**Nanobio Technology Enabled Point-of-Care Devices**
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**Lecture - 37**
**Tutorial - 04**

Dear students. So, today is the last lecture on the theoretical part. So, let us tell you again some Tutorials. So, in this tutorial you know mostly I covered everything like whatever I taught like questions and answer wise. Now, this times I will again cover some very important basic fundamental part that is that was remaining in this tutorial I will cover.

Then after this lecture I will start some experimental part in the lab where you can see how you can fabricate the sensor surface, how we are measuring the cyclic voltammetry, chronoamperometry, chronoamperometry like all the technologies that you can see practically there.

So, all the lab base weight lab base work, experimental work that that I am going to show you after this lecture, ok. So, in that experimental lecture you try to you can imagine how we are developing and also you can interact with us during your live chat, like you can ask the questions that yes, it is really feasible, whatever I am telling you yes, it is possible.

We can develop in the lab and I will show you all the fabrication technology very small small chip we can develop that also we can bring in the market that is possible that also you can learn after this theoretical lecture. So, practical lecture we will see after this. So, this lecture is the end of the theoretical part.

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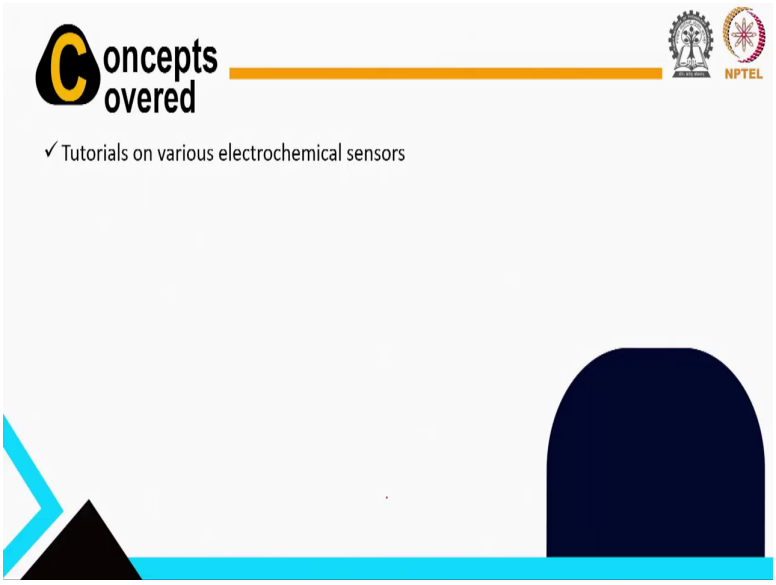

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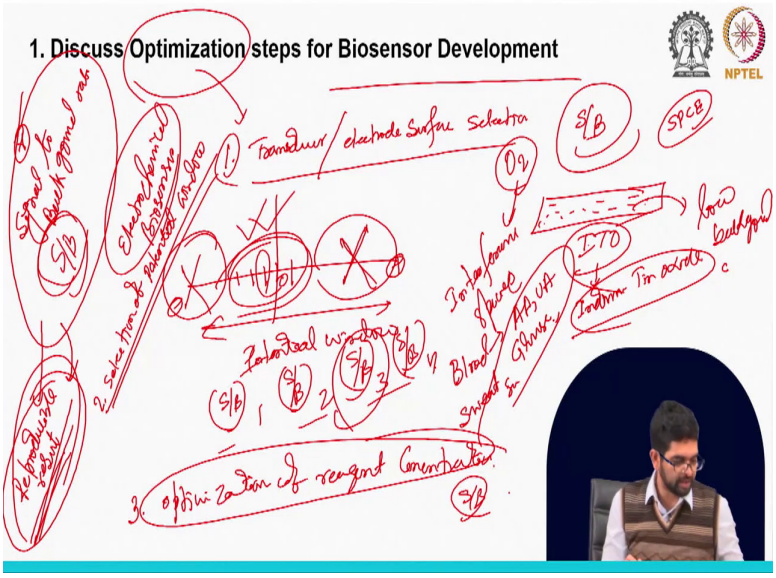


So, today in this tutorial mainly let us cover the very important things that is the optimization that is the remain. That actually I wanted to discuss with all of you. So, what is this optimizations? Ok. Yes, this is a really important part without this part you cannot make the whole sensor device.

You made you can say, you can make plan many say many people they are making so many plans, but finally, why all technology not in the market, why? Because not properly optimized ok, without proper optimizations it is very difficult to make a successful work in the sensing biosensor device.

