Nanobio Technology Enabled Point-of-Care Devices
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Lecture - 23
Effect of pretreatment on PCB and biosensor development

Ok students, last few classes I taught you the different pre treatment methods and how we can clean the surface and how this enhanced activity that we are getting after the pretreatment, it can be effected by aging effect. Now, let us use all those electrodes after the pre-treatment for biosensor development. For example, I will give you one for printed circuit board like PCB.

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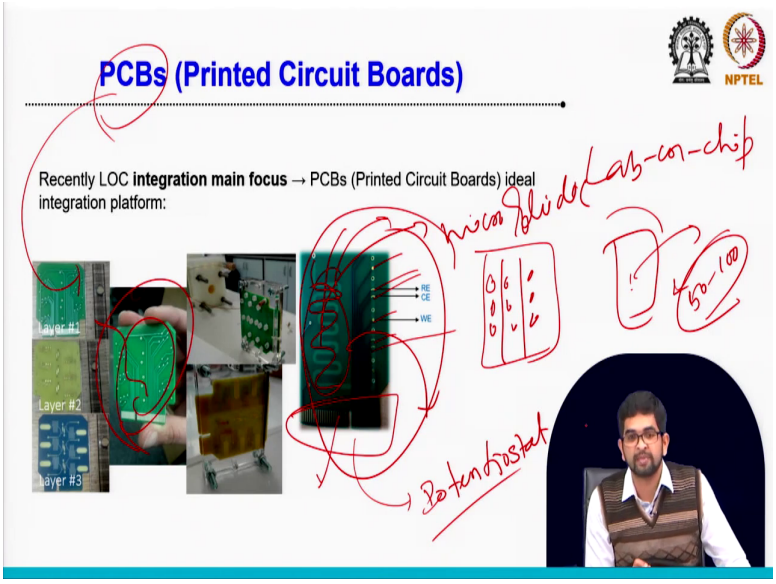

As you know like printed circuit board that recently this is it has a huge impact with for the biosensor development. Why? Because PCB based electrodes, we can easily integrate like

other stuff that we need for lab on a chip development. You can remember lab on a chip means all the lab work that we wanted to bring on a single chip.

So, there you may need like sample processing, sample purifications, maybe sample amplification, sample delivery. So, everything that you that is why you need some very micro fluidics right you needs electronics. So, everything can be definitely it can be integrated on the PCB. But if you want to use the PCB for your biosensor development you may face some challenges.

Let us show you the challenges that you may face first of all you know basically in industry level when we are developing the PCB, we have very good fabrication facility all over the India. You know all the electronics they have the printed circuit board. But the new thing why we wanted to use the PCB? Because as the fabrication method everything all the procedures is well established and we can make this printed circuit board in a large scale.

And if you can make the although your PCB the gold coated, but you may think as it is the gold coated it can be the highly expensive, but no. If you can make it in the large scale, you can reduce the cost although you may need the gold coated coat on the surface. But you are making like everyday like million of chip in a industry level then definitely the price will be reduced.

But if you make only single chip it may be the cost around maybe few thousand rupees. But you can reduced it just like the 50 to 100 rupees because you are developing you are fabricating this in a large scale. So, this is the one important thing that PCB can be used for biosensor development. But when we receive the PCB from the industry this surface is huge contamination there. I taught you know last few class the contamination effect pre-treatment pretreatment methods.

Let us use all the concept now for biosensor development and for example, we are using the printed circuit board. So, as I say is the very very ideal conditions let us show you first some PCBs like you can see if you open the computers or the mobile phone you can see this kind of

a electronic part electronic circuit you can see that is all are on the substrate then we are using some PCB substrates.

And those substrate those PCB design you can use for sensor development that I am going to tell you. You can see in the right hand side I am showing one a PCB. Here see the channel this is the simple micro fluidic channel micro fluidic channel and you can see here this is the spot this is all the spot this is all the spot we can use as a electrode.

So, this PCB design we can easily PCB designer you can easily compose your own design like if you need something like this way or something like this way or maybe you may need for multiplex detection like one chamber one here one here one here that also you can design its up completely up to you.

