Nanobio Technology Enabled Point-of-Care Devices

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Lecture - 10
Limit of Detection and Wash-Free Detection for Biosensors

Dear students, today this class I will teach you the Limit of Detections and Wash-Free Biosensor. So, last class I taught you different technique like cyclic voltammetry, chronocoulometry, chronoamperometry. Now, using this technique we have to find out the sensitivity, how sensitive this biosensor. So, we will have to we have to understands the calculation like how we can calculate the sensitivity of the biosensor.

Then, next topic I will go to the wash-free biosensors, this is a very important technique of biosensor, so that we can apply this novel technique for point of care device development that I will teach you today.

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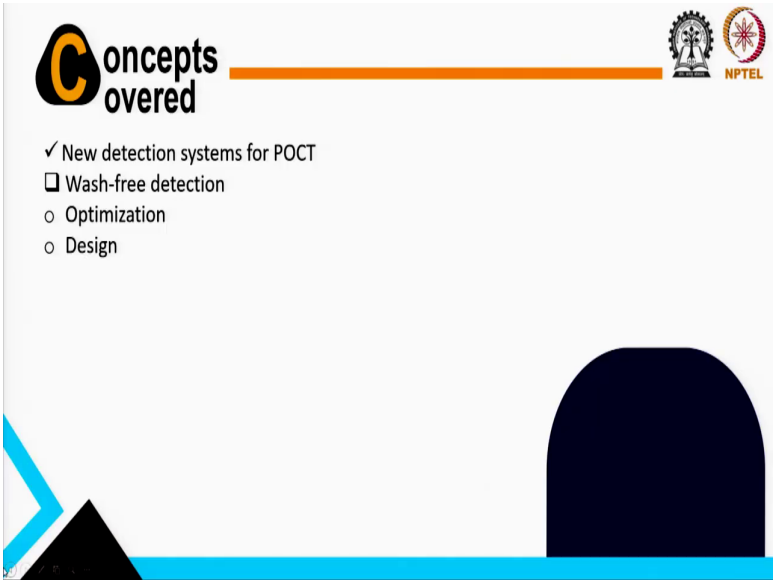

So, our main concept we will cover today, the limit of detections that is the very parameter just very basic things, then I will cover wash-free and its optimizations, its design used for the biosensor development, ok.

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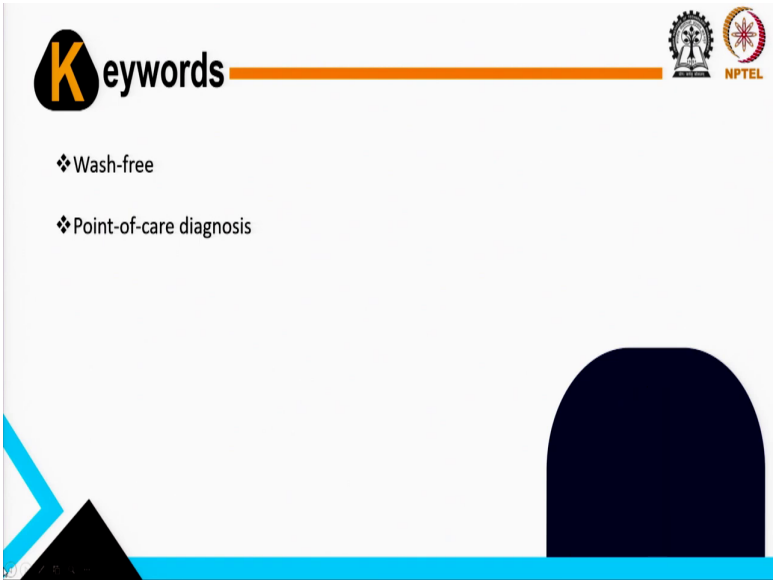

So, the main keyword of this teaching was the wash-free technique and point of care diagnosis. Another thing you can consider the limit of detection, these keywords you can search and you will get lot of informations for the biosensor development, ok.

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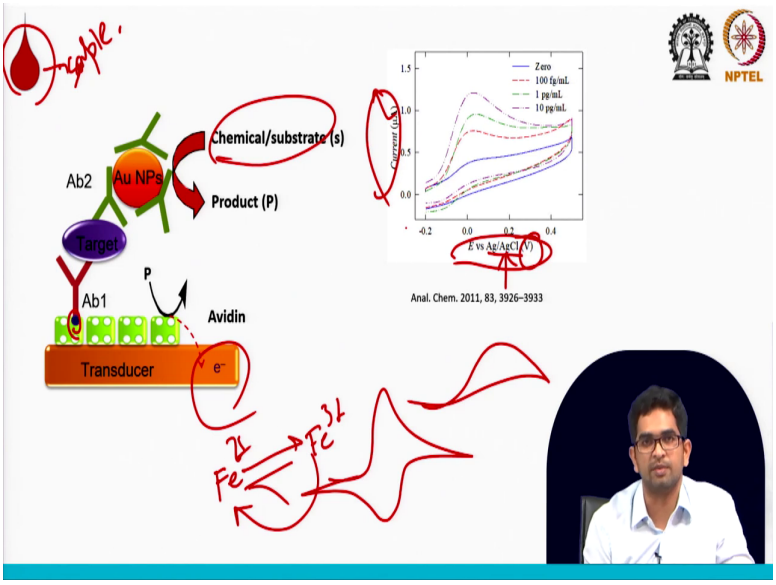

Now, let us come the biosensor, how we can use this one? By using our whatever you learned last few classes, then apply your knowledge of cyclic voltammetry, amperometry and coulometry and detect the signal, ok. Suppose, you have a transducer surface, right and this transducer surface is modified with avidin.