So, let us come, let us summarize all the optimization technology one by one while you will make the plan. Let us keep in mind this optimization topic, let us summarize and this is the

end of this your I think you are learning in this course. So, I will say the optimizations can be first your transducer or electrode surface selection; this is the kind of optimization.

So, transducer or I can say electrode surface selection. Why I am saying this one? Because which surface may so high signal to background ratio. So, finally, we have to get the high signal to background ratio. So, this is the star mark like signal to background ratio S by B. So, that is our main goal to get the high signal to background ratio and definitely you have to get the reproducible result reproducible result.

So, so high signal to background ratio first thing then you definitely you have to get reproducible result all these are getting very good signal, but if that condition that optimist condition if you knows reproducible then that plan will not work. Well, you cannot this is the not reliable you cannot bring this things in the market. So, reproducible result finally, is your milestone means your this is the root you have to follow definitely.

So, transducer is ready how you can make your transducer ready? Suppose you have a glass surface and definitely glass is not the conductive, you have should have some nanomaterial conductive nanomaterial. Generally, people are making ITO coated ITO means Indium Tin Oxide nanoparticle, right. Indium tin oxide coated nanoparticle that this is very much conductive. And this surface can be useful to get the low background current low background low background like your signal.

But you can you may ask why not gold, why not platinum, why not others? Because in that case other interface species as I mentioned you know like you have the dissolved oxygen in the solution. So, they can reduce easily the platinum case platinum is very very active for the oxygen reduction.

If it not only that some other interference species may present in the real sample they may show the very high contributions, ok. So, that is why selections of transducer is really important. Maybe you can choose like some screen printed carbon electrode a species screen printed carbon electrode, that is also good selections. Because there is also not that much

interference species contributions or oxygen direction (Refer Time: 05:48) not then much if it you can see ok, that also you can try.

So, mainly I am saying here do not use that kind of electrode which can show very fast other that is unwanted reactions, mainly oxygen can be one unwanted species that means, they can show some background current. And the interference species then interference species that present in the real sample, real sample means think about the blood sample.

So, if you have the blood sample, what is the interference species in the blood? Ascorbic acid, uric acid, glucose acetaminophen like so many things it is present, right. So, any ions, but if you have like sweat sample. Means it may contain different different so many ions right, if they are interfering or not that you have to check properly. That is called the optimization.

So, optimizations not only the selection also same time, which potential or which potential window those interference species not showing any contributions that you have to think first. Without optimizing these conditions, it is very difficult to get a very good biosensor device. So, you have to that is why as you are going to develop a electrochemical biosensor mainly.

So, this class mainly electrochemical biosensor so means electrochemical property. So, which potential window and this is the potential window you know right, here 0 here minus this is the plus. So, this is the whole potential window maybe in this area is normally not useful because maybe high and high potential all the interference species can be oxidized.

At the very low potential it is not good because at that potential oxygen can easily reduce. So, maybe this potential near 0 potential is the best that, but in this range also for which when you have to determine which particular potential you can use for a good biosensor development. So, that you may avoid all the other interference species effect at the same time you will get highest best signal to background ratio, that you have to predict.

So, it means selections of the potential right, number 2 selections of potential window. That is also very important part for the optimizations of the biosensor, ok. And I will say this is the very very important part. So, after this so, but I one thing I think I already taught you the

selection of potential how you will measure? Let us when that just I just mentioned like this potential is not good, this potential area not good, but this area is good, right.

Then how will determine which potential is the best let us try like 1, 2, 3, 4, 5 like few potential let us select randomly and get S by B 1, S by B 2, S by B 3. So, like this you have to calculate 1, 2, 3, 4 like few potential. And then the thing which potential is the best like maybe S by B 3 is the best then choose this potential only for your whole biosensor development, ok.

So, transducer, then potential window number 3 optimization of the reagent concentration that you have to do properly, why? See in sometime you may use like few reagent the very high concentration. Because of the high concentration you may get very high background because of the very high background your S by B value may not be very high.

But may not be the best one your potential is the best, but because some reason you are using very very high concentration. So, you have to optimize maybe sometime very because electrochemical sensor is a very very sensitive, sometime by using very low amount of like electron mediated concentration can be enough to get the your single to background ratio is a higher. So, you have to optimize properly like, which concentrations you can try for the suppose I will show you in this one example.