That is why you can play around the design here and you can integrate this kind of micro fluidics and there is a connector you can use something like a corrector and you can insert your chip then the connector and then you can apply all your like potential you may need to apply or some current you may need to apply that you can apply by using some potentiostat potentiostat that you can do.

That is why we are saying the PCB is a very ideal condition very ideal platform for biosensor development.

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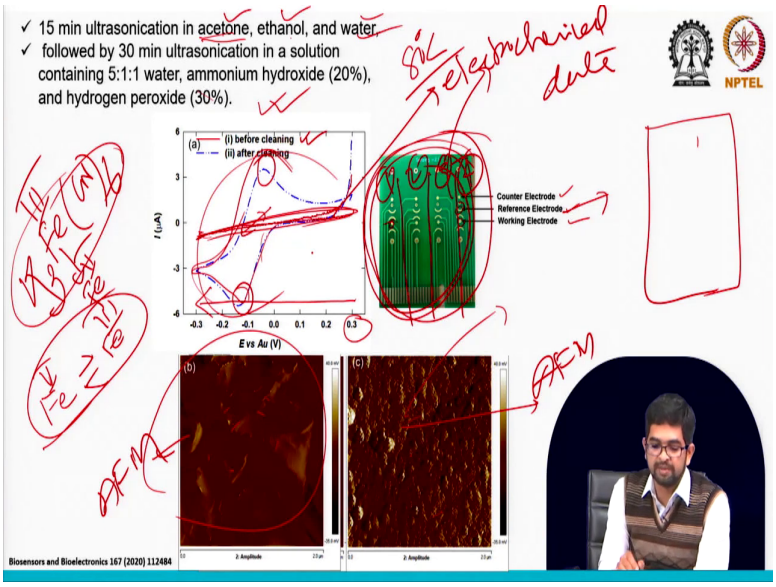

But now, I will show you few information here when suppose this is a example of the printed circuit board you can see like this is a multiplex printed circuit board 1, 2, 3, 4 like 4 channel.

So, here maybe you can drop cast your antibodies and one electrode I mean that is saying that your working electrode this is your working electrode and this one you can use the reference electrode this one use the counter electrode. Actually, here we are using gold as a reference electrode I think last class I taught you generally we are using reference electrode silver chloride or calomel electrodes something like this.

But here to make your chip very unique and to very user friendly and not to make very complications we are using some noble metal as a reference electrode. Actually, this kind of reference electrode is not mass recommended, but still if you see the gold is a noble metal

they cannot be oxidized easily. So, in this case this kind of electrode you can use as a reference electrode.

But if you see any change time to time it happen because of your noble metal although you are using gold then you have to think about the problem maybe this reference electrode somehow can be effected by your nearby some other may be below of this gold electrode you may have some other layer copper layer also there.

So, it may be exposed it can effect also the potential that you have to keep in mind. That all the story I am going to show you in the next slide again, you know because you have in the case of the PCB electrode first we are using a copper layer on the copper layer we are using the gold. So, if somehow during the pre treatment during the cleaning is somehow copper exposed, then it can effect on your data because copper can easily oxidize right it can effect on the potential what whatever you are applying.

So, let us see. When you receive this kind of PCB electrode from industry from the fabrication like where you can send to your design they can fabricate easily and after that if you measure the cyclic voltammetry.

So, you can see here actually measure the cyclic voltammetry in a mixture of potassium say you we measure from left sorry we measure from here from right 0.3 to minus 0.3 means from first oxidation then reduction and it is then you have to use K 3 F e C N whole 6 I mean here iron III stage right I mean iron first reducing F e 3 to F e 2 then again oxidizing right.

See in this red one the first one this is the before cleaning. So, when we did not use any kind of pre treatment method any cleaning method you can see there is no peak almost there is no iron F e 2 F e 3 reversible this peak you cannot see because your surface is too much contaminant when you received from industry from PCB factory. When they are fabricating the PCB there is lots of contamination there that is why I will just show wanted to show you here a AFM image of the printed circuit board that we received from the PCB factory.

See immediately before cleaning if you check you can see almost the surface is cover by some contaminant here you can see that the from this shape and from the electrochemical data like 2 way one is the AFM data and another is the electrochemical data. So, these two data can help you to predict the surface impurities you have.