Why we need avidin? To modify the primary antibody right, because avidin and biotin they have strong interactions. Your primary antibody already conjugated with your primary antibody will conjure with the biotin, right, the biotin. So, avidin biotin is a strong interaction that I already taught you. So, this is just for conjugation chemistry and we will drop the sample, here is the sample.

So, if you drop the sample, then your target will bind with your specific antibody and then drop your secondary antibody that I taught you, the secondary antibody already have the tag

like gold nanoparticle and then you can use some chemical substrate and that can react to form the product and that will release the electron, right. This is the very basic biosensor.

Now, get the output, how we can measure this output, just last class I taught you know different technique, let us use those technique. So, this is just for your applications of your technique. So, just I want to apply cyclic voltammetry. You can remember this is the cyclic voltammetry technique, where in the x-axis is the potential window and we applied, see we mentioned silver, silver chloride.

Silver, silver chloride is the reference electrode because we are applying some potential with respect to some reference electrode. Which reference electrode? That you have to mention here all that is. So, E versus Ag AgCl. So, an unit is the volt and y-axis is the current where unit is the micro amps.

As I said if this chemical this chemical is formed like a very good reversible redox process like Fe 2 plus, Fe 3 plus, they are very reversible reaction. In this case you will get this kind of cyclical voltammetry shape, voltammogram like it would be like this. But, if it is not like a reversible reaction, something irreversible, suppose you have glucose as I mentioned you know.

So, in this case you will get something like this or some substrate if the oxidations is not favorable much, then you will get something like this. So, in this case we use some chemical, the oxidation is favorable, but induction is not that much favorable ok done.

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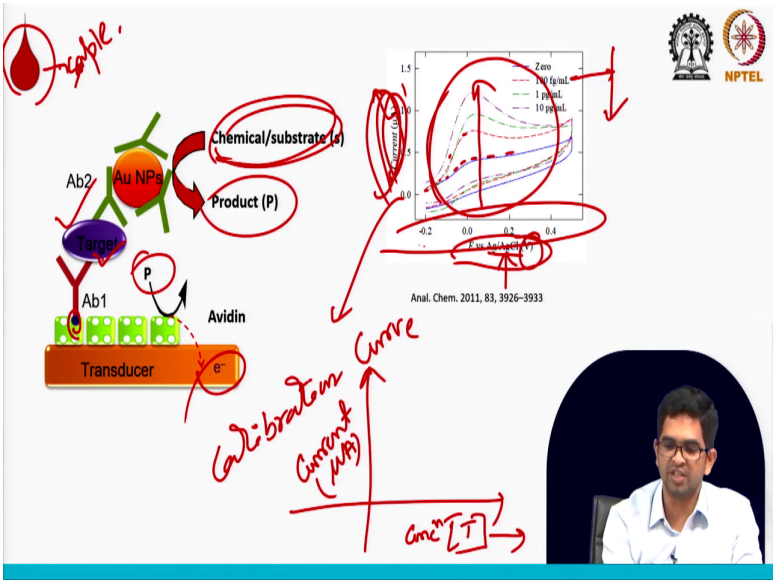

Now, I will come how we can measure this electron. See, in the cyclic voltammogram case, we will scan in a potential window like 0.2 to 0.4 and we will measure the different different potential we will measure the current. See when you oxidize from the negative potential to positive potential, the current increase and then it is become like this.

Now, if we increase the target concentration, what happen in the sensor surface, what happen when you increase the target concentration? More target means more gold nanoparticle will be on the surface. So, more gold nanoparticle means more chemical react will form the more product. So, more product means more electron. So, your current of the cyclic voltammetry will increase.

See so, as you increase the concentration from this to this, when you are increasing the concentration, see the current also slowly increase, right? So, your cyclic voltammetry

helping you to get the output in the form of the current with respect to potential and by using this current, you can determine the concentrations of the target present on your biosensor surface.

How? So, you can make a calibration curve basically by using this cyclic voltammetry, this is just in the form of current. Now, make a calibration curve. How will make calibration curve? Calibration curve will be something like this in the y axis, you are getting the current, right.

So, y-axis your output current microamps in the x-axis is the concentrations of your target, your target concentration that you can put in the x-axis. So, let us measure the all the current with changing the with changing the target concentrations ok. So, from here, let us remove.

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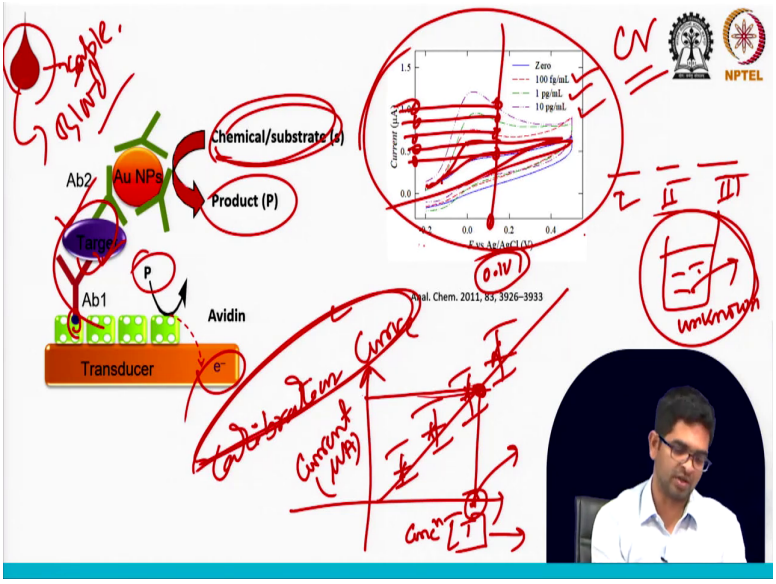