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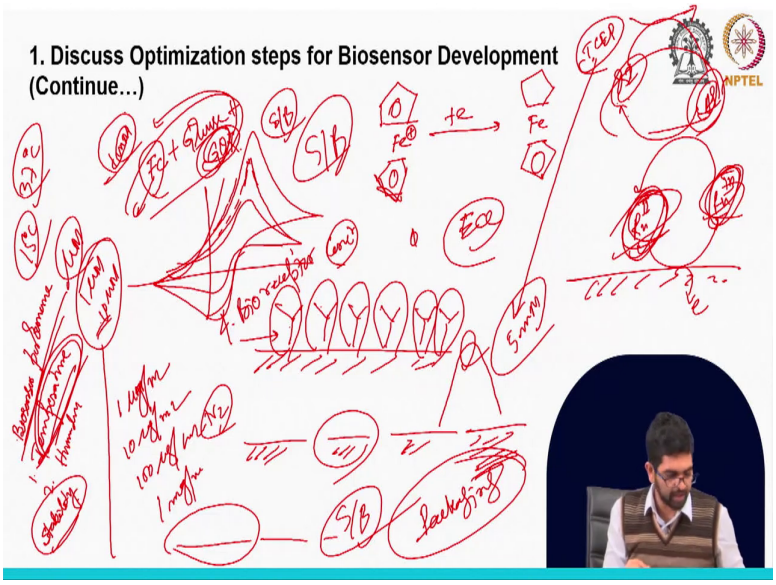


Suppose you are using ferrocene, you can you remember the ferrocene like iron this 5 member, right. So, this is the ferrocene plus and if it take one electron then it become ferrocene ferrocenium ion and ferrocene, right. So, you can how you will means I already taught you like ferrocene plus glucose plus glucose oxidase it is also very good enhancement of the enzymatic redox cyclic reactions, right.

So, like all the ferrocenes may show like the signal, but all the ferrocene glucose and glucose oxidase it may show very high signal amplifications, but if you use here like 1 millimolar ferrocene may be you may get the very high current, right. But at this very high current because it is the background related, right.

What is the signal? If you have the glucose oxidase then you will get the signal, but if you use the very high concentration suppose 1 millimolar or 10 millimolar, if the high concentration,

then you are getting a very high current, but when you are using glucose oxidase, I mean you will get the amplifications.

But as the background is very high maybe your S by B value not be that much high, but it is kind of the mediator I will recommend do not use too much high concentration of electron mediator.

Let us try like instead of millimolar you can go to the micromolar range like 1 micromolar, 10 micromolar like this where you can try. And let see maybe your background will be very very low and let us add glucose oxidase, you may get the very high signal and signal to background ratio will be very high.

So, that is optimizations of this kind of reagent is really important. And ECC redox cycling case you can remember I let us show you one ECC redox cycling. So, suppose electrode surface you have like ruthenium right, ruthenium 2, oxidized into ruthenium 3, then ruthenium 3 to ruthenium 3 to in a ruthenium 3 to ruthenium 2, the ruthenium 3 can be reduced to ruthenium 2 by the help of like aminophenol, right. Quinoneimine and then again quinoneimine aminophenol here you can use TCEP right, can you remember TCEP.

So, in this case I will show see ruthenium is the electrode mediator. Definitely, you have to optimize this concentration ruthenium 3 mediator and aminophenol quinoneimine kind because of the enzymatic reaction, right. So, no need to optimize this one because this only can control the whole redox cycling reduction, if you have the more target. So, more aminophenol will generate and more aminophenol will react with the ruthenium 3 will more ruthenium 2 will generate.

But TCEP, it is the fixed one, right. So, maybe this and TCEP always consuming it is not cyclically reproducing see ruthenium 2 cyclically reproducing ruthenium 2 and 3, but TCEP is not. So, let us you have to optimize this TCEP concentration, you may need some high concentration of TCEP not very very low concentration of the TCEP.

Generally, we are using here 5 millimolar TCEP that concentrations you have to optimize, ok. So, reagent optimizations is really important for the biosensor development. So, this is the upper part of the sensor. Now, come to the sensor surface again. Sensor surface we are immobilizing the antibody right, always I am saying. Sometime this antibody optimizations also required to get a very good biosensor.

So, see as I told you like in my last class in my last tutorial also, you have to develop a biosensor so that it can detect a large range of the concentrations of your marker. So, if you can detect like nanomolar range, but if, but some patients may have like micro molar or like millimolar range of that target like biomarker, then it will be difficult for that biosensors to detect that much high concentration.