Now, you can go for some surface cleaning method surface pre treatment method. What pre treatment we are following in this case that I already taught you some different different pre treatment method. So, one pre treatment method you can follow like 15 minute ultrasonication in acetone ethanol and water.

So, first like very non-polar solvent like acetone then you can go to the little polar like ethanol then polar water you can sonicate it for 15 minute 15 minute 15 minute. Followed by you can make a mixture like 5 is to 1 is to 1 ratio like water hydrogen peroxide and ammonium hydroxide then you can sonicate it or even you can heat the solutions around 80 degree Celsius and for like 1 hour then you can clean the surface with d i water and let us see the electrochemical behavior.

See immediately when you clean the surface you see you can see you can observe the reductions and oxidation very good reversible peak, but at the very beginning there is no almost no peak only just a very small current you are getting little bit current you are getting, but immediately after cleaning you are getting like this very good say if like F e II, F e III conversions happening now properly this reversible reaction happening now on the surface properly.

So, this I mean this information that is why you should have whenever you receive this kind of PCB electrodes from PCB factory lets go for this kind of cleaning and let us remove all the organic stuff on the surface there is huge organic stuff on the surface I will show you my XPS data you can see the how much contaminant there. And you can see this is the AFM image again see and this just after cleaning surface you can almost exposed now you can and in the gold surface now, I means outer surface is the gold.

But before that there is not a mass gold that is why you can see almost no current no peak, but when you clean the surface by this method you can see the very good peak. This is very good informations when you will use your PCB electrodes for biosensor development.

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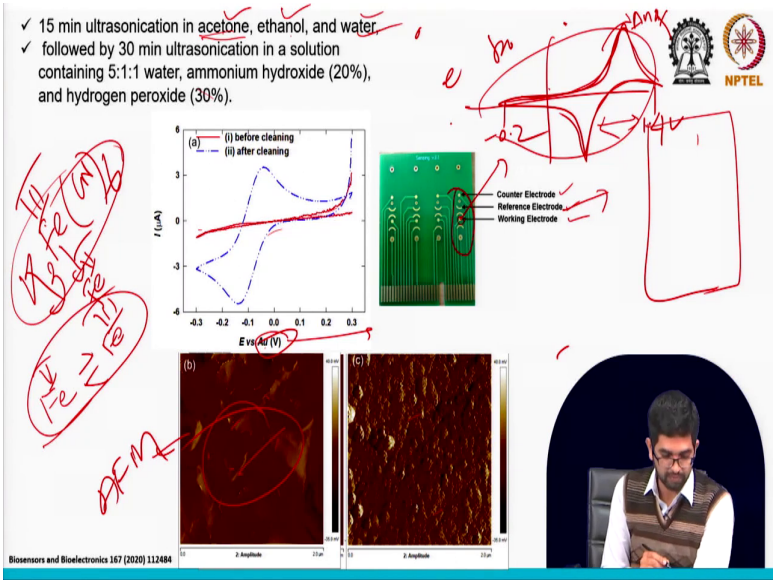

Now, let us come the XPS study photoelectron spectroscopy study you can see I means you know. So, BC means before cleaning AC means just after cleaning you see just this is the gold peak right this gold peak very very you can the intensity was very very low because it has covered by the contaminant what contaminant? Organic contaminant then you can see the carbon peak here see before cleaning you have huge carbon peak why? Because that is why you can see the AFM image is almost covered in organic contaminant ok.

That you can clearly you can prove that it has a huge contaminant of the carbon material there and you can see here for the before cleaning case for the gold peak in the 4 f region its very

low and when you go for the cleaning you can see the very good peak you can observe and you see almost after cleaning the carbon peak almost diminished means almost there is no contaminant now and carbon almost removed from the surface.

That is why you are getting mean very good reversible Fe 2 Fe 3 cyclic voltammetry here. So, like this way whenever you want to develop the biosensor lets catalyze the surface very systematically first go for the electrochemical measurement then go for AFM measurement XPS measurement and you see the contamination.

See the copper peak one story I told you know last time when like PCB factory they are designing based on your design you send your design to the PCB factory they use below a carbon a copper layer and then on this copper on that copper there is the gold.

So, if this copper is exposed during the pretreatment process because very thin gold layer we are using on the top of the copper few nanometer gold only. So, if somehow it exposed it can impact the result that is why see here you can see some copper peak actually after cleaning, we are getting.