Suppose, in the 0, so, you have to fixed a potential, suppose at the 0.1. So, 0.1 volt, in this potential, you want to measure all the current. So, 0 cases you are getting this much current, in that 100 femtogram case you are getting this much current and 1 picocase you are getting this much current and 10 picocase you are getting this much current, right.

So, these all the current you just measured and plot it, suppose 0 case you are getting here, 100 case you are getting here, then 1 case you are getting here and 10 case you are getting here. So, something like this. So, then you can make a something like this a calibration plot, but not one data, then it is rule at least you have to make a three data for each concentrations.

Then you have to check the error bar, for one concentration you have to make three sensor surface to get the reproducibility, to get a reliable sensor at least three sensor, same concentration you have to make and you have to see they are reproducible or not. So, 0 cases just you have to check like 3 times like by measuring three different different electrode surface, same case 100k, 100 femto, 1 pico, 10 pico, efficacy we have to make 3 3 electrodes.

But specially we favored zero data your basic data right, then we are taking sometimes 7 times also 0 data, so that at least your base value we should be more reproducible right, it should not be like up and down. Then it will difficult to obtain the sensitivity means your limit of detection for the sensor. That is why we are measuring more data of the 0, then we are getting the some error value, this is the error value error bar.

And, if you make the straight line something like this so, you can see your full calibration curve will different different concentration. So, this is called calibration, this is the your output value by using cyclic voltammetry. Now, suppose you have a sample a unknown sample you have, unknown sample your you use here blood sample. So, you have a one unknown blood sample you do not know its concentrations of target that you do not know.

So, how will measure? Let us drop this sample here and measure just run a cyclic voltammetry suppose it your cyclic voltammetry came here. So, how much current you are getting in this potential? Suppose here 0.5. So, suppose this one here, here. Then you just

draw a straight line up to the straight the up to this line, then draw again the straight perpendicular line, then you can measure your unknown concentration ok this much. So, this much your unknown concentration.

So, by applying your calibration curve you can determine your unknown concentrations of a solution, ok. So, this is just for using a cyclic voltammogram technique. Now, let us use chronoamperometry, let us use the chronocoulometry all the technique. Now, I will teach you, I will show you this is just for just one example I will show you, then next I will come the another topic ok.

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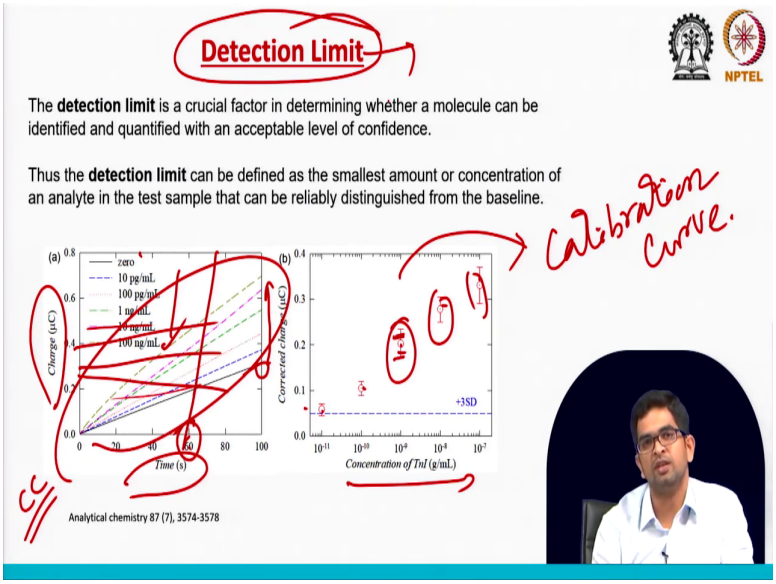

So, for getting the detection limit here I am showing the another example this is what is this unit? This technique remember here in the y-axis we are using charge x-axis with respect to time. So, this is chrono-culometry, right.

So, we are using chronoculometry with different concentration of the target that is we go back. So, here we use the cyclic voltammetry with changing the with changing the concentration and here we are using the chronocoulometry with respect to charge and time.

So, zero data here now change the concentration 10 pico, 100 pico, 1 nano, 10 nano, 100 nano, right like this way we are increasing the concentration see we are increasing the what we are increasing? Charge we are increasing. Why charge will increase because you are using something like this biosensor a sandwich biosensor sandwich (Refer Time: 10:53) type of biosensor develop.

So, if you increase the target so, more nanoparticles will form on the sensor surface more nanoparticle means more product will form you will get the more and more electron on the surface. So, charge will increase see that is why charge we are increasing and you measure and here see this is the calibration curve. So, based on this output based on chronocoulometry so, based on this output we measure this calibration curve.

