, maybe you can use some glucose. So, in ferrocene is oxidized on

the surface and that glucose actually you are trying. So, for this kind of this is the enzymatic this is EN redox cycling and EC redox if you want to EC redox cycling there is no enzyme without enzyme you can try like electrochemical and chemical.

So, for that reactions also you may need some time, because for this reactions may not happen immediately or it may happen immediately. But if you incubate it for some time like 5 minute or 10 minute, then you may get the very high current your signal to background ratio will be much higher than if you change immediately. Then let us incubate for some time, that incubations also you can optimize ok.

So, though these all the things you have to consider while you are developing a biosensor. So, in this see in this tutorial I just ask the questions like this way, like describe the optimizations of immunosensing conditions. So, you have to describe like their how they are behaving, first you have to measure the cyclic-voltammetry for your ECC redox cycling. So, like just without reagent you may get one cyclic-voltammogram, after adding one reagent you may get like different cyclic-voltammogram.

When you add all the reagent for this cyclic you may get the amplification that cyclic-voltammogram you can see right. Then from that cyclic-voltammogram you can predict, let us take one more cyclic-voltammogram with some real sample. Because, finally, anyway you are going to the real sample and in that real sample this all the chemicals is behave same or maybe there is some change that you can check.

Then you can decide based on the cyclic-voltammogram which area actually is the best area for the measurement. Suppose in this case you can see this area actually there is not much interference species effect. Accordingly, we just optimize right, like this way you can explain your optimization of the biosensor clear.

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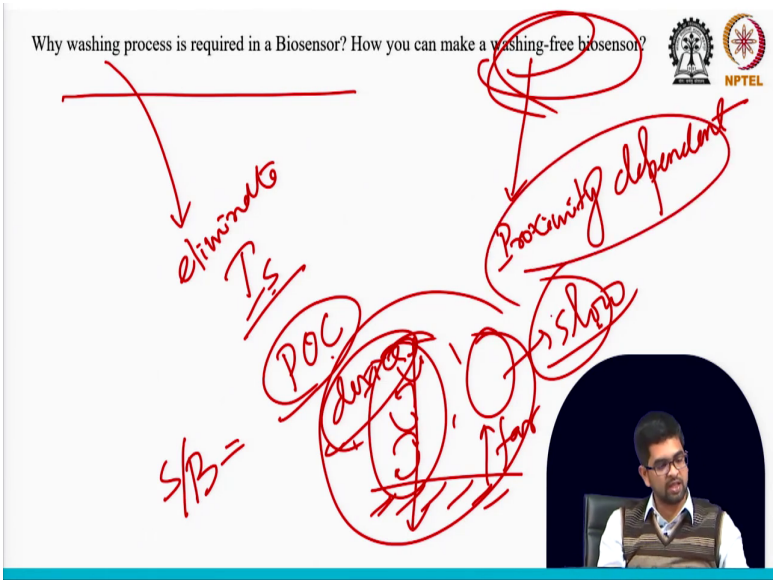

Then again, the next questions come and there is very important questions for your biosensing study that is the in the exam also may come this kind of question the washing process why require. I think the last time also I told that washing process always require to eliminate the interference species effect right, eliminate interference species effect.

But this washing processing is not always possible if you want to develop a point of care testing device. Because, our this course is a point of care development some device, but washing layman they cannot they can they cannot do on site. So, we have to develop some washing free biosensors that I already taught you.

So, how you can develop the washing free biosensor that is proximity dependent right, proximity dependent I think you can remember like on the sensor surface we can develop the redox cycling right. So, if this redox cycling happen very close to the surface, then we get the

higher current. And if this redox cycling happen in the far from the surface if it is far then the reaction rate will be slow then you will get the lower current.

So, that washing free concept you have to discuss and so washing process always required to eliminate the interference species effect. But we can design a washing free sensor based on proximity effect, but that time you have to very much careful that although you are not using the elimination of interference species effect. But they should not contribute much on a background current, otherwise your signal to background ratio will be low means that you can minimize.