So, before cleaning there you almost no copper peak, but after cleaning we are getting some copper peak it means you have to think about which pretreatment method will follow for your removal of the carbon contaminations on your PCB surface, I taught you many pretreatment methods right.

Now, you can link all the pretreatment method for this removal of the contaminations of your transistor surface right that is a see last few classes whatever I taught there all are equally important for the development of the sensor surface right. So, you may use this very means very strong cleaning method like piranha you cannot use piranha because it can dissolve almost the gold from the upper surface then copper can be exposed

And in this case, you can see here we measure the peak with respect to gold reference electrode right ok. I will show you very clearly now because today I am showing you the

another reference electrode for the PCB right generally I was showing the reference electrode that is silver chloride.

But here I am showing the reference electrode is the gold, but this is actually pseudo reference electrode because this this is not very much ideal, but if the potential is constant means if there is no exposure of the copper can happen then only it will be suitable.

And you have to think about there is gold dissolution should not be there suppose in this PCB surface you want to scan like anodic treatment you want to use right anodic treatment you can remember like gold will be oxidized then gold will be reduced then like this right. So, maybe minus 0.2 to 1.4 volt right.

So, gold can be dissolve oxidize means somehow gold oxide forming right AOX gold oxide forming and then that gold oxide again reducing during this process if the gold dissolve and copper can be exposed it can effect on your sensor development right that is why you have to keep in mind that can we use this anodic treatment for this cleaning method of the gold PCB or not that you have to keep in mind or if you can use then how many cycle you can try and what is the thickness of the gold you should have the all the information.

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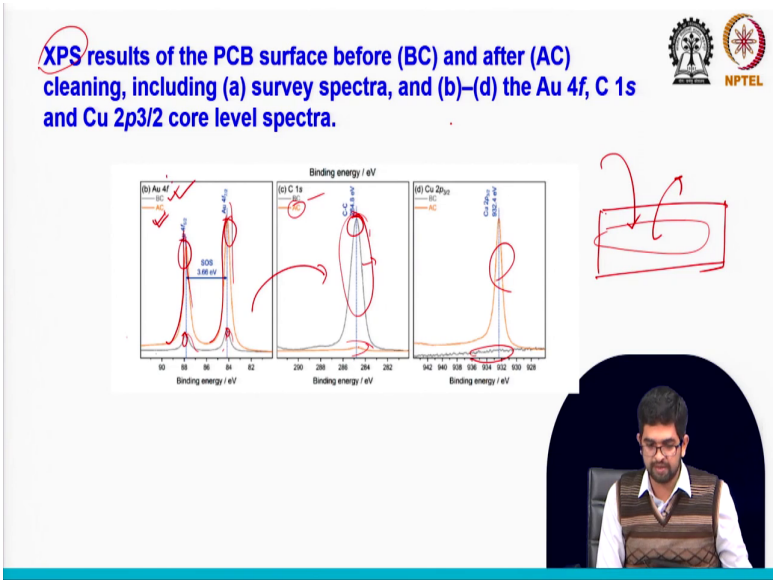

Ok this is very good means information for you for the PCB based biosensor development.

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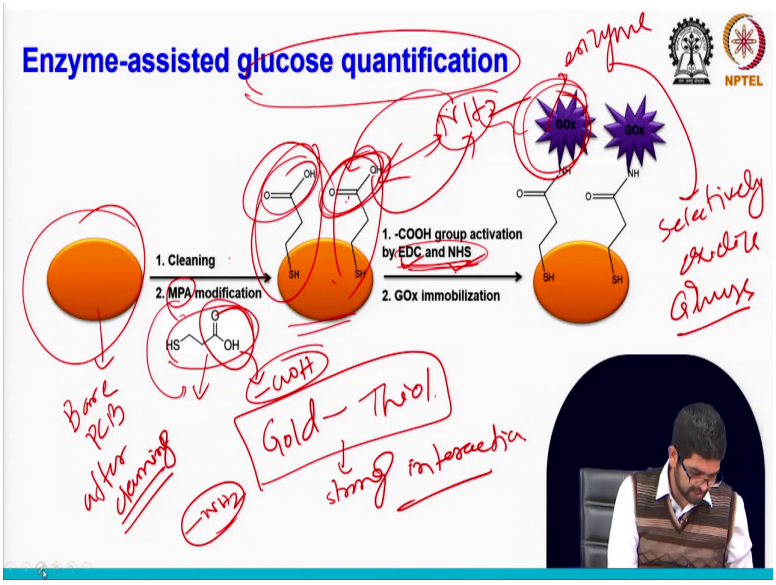

Now, let us come a PCB based biosensor development for glucose quantifications. So, your sensor surface now clean and it is ready for like some organic molecule immobilization.