So, for that you have to just fix a time like suppose 60 second, in the 60 second we will measure all the charge all the charge we will measure for each concentration. See for the each concentration suppose in the 67 we fix all the charge and which this concentration case we are getting this much of this concentration, this concentration, this concentration and we measure 3 3 times that is why we are getting this error bar each concentration we measure 3 3 times, ok.

So, if your sensor surface is very reproducible then this error bar will be it is very short and your sensor will most reliable if this error bar is very short, ok. Now, let us come the detection limit. This is a very very important parameter to understand your sensitivity of your developed biosensor. Let us teach you the detection limit. So, what is the definitions of the detection limit?

Detection limit is nothing, but this is a whether we can detect a molecule in a certain limit like accept it will show you the acceptable value your sensor can detect this is the lowest

amount you can detect by using your biosensor that is the LOD. It is called limit of detection or the detection limit LOD.

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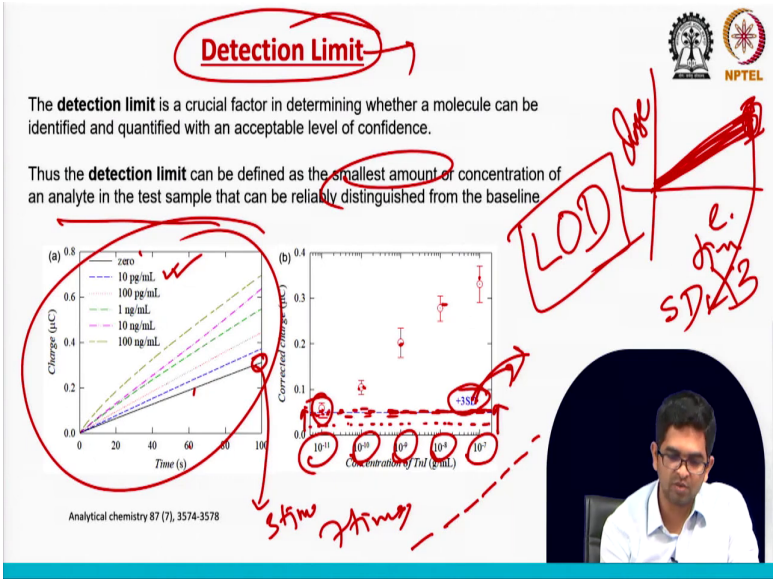

The detection limit can be defined as a smallest amount of the concentration of any analyte or in your test sample that can reliably, we can distinguish. See, so, from this biosensor we can see that we can determine 0, 10 like this way, then we made a calibration curve. So, in this calibration curve you can see we put all the concentration all the different different concentrations, so, this is the 10 pico, then 100 pico, this is 1 nano, 10 nano, 100 nano in this case you are getting this much charge.

Now, here I did not put the zero data I put the zero data something like this way and we put plus 3 SD. So, what is this? Let us understands this one to calculate limit of detection, ok. So, as I mentioned your zero data is very very important to get the limit of very if you want to

develop very good biosensor or very sensitive biosensor your zero data also should be very very predictable.

That is why sometime we do not we are not measuring 3 times also or 7 times you can measure means 7 times means 7 different means seven sensor you have developed means similar your zero data sensor you can develop. Then measure there all the zero data suppose in the 0 like chrono coulometric is zero data you are getting this like this way charge versus time like seven data it can be like this.

So, there is some deviation there. So, these error were we have to calculate means standard deviation. You have to calculate from here standard deviations of the zero data 7 zero data you can measure and calculate the standard deviation and you can multiply with this 3. So, maybe your standard deviations of your zero data is here. But we are multiplying with 3 then it come here. So, why we are multiplying 3? To minimize the human error.

So, see suppose I am developing a biosensor using the all the protocol. So, I am getting this much 0 current 0 when background that is your background current zero data case, but you are developing and you are getting maybe something different little bit different. So, it is depend man to man sometime it can vary also, ok.

So, to minimize this one and to make the most reliable sensor, so, we are multiplying with this 3 and see and this 3 times standard deviation this is the mathematically proof I mean mathematically acceptable if we can multiply with 3 and see it come here and then it will decide your sensitivity of the biosensor. See these 3 times standard deviation now it is it already tasked with the your concentration that detected with 10 picogram.

So, we cannot go below of this concentration with your biosensor because this value already touched your 10 picogram per ml concentration, this charge value. So, this one will be your detection limit because it already touched.

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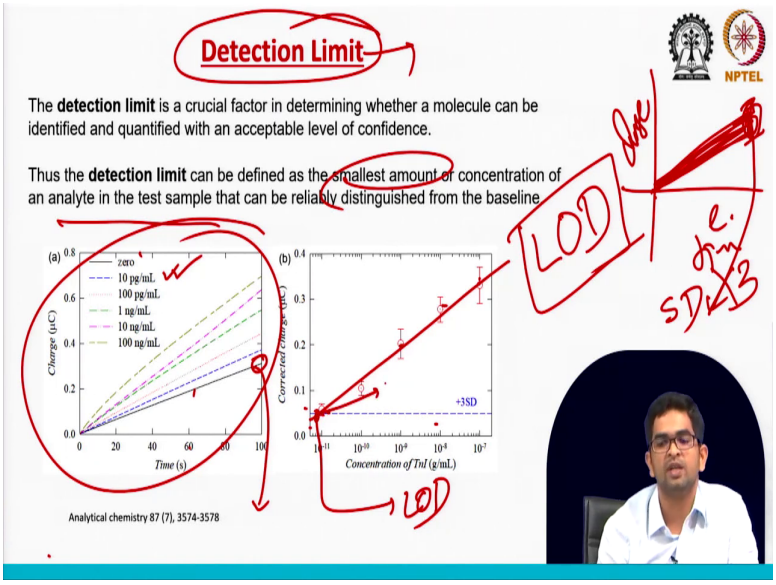

Generally, what we are doing? So, let us remove this thing we are actually making a straight line. We are making a straight line while using all this concentration and where it intersect this one, this one will be your LOD, ok. So, you cannot go below than this because it is already touched and this line and if you make the straight line and this is already touched this is your limit of detection.

