Nanobio Technology Enabled Point-of-Care Devices

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Lecture - 09
Different Measurement Techniques for Electrochemical Biosensors

Hello students. So, today we will discuss on Different Measurement Techniques for the Biosensor Development. So, I describe the development of biosensors. Now, let us collect the signal using different techniques. Like which technique? Like, how we will measure the electrochemical signal, right.

So, as I said like when we increase the like different concentration of target, then we can see the change of signal to background ratio. Well, let us measure the signal, let us measure the background, how we can measure. So, those techniques I will teach you today.

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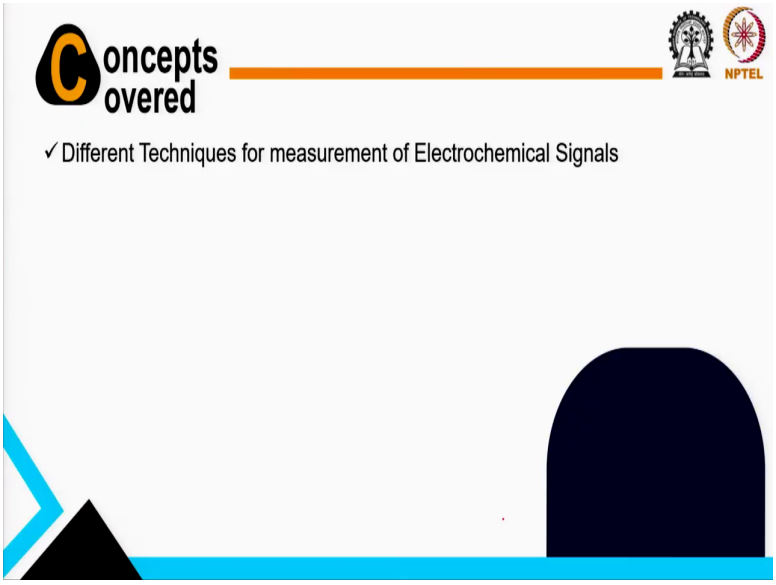

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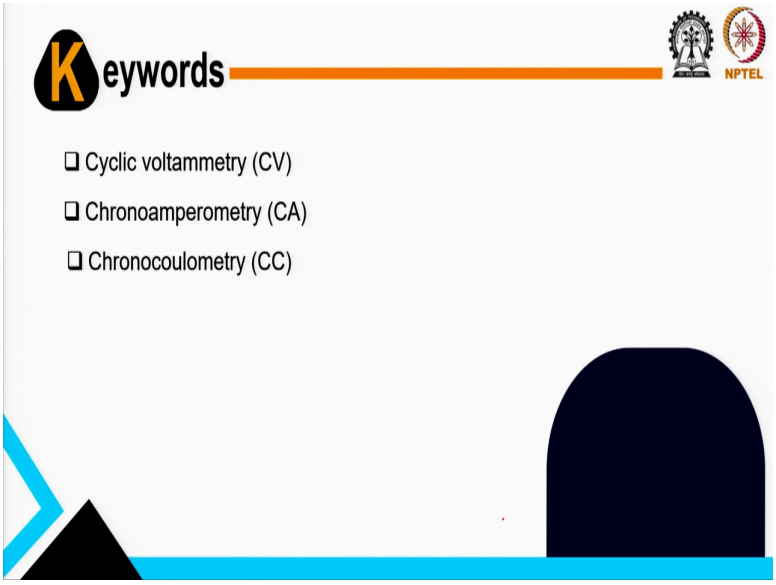

So, the main technique of this different electrochemical technique method that I will cover today is the first cyclic-voltammetry that is called the CV, and then chrono-amperometry or CA, or chrono-coulometry CC. So, these 3 techniques I will cover today, ok.