You can see this image this is just bare PCB surface this is bare after cleaning. So, who is cleaning that you have to decide that I already taught you and then you have to check which is the best cleaning method. So, there should not be like this too much copper exposure should not be happened that you have to keep in mind.

So, after the cleaning let us MPA mercaptopropionic acid why we use mercaptopropionic acid for PCB biosensor fabrications? I think you can remember at the very beginning I taught you that gold and thiol they have very strong interaction right strong interaction.

So, if you drop your thiolate in molecules on your gold sub surface, then it will easily bind. So, mercaptopropionic acid MPA is something like this is a linker that will help to bind some other receptor that you wanted to use on the PCB based biosensor. So, this is your PCB based biosensor we just drop cast it your mercaptopropionic acid then you wash the surface then we got this carboxylic acid group on the sensor surface.

So, mercaptopropionic acid it has a functional group this functional group is C double O H. So, this is a very important functional group for biosensor development you know C double O H or maybe amine group you can functionalize. So, in this case we tried carboxylic group and we use MPA.

If you remember if you have the carboxylic group on your surface, then you can try EDC-NHS activations process like EDC-NHS that you can remember that I think you know that for the glucose quantifications you want to modify on the sensor surface glucose oxidase. So, glucose oxidase is a enzyme it will selectively oxidize the glucose.

So, this is the enzymatic biosensor, but here we are using some process of the modifications for MPA, for carboxylic group first you brought the carboxylic group on the sensor surface, then you activated this carboxylic group with EDC-NHS because this is just a catalyst, it can activate this carboxylic group see glucose oxidase it has the amine group pre amine group.

So, carboxylic group amine group they can bind they can form amide bond, but they are thermodynamically they are fine they can react, but kinetically they are very very slow. So, how you can make faster reaction for that you need EDC-NHS. So, they will activate the carboxylic group and then these glucose oxidase will bind on the sensor surface. This activated carboxylic group will react with the amine group or you can say the amine group will react with this activated carboxylic group and then glucose oxidase will be attached on your sensor surface.

So, your sensor surface now ready for glucose detections, but you have to keep it mind.

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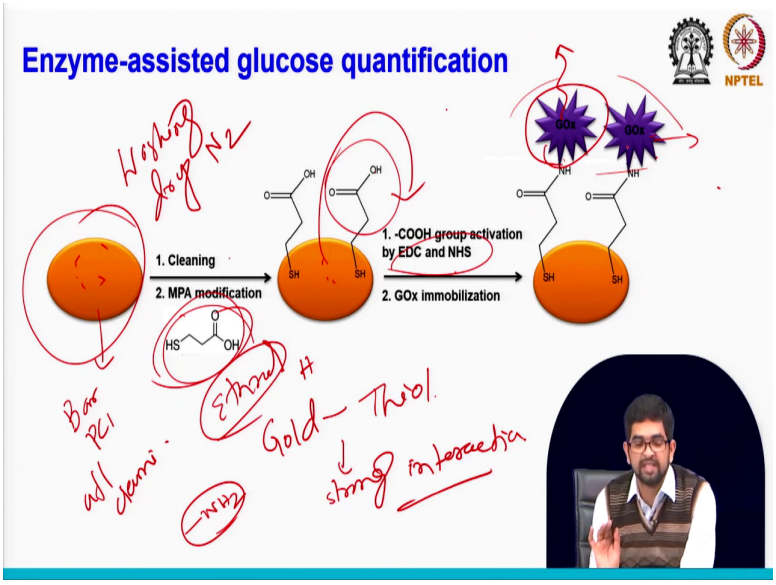

As you know now slowly you become the expert of the sensor development of the surface. So, you may need many time the washing of the sensor surface. So, you have to very much careful of the washing the step like after the cleaning definitely you have to go first thoroughly wash your surface if there is some contaminant pressure it may impact on your sensor reproducibility.