But if you have one calibration curve suppose I am I want to show you suppose your calibration curve this one maybe here right maybe this concentration here. So, we have some gap with this 3 times standard deviation then you can go below of this concentration that will be your limit of detection. This is not your limit of detection if it is off of this line.

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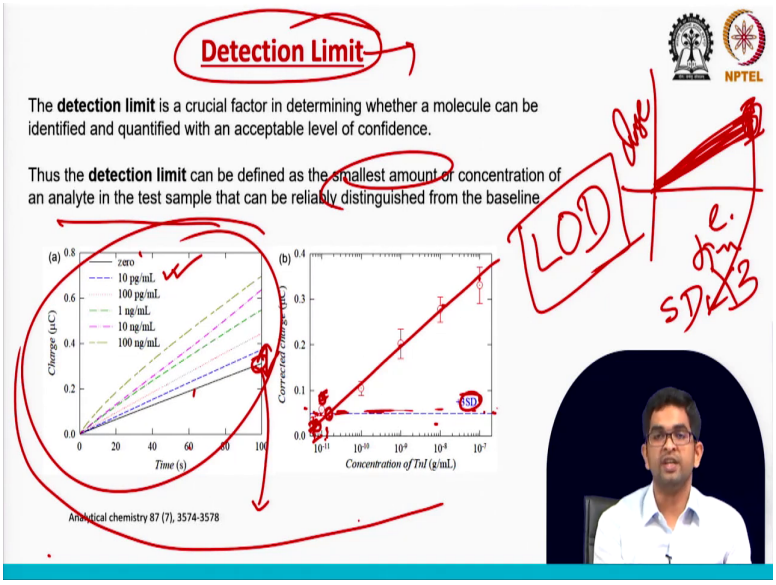

But, if suppose you have one biosensor and it can measure means you can it can measure 10 picogram per ml, here you can see I mean here you can see difference here 10 picogram ml here it is showing the difference. But, after calculating standard deviation multiplying with 3 everything, but your 3 times standard deviation line here and your 10 picogram per ml this charge is point is showing here then this is not your limit of detection.

Then you have to as you plot put the make the straight line and if it cross here this will be your limit of detection, this is not your limit detections ok. So, you have to first measure the 0 data, you have to make the standard deviation and multiply with 3 and then you have to make this base straight line. This will guide you to determine the limit of detection.

So, this one the lowest amount you can detect that this parameter detection then it will guide you ok. So, this is the whole things, this informations we are getting from this limit of detection this parameter and we can determine like this way.

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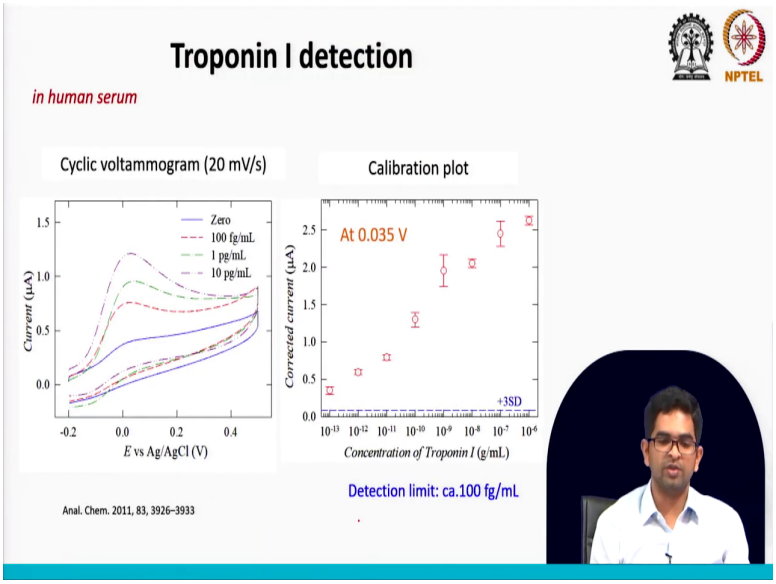

Now, I will show you on more example of limit of detection by using the cyclic voltammetry that I taught you already before. So, last class I showed you just a chronocoulometry where here just a cyclic voltammetry. I think I have already mentioned see in this case in the cyclic different different concentration we are getting different different cyclic voltammetry like 1, 2, 3, 4 different different cyclic voltammetry we measured.

Now, other data I did not show you. So, in this case now you can see you can measure so many other concentrations also. So, many other concentration you get the cyclic voltammetry something like this and you have to fix a potential. So, here is a fixed a potential like 0 point

0 0.035 volt. In this potential, we can measure all the current and those all the current you can put here with respect to the concentration.