But if you use some optimized concentrations of your bioreceptor that can help to go to the high level of detections. So, that is why we sometime you have to detect the this biosensor concentration because this only is a driving force. It is only giving the information like how much you can go in your calibrations, right.

That is why we use the antibody? So, we will generally recommend. Let us use like 1 micro molar or 1 microgram per ml, 10 microgram per ml, 100 microgram per ml or 1 milligram per m. So, like this way you can increase one by one like few sensors you can develop.

Then let see which sensor is the best for the signal to background ratio that you have to calculate. So, this is one more optimizations steps, ok. So, chemical so I told. So, first optimization transducer surface, then you can optimize the potential, then you can optimize after the potential that the concentrations and then is the bioreceptor concentrations also. So, number 3 is the 3 then number 4 is the bioreceptor concentration that is also very important.

And again, so many factor means everything like the kind of factors may there for the, when this is the main factors you can optimize. Now, you have to think like there is any other factor present or not, they can affect the biosensor and performance. So, biosensor performance can be affected may be another parameter is called temperature, right.

So, in case of why is the temperature? Because there is some low, we can see in a glucose oxidase case, they are the enzymatic reaction. So, there may be temperature because of the different different temperature, their kinetics can be different. So that things you have to check, means there may be temperature may not be.

But still, you have to, if you want to really produce a reliable biosensor, this factor you have to check temperature, then humidity. So, humidity temperature, this kind of factor may come. There are different biological like reactions can happen very, 37 degree celsius in the, but if you are measuring like 15 degree celsius, maybe you are getting the very slow reactions, slow kinetics.

Again, if you increase the sometime more like around the more than 37, also reaction can go much faster. And the certain temperature may be faster, faster, faster, then, again some certain, it can go down. So, you have to check which temperature is the best for this sensing reaction, that temperature you can select.

But it is again some point, one point may come, if it is very much dependent on the temperature, then it will not good for your point of care device development, specially in the extreme point of care. Like if you think like the very high temperature in the, some rural area setting, where you do not have refrigerator this condition.

So, there you cannot use this kind of scheme, this kind of biosensing device you cannot use, if this is a too much sensitive towards the temperature and humidity, right. So, though that is why you have to check, ok. So, those are the parameters. So, transducer, electrode surface, potential, after that reagent concentrations, then come to the bioreceptor, then come to the other parameter like temperature, humidity, those things, ok.

Now, let us think the other some, like small small parameter may come. So, you have to check the another things is the stability. So, while you will develop the whole sensor at the end of your study, you have to check the stability, right. Why? As those factor I am saying, may be you optimize everything, right antibody concentration, you optimize, other

concentration, you optimize and your sensor is not that much sensitive to our temperature humidity.

But still if you there is may be some very slow reaction, slow reaction means, although you check there is not that much temperature effect, humidity effect. But some slow reactions or storage effect or I told you like you can remember like where I taught you like tuning of the electrode surface, using effect that also can effect on your sensor performance. So, that also can be considered, ok.

So, what you have to do, you have to store some electrodes in a very best conditions like maybe in the nitrogen environment, maybe in the vacuum condition, let store it or maybe even you can try the open conditions, then compare their stability. If you see that in the open condition, still also you can store that is then really good. But if it is not, then if you have to store it, you need a very good packaging.

Packaging of your sensor device is really important again. This is a very crucial part. Finally, it can help to increase the stability anyway, because simply storing sometimes sensor is not recommended. Although, maybe sometime may stable, but it is not recommended, let us store any good packaging, let us go for a good packing system.

So, what generally we were doing, if you like I will recommend let us go to the like any shopping store and buy some glucometer things, or may be pregnancy test kit, you can buy and open it, check it properly, how they are storing, they are not that much expensive, just check the storage condition, then you will get some information.

So, Very good way we have to store it, mainly the nitrogen environment or you can put the vacuum conditions. So, this is the you know again the factor for the to I mean to get the best quality of the sensor, ok.