So, after treatment very very you have to be careful the washing steps thoroughly clean and then dry the surface with some nitrogen gas you can dry with some nitrogen flow you can flow with the nitrogen then dry it then use this MPA then again after the modifications of the MPA this is the organic molecules then you may need the ethanol here.

So, ethanol solution after the modification then again was it thoroughly then dry it and then again you can dip this surface in the EDC NHS solution after that you have to clean it

properly there should not be any unbound any other molecule. Otherwise, when we will add the glucose oxidase they may bind with the other unbound molecules or maybe some may be other some unbound stuff there they can bind.

So, you have to be very very careful for the washing step there is the modifications of the biosensor surface ok. So, this biosensor now ready for glucose detections and why we choose the glucose oxidase? Because glucose oxidase is very very specific for the glucose.

Later I will teach you without glucose oxidase also we can modify the sensor with some nano particle because nano if we can develop very specific nano particle that can oxidize specifically glucose, it also has impact because there is some drawback of the glucose oxidase whether it is a protein you cannot store longer time in the room temperature right.

So, that is why if you want to really wanted to form a point of care device you have to think about extreme conditions like at the very high temperature in the rural conditions if you want to store your electrode surface then there may not be available in refrigerator not available right.

So, how will store this at the 45 degree Celsius in the summer time in the rural area? That time this kind of enzyme may be denatured easily. So, if you have to think about one maybe you have to stabilize the surface anyhow otherwise go for some non enzymatic process that is the drawback, but what is the advantage of the glucose oxidase? Advantage although they are protein, they are very very specific for the glucose that is the advantage.

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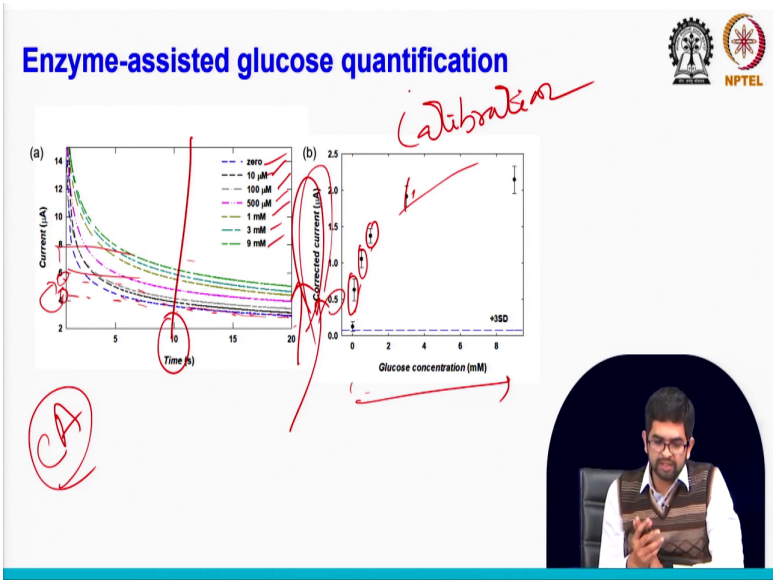

So, enzyme assisted glucose now quantifications you can see this this is a chrono-amp program chronoamperometric study we did and you see different different concentration like the 0 data. So, what is your zero data? Means, this is the surface for the 0 data. So, you can drop here just your without glucose solution you can add and then you will see like this blue one this is the 0 data.

Now, slowly increase like 10 micromolar, 100 micromolar, 51 millimolar, 3 millimolar, 9 millimolar we are increasing the concentrations and current also can be increased on your sensor surface ok. So, enzyme that already mobilized on your PCB surface now it is showing the oxidations of glucose and you can like I already taught you this is a calibration curve how to develop how to make a calibration curve that you know.

So, maybe you have to fix a time the suppose 10 second in this time you can measure all the current for each concentrations and for each concentration you have to measure 3 concentration see this is the error bar for 3 data and for each concentrations we just put all the current for the different different concentration and this is the different concentration of the glucose is the x axis y axis is the current.