So, this is called the calibrations and then you have to determine the standard deviation of the zero data and make this value and then make a straight line and where it intersect that will be your limit of detection, ok. So, like this way we can determine. So, this thing is done limit of detection this part I think it is very clear to you.

Now, I will show you some biosensor where we can use this all the concept to determine the limit of detection and use this all the cyclic voltammetry, coulometry, amperometry all the cyclic voltammetry, chronocoulometry, chronoamperometry, all the thing we will use ok.

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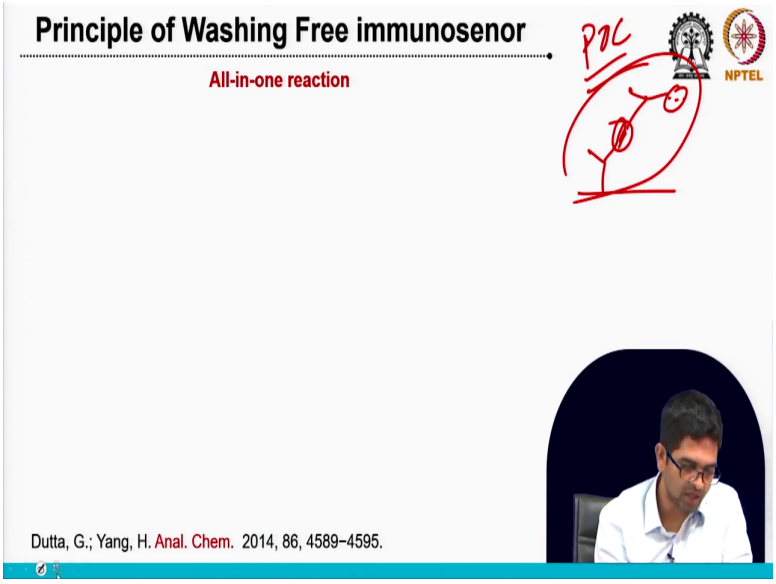

Now, today, I will show you one more important technique for point of care device development that is washing-free biosensor or immunosensor. So, this washing-free immunosensors is very very important to develop a point of care device. What it is? Washing-free as I mentioned when you develop some biosensor you need many steps washing, right on the primary antibody surface they may be you will drop a what? Your target.

Then you have to wash it, then secondary antibody that conjugate with the level then you have to wash it, but that cannot be done by end user, right. If you want to develop a device for point of care may be for field testing. So, end user cannot do this all the step. That is why you if you can develop a sensor that is wash-free just drop the sample you can get the value that will be very impactful for the point of care device development.

Let us come the principle for this wash-free device development ok. So, let us remove this I will now show you one by one steps ok.

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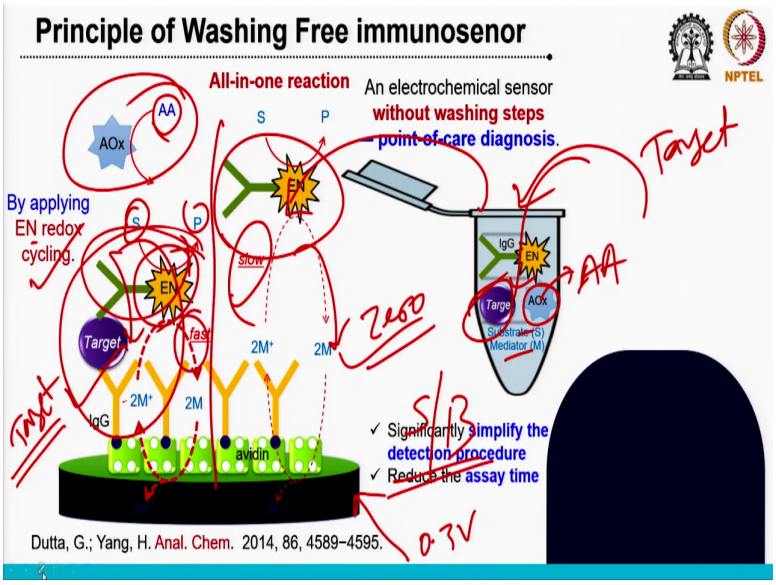

So, washing free technique this is the very important where all environments all the reactions will happen on the sensor surface you do not need any extra steps. Suppose you have a like this a sensor surface that already modified with some primary antibody that where I use the avidin and biotin avidin antibody this conjugation protocol.

Now, what I will do, in a sample tube I will put your all the chemicals like secondary antibody we need some substrate we need some mediator everything I will put on a sample tube. And, here also I will draw here also what I will do? I will draw my target solutions target solution. So, this is your target I will drop the target solution. Now, I will drop these solutions on the sensor surface ok.

What will happen? What will happen if you have the target this will bind on the sensor surface right with primary antibody and your secondary antibody that is already labeled that

also will bind with the target, right. And all the other coming chemical like substrate, mediator here AOX; AOX is the ascorbate oxidase one chemical that can react with the ascorbic acid and it will remove the ascorbic acid by reaction.

So, why we need ascorbic acid because ascorbic acid is very very means like bad it is a interference species that can increase the background current. So, we have to remove them. So, that is why I use the AOX that will react with ascorbic acid you will remove. So, all the chemicals I will use in this sample tube. I will drop it here, then reaction will start.