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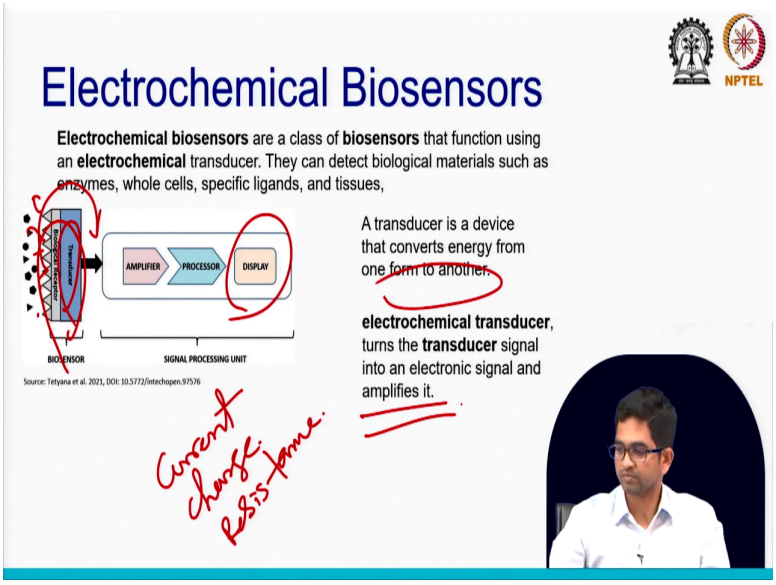

See, the electrochemical biosensors that mainly will be measured by different electrochemical technique. So, first give you one basic introductions. Like, I told you that electrochemical biosensors, this is the area of the biosensors that functions the electrochemical that we are functioning the electrochemical transducers. As I told you, the like this is the transducers where we are modifying the different kind of bioreceptor, right, these are all the bio receptor. And different like only specific analyte will bind on the bioreceptor on the transducer surface.

Now, what is the role of the transducer? The transducer is a device that converts the energy from one form to another form, right. So, here may be your chemical energy will transform to a electrochemical energy. I mean they will converts this is the number of the electrons. So, then we will amplify, we will process it, and we will display on a detector.

So, how we will see the output? Right. The may be in the form of may be it can be in the form of current, right, may be in the form of charge or may be in the form of resistance, right. So, something like this. So, the different different parameter we can measure. So, electrochemical transducer, the transducer signal into the electronic signal and then it will amplify it.

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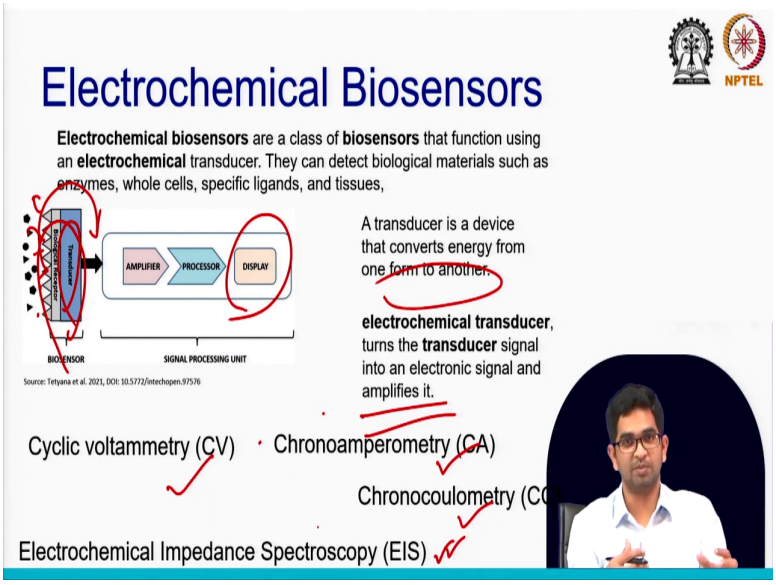

See, so, these 4 techniques I will mainly cover today's this lectures. First is the cyclic-voltammetry, means what is I will describe very briefly first today. What is the this kind of what is the cyclic-voltammetry, what is chrono-amperometry, then what is the chrono-coulometry and impedance spectroscopy.