And why I am saying here corrected current corrected current because we are subtracting the zero data from the say suppose you are getting this much 0 data right and your 10 micromolar data is this much. So, if you subtract the 0 current from the 10 micromolar current then actually how much current you are getting for the 10 micromolar that you can get that corrected current I have shown you here.

That all the corrected current for the all the concentrations we plot it and then we got the calibration curve. So, your PCB now is ready for the glucose quantifications.

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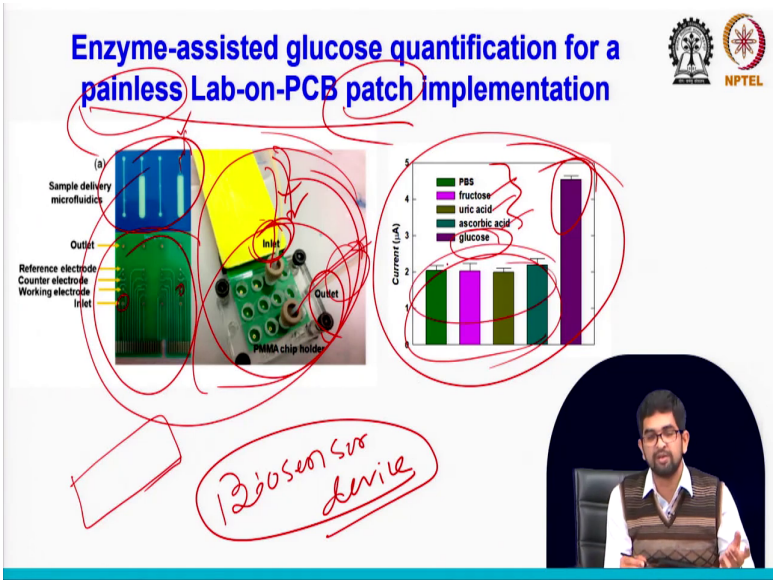

So, this is really really important biosensor for specially where we can use this PCB based glucose sensor. One is painless lab on a chip patch that I wanted to show you this applications.

Now, slowly I am entering again use this kind of biosensor or extreme point of care or develops some biosensor device. So, now we are looking for some biosensor device. So, that these after pre-treatment after cleaning your PCB sensor ready for glucose quantifications now let us use for some patch development.

You can see on this PCB we immobilize the glucose oxidase on the working electrode, we can integrate some micro fluidics. And from here you can pass your real sample like if you want to use the sweat sample or may be if you want to use some serum sample then you can pass. And then like here you can see one inlet one outlet mean we can make a device when

this is just the laboratory setup maybe next class, I will show you the actual patch how it looks like.

But first let us develop the prototype concept in the laboratory based. Let us try to learn how we are developing the prototype in the laboratory conditions. And then use that prototype for actual patch that you can commercialize this this part you cannot commercialize this is just for the laboratory setup various miniaturized chip we develop using the PCB.

Now, let us use the inlet on outlet in the inlet like a artificial sweat you can make and that artificial sweat let us pass through. Then outlet from there your all excess artificial sweat can pass out and then you can check and their concentrations of the different glucose.

Then similar kind of daytime may get in this setup also it means your PCB setup is ready for patch development a painless patch why I said because here I wanted to be sweat sample and one important thing you have observed here see 10 micromolar glucose. We can detect from this setup and our sweat glucose level is really very very low, but your serum or blood glucose is very high millimolar range, but your sweat glucose level is micromolar range and your sensor can detect in the micromolar range.

So, this sensor can be useful for painless Lab-on a PCB patch implementations that is why. So, now this whole systems in the next class I will show you a very small patch we can develop just like a band aid and we can put on a screen on a scheme and then you can measure the concentrations of glucose on like in your sweat sample and using your IOT concept mean internet of things IOT concept you can transfer this data to your mobile phone also.

That all the advantage disadvantage and still you have we have some other mechanism for this patch development that I will teach you in the next class. So, you just keep in mind when you develop some kind of patch sensor or a any bio real sample based biosensor, you have to think about interference study.

You because with the real sample you may have fructose, uric acid ascorbic acid with glucose you are detecting glucose, but you may have some other interference species this should not show much background current right that is why we measure some other also see.