See, yours sandwich already formed on the sensor surface and now, if you have the chemicals everything then you can apply the a redox cycling reaction like EN means enzymatic redox cycling because your tag is a enzyme. So, you can apply enzymatic redox cycling. See, so, M plus means your mediator oxidation state, M means your mediator in the reduction state like ruthenium 3, ruthenium 3 plus ruthenium 2 plus something like this.

So, your substrate product glucose it will be the glucose oxidase it will react, right. So, this kind of redox cycling reaction will start on the sensor surface and if you have do not target see there is a two part in this sensor. In the left hand side if you have the target in the right hand side if it is 0 data; like if you do not have target so, if do not have target then your secondary antibody that conjugate with enzyme that will not bind on the sensor surface right if you target then it will bind, right.

So, when you apply some potential on the sensor surface suppose 0.3 volt you are applying and you started the redox cycling reaction these redox cycling reactions will start in the left hand side that will be very very fast that will be very very fast. Why? Because it is very close to the sensor surface. It is called the close it is called proximity dependent this is proximity dependent electron transfer.

So, this redox cycling electron transfer because of the closed proximity it will be very fast, but in the other case it will be very slow because it is far from the sensor surface. So, this electron transfer rate will be slow. So, easily that is why you can differentiate target and zero

data like signal and background data. You can easily differentiate because of this proximity dependent electron transfer rate is the different, clear?

So, see here I am not using any washing just we drop all the chemicals here then we are applying some potential and we are getting the different. Why it is we are getting the difference because of this principle proximity dependent electron transfer this difference this principle we are applying.

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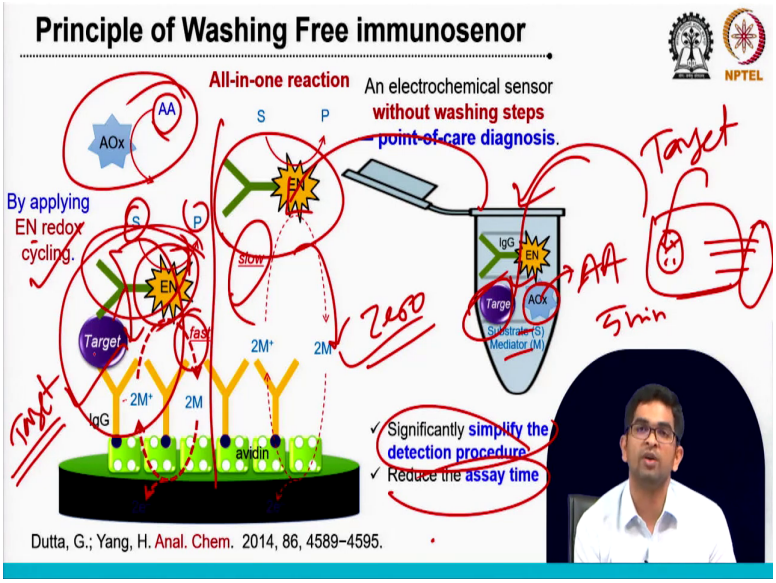

See, so, this actually basically you do not need any extra steps of the washing steps that is why it is significantly can reduce. It significantly can reduce the detection protocol and detection time, you just you can develop a sensor surface, there will be just as sample zone you can drop and you just apply some potential using some portable potential strand and that is done.

May be you can wait for 5 minute for antigen antibody interaction and you can see the value. So, this kind of washing free sensor is really impactful for development of point of care device that is all.

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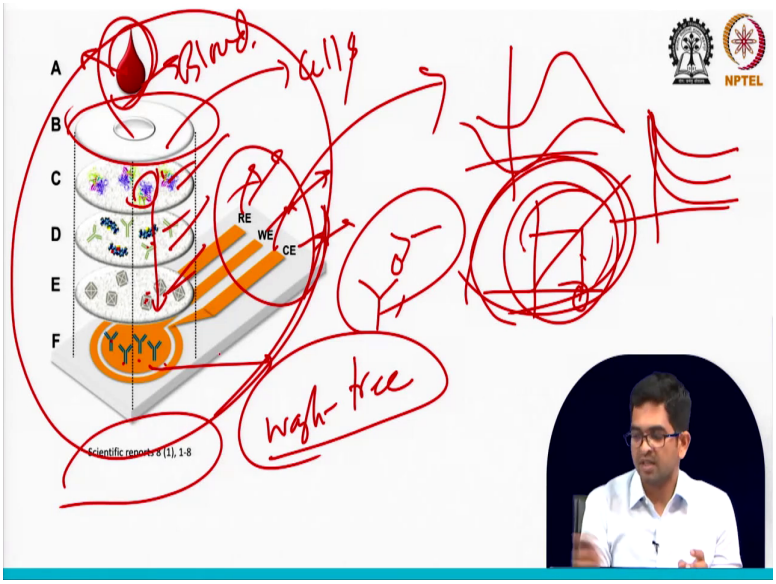

So, I will show you this kind of wash-free sensor can be useful very cheap base or lab on a cheap based biosensor like something like this I want to show you one basic very very basic example that is reported in this general. You can see here or your primary antibody coated your three electrode system, right? Reference electrode, working electrode, counter electrode – this is your three electrode system.

And, offer of this antibody coat it is working electrode surface you can use few membrane that is paper based membrane you can use where you can use all the chemicals. So, here may be some ruthenium based chemical here, some methylene blue or may be a secondary

antibody. So, here also some chemicals you can use for redox cycling formation to get the ultra-sensitive biosensor, right.