Because, see in your washing free sensor case you have lots of interference species present. So, your application potential is really important role that you have to mainly consider for the washing free biosensor application potential. Proximity dependent effect that you have to consider, how fast is this proximity dependent effect is possible that you have to consider clear.

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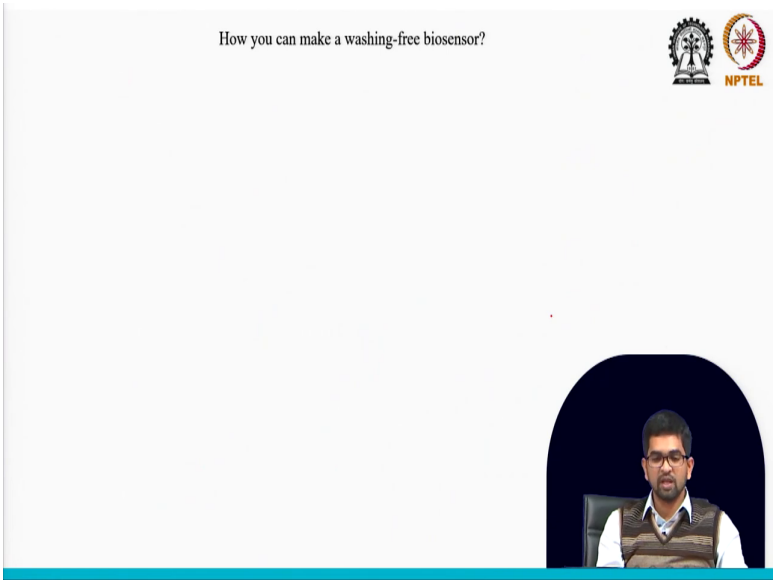

So, how you can make the washing free biosensor that proximity dependent biosensor you just you have to explain properly that I taught you already ok.

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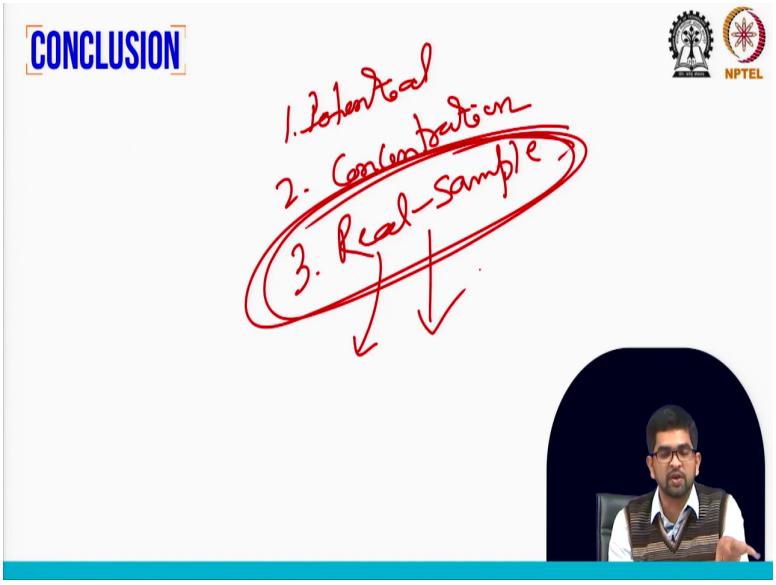

So, the conclusions today all the tutorial that I told you that let us think about like limit of detections how to calculate. Let us think about the optimizations of the biosensor like all the steps like potential optimization is very very crucial factor. Then concentration optimization, concentration optimization that is a very important factor and always you have to try the real sample.

So, always think how to handle the real sample on your sensor surface, then immediately once you fabricate your sensor try real sample. And let us check your all the chemicals all the potential that you have optimized there that you can validate with real sample or not if your biosensor cannot varied the real sample then no use; so, always you have to try the real sample ok.

So, that is all for today's tutorial, the next tutorial again I will discuss few more concepts, few more discussions on the this kind of new like technology also I will bring slowly based on this discussions that I will taught until now all the classes and you may think independently that is all.

Thank you.