So, your glucose is here, but your fructose your uric acid, ascorbic acid others they are not showing much background current. So, your sensor that is why ready for glucose detection because in this real sample not much interference have been present ok.

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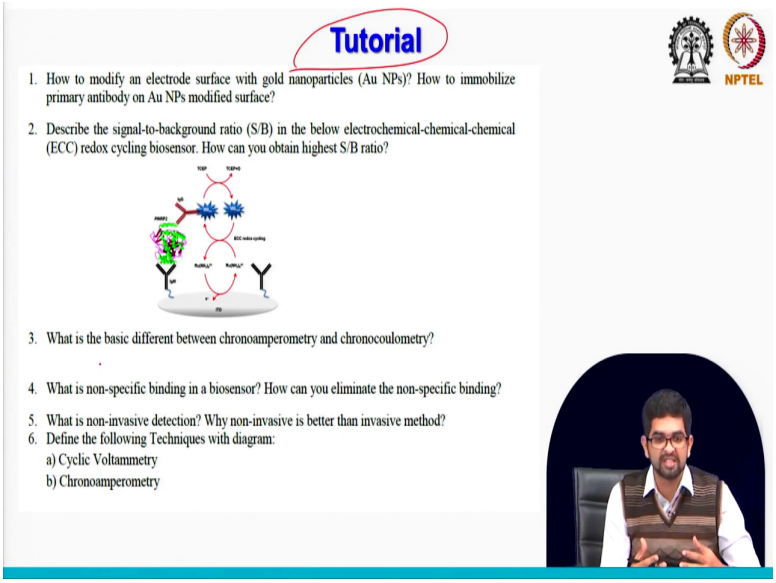

So, that is all for today's, but next class I will take a tutorial also I will give you some problem and I will discuss this all the tutorial problems how we can solve.

So, one by one I will tell you how to solve this problem and I will discuss and also next class I will discuss the patch development and then I will come to the next some wearable

biosensor device development. So, this patch kind of biosensor is the wearable sensor. So, this concept I just started today then I will cover next class also I will give you I will discuss some problem so, that you can design your own biosensor like this like six problems I just made that I will discuss one by one all the problems.

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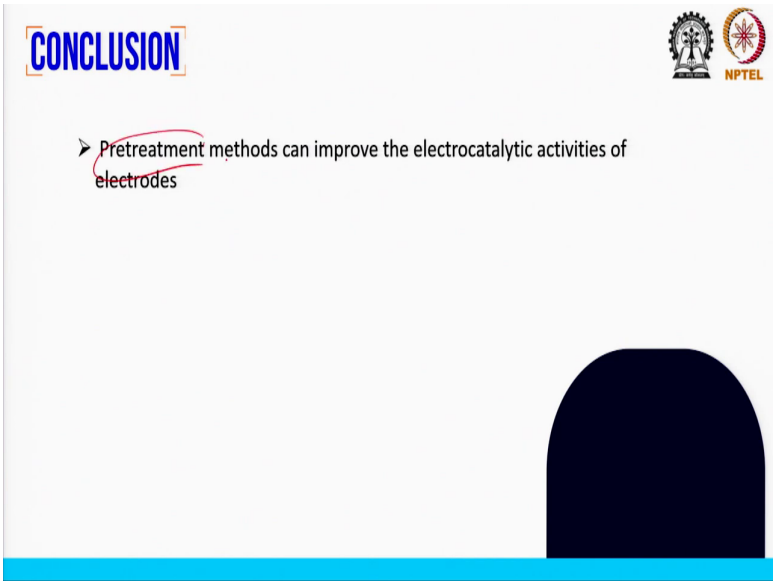

And then finally, I will go to the another biosensor that is wearable biosensor for other analyte detection. This this wearable sensor that I started today that is glucose detection using some wearable patch.

So, today's main conclusion just try to learn that is the pre-treatment procedure is very very important for biosensor development because it can remove all the contaminations. Although it can remove the contaminations that to which pretreatment is the best for biosensor

development for which substrate and then modify like your common substrate for biosensor development and use some really important target detection.

Because right now we have glucometer, but we need the we need blood sample, but today I started like how we can develop a glucometer type of sensor from the sweat sample ok. that is all for today.

Thank you very much.