All the chemical I taught you know we need some different chemicals for ultra-sensitive biosensor formation so, those chemicals you can keep here all the this membrane. Now, we can drop the sample, ok. So, if you drop the sample then this suppose it is blood sample, but blood contains so many blood cells. You have to separate all the blood cell.

So, here we can use a blood cell separation membrane. So, it will remove all the cells, then you will get on the plasma. So, this plasma will go through this all the reagent. So, this all the reagent will dissolve your primary and your secondary antibody everything will come and they will form now a this kind of sandwich (Refer Time: 27:49) on your sensor surface, clear?

Now, you can apply the potential. Here you can apply the potential and you can see the output may be may be in the form of cyclic voltammetry case you will get output something like this.

Now, if you it is chronoamperometry then you will get the output something like this with different different concentration, you can see different different value we can see and based on the calibration curve you already have a known calibrations curve, right that I told you and based on unknown solution you know you can determine the unknown sample concentrations.

It is very and that everything we can make a mobile phone based sensors. So, your all data like your all the calibration data everything will be stored and we can make IOT enabled biosensor and in a app all the information will be stored. And, this how much current is generating via Bluetooth it can send to your mobile phone ok then this current is corresponds to how much concentrations it can automatically it can predict and it will show on your app display you can see, ok.

So, if it is like malaria if you want to detect so, it can show you how much malaria present in the blood samples, you can see easily. So, like this way you do not need any sample pre-processing, you do not need many step sample handling, mainly you do not need any

expert persons by developing something very simple sensor surface you can use this one for point of care device development.

So, this is. So, here I use some wash-free technique, wash-free biosensor this is called that is why and that one will be useful it is very very small chip also you can develop. It can be useful for point of care device development ok. So, in the next class again I will show you like a different different optimization parameter how we can optimize like here I showed you the principle of the washing free biosensor development, but I just principle I taught you today.

Now, let us find out how we can optimize a wash-free sensor there is different parameter existing parameter are there. So, we have to optimize all the parameter because there is otherwise, we can face lots of interference effect. Maybe your signal and background we cannot differentiate if we cannot optimize all the parameter, it is very very important things. So, next class I will teach you all the parameter select sanction we can optimize ok.

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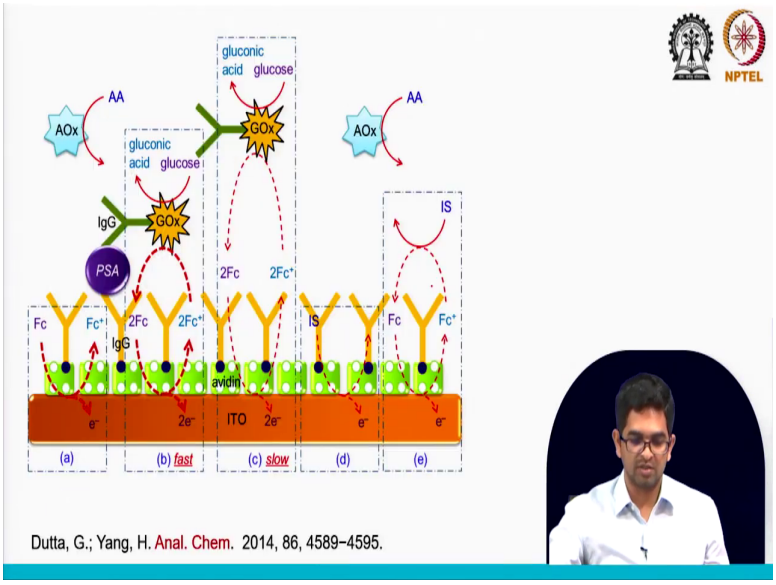

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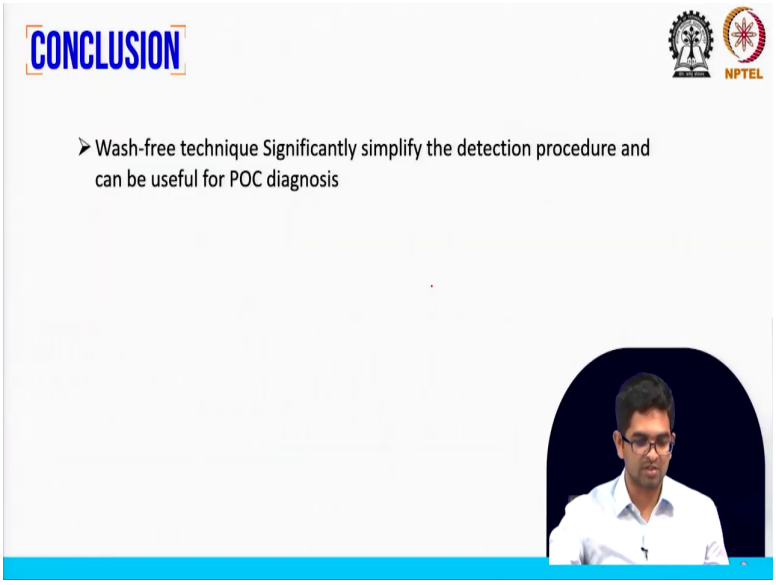

So, that is all for today. So, here I just taught you the wash-free development, seen a signal measurement systems and this will be very much useful for point-of-care devices ok.

So, thanks for today. Next class I will teach you the optimization for wash-free.

Thank you very much.