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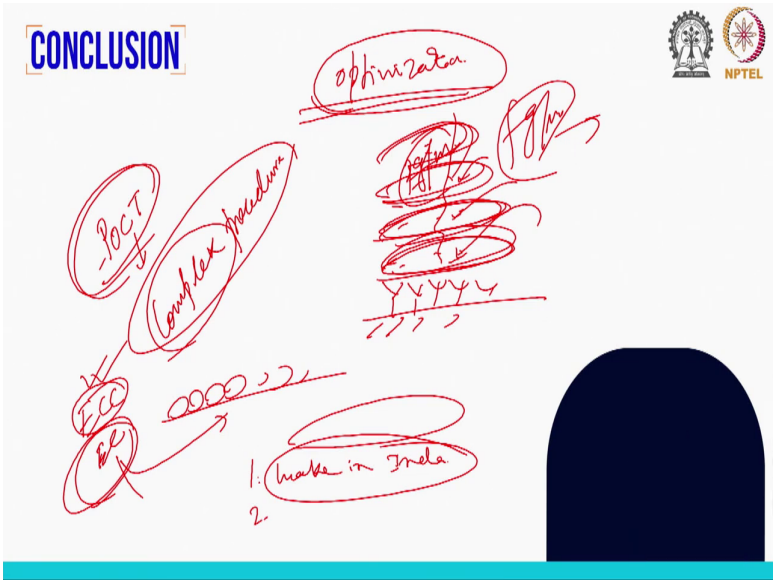


So, let us, I will summarize and again some conditions, all the things and conclude. So, optimizations, this is my main motto in this lectures and then I want to conclude this whole theoretical lecture that optimizations let us follow properly. Means once you decide your plan, let us go ahead one by one on the optimization steps.

So, first optimization steps, the electrode surface, then all the steps that I told you. And I will definitely recommend just think when as this is my concluding remark for this course, I will say this course is mainly point of care testing device development. Do not go very complex procedure.

Complex procedure means I taught you many scheme like ECC scheme, EC scheme, then fabrications of the sensor surface with different way, but try to see which condition, I mean if

somehow very simply, like instead of going ECC, if you can make some sensor by EC, very less number of chemical you can use.

That will be the best for the commercialization for point of care testing device, because if you have the many chemicals, then is sometime there is no way, you may have to use the many chemicals. Because few analyte in our bodies really really low concentration, maybe some picogram per ml, or maybe femtogram per ml.

So, in that case, there is no way you have to use the ECC redox cycling, but then it will be little complex than procedure. But you have to think about then how to use the reagent. I already taught you know one lab on a chip, like you can like bio receptor here, but you can use some membrane like 1, 2, 3, 4, few membrane you can use, all the membrane you can keep all the chemicals in the dry condition, lyophilized condition that is again another optimization.

Because simply you cannot store all the reagent in the I mean solution phase for the longer time. So, it is better just drop it all the reagent, then dry it or lyophilized it properly. So, your biochemical so the reagent will be not react each other, their conformation means the structure changes will not happen.

It is better that so store it properly, that is also kind of another optimization technique. Like then how you will store keep all the reagents on the top of the sensor, that is also things you have to optimize, right. This is my like concluding message to everyone. So, whenever you will design any sensor, just try to make a simple case.

But if not possible, if we have to go complex steps, because sometimes you may need a very low limit of detection, that time you may design some like biosensor like many paper membrane you can use. But you have to think how to store them, then try to go to dry condition, lyophilized condition to store all the chemicals, ok.

So, point of care testing's means you just think like drop and sense kind of technology, drop and sense kind of technology like the glucometer you know, that kind of technology you have

to think. So, that will be very easy and easily you can commercialize. And nowadays because of the smart phone related technology, nowadays you can bring in your sensors, in your point of care testing device.

Because now there is everyone maximum case, you have the smart phone. If you can easily may develop some app and integrate your device with the smartphone, you can easily get the value and continuously you can monitor. This is the very important part are IOT enabled biosensor development.

And really like this is now our in India our main motive like digital India or make in India kind of concept, we can really make it based on this course. See it is very simple, just few chemicals you need on the sensor, all the ingredients is very much really available inside our India and just try to develop this very common technology and try to commercialize it, make your own start-up try to be independent.

So, this kind of course, that is why this really help you to become independent. And this kind of like our Government of India main concept like Make in India Make in India concept Startup India or like Swachh Bharat Swachh Swachh Bharat. So, this kind of concept is really, really means can be implicate, I mean can incorporate in this kind of the course, ok.

So, that is the message for everyone, please try to I mean get all the concept that I taught you and try to be independent after this course. And I will start now after that few lab based experiment where you can easily see how we are developing all the technology inside lab and it is really possible to bring in the market. So, that I will show you after this lecture ok, that is all for all of you and good luck.

Thank you.