So, mainly, I will focus this 3 part first cyclic-voltammetry, chrono-amperometry, and chrono-coulometry because they are very inter related to each other and easily we can apply

this one for biosensor applications to get the output signal from the biosensor surface. So, you should know this 3, main this 3 technique, ok. So, where we mentioned it and we are applying some potential we are getting the output in the form of current or in the form of charge, right. So, let us try to understands now this technique.

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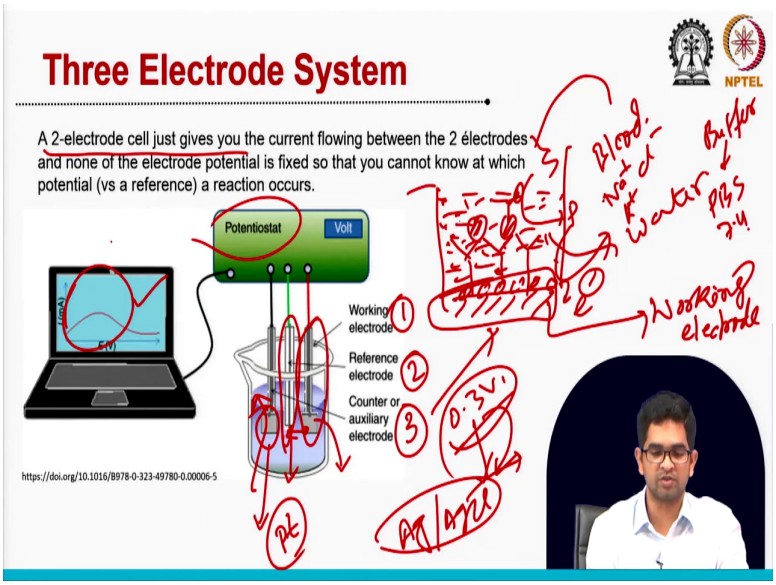

So, first you have to understands that how we can fit your biosensor surface so, that we can apply some technique like cyclic-voltammetry or chrono-amperometry or chrono-coulometry whatever. So, we are using biosensor surface as like kind of 3 electrode system. So, you will have a sensor surface as I told you, this is the sensor surface where you are like bioreceptor antibody already mobilized, right.

Now, your target will bind, then you will apply something some chemical, some substrate and it will I mean after reacting with secondary antibody is labelled then substrate will react and it will form some product. And this will give some electrochemical signal, right.

So, here as I always saying we are applying some potential, right, suppose 0.3 volt, right. So, with respect to what this potential we are applying? So, we need some reference that is why some electrode, right. This is your main primary electrode that is your working electrode. Just keep in mind, this is working electrode.

Why; it is working here we are working. Mainly we are modifying the sensor surface with some nanomaterial, right or we are modifying this sensor surface with an antibody, then we are applying your test solutions. So, here we are working, right. So, this is the working electrode then, this surface not the dry condition, right.

So, if this solution should have a like in a analyte solutions, like you are dropping some maybe blood samples. So, it contain like so many like ions or maybe we are using some buffer sample. So, if you use the blood sample you know your sample content may be sodium plus chloride minus potassium plus and water, right there is a water or if you apply some buffer.

Buffer sample, suppose for example, phosphate buffer saline p is around 7.4 we are using and here it is also content like phosphates ion or sodium ion, potassium ion, chloride ion. So, it is kind of your solution the conductive, right. So, we are using a conductive solution.

Not in the dry form. We cannot get the electron transfer in the fully dry condition, right. So, when you are when you are dropping the solution something so, maybe your all the electrode will be connected. So, all the electrode means, how many electrodes we are using?

We are using 3 electrode system. So, why we need 3 electrode that are incoming so, here we are using 1 is the working electrode, 2 is the reference electrode, and 3 is the counter or auxiliary electrode. So, now let us come to this question. Why we need 3 electrode system?

Generally, in the two electrode systems case, the flowing the current between the two electrodes and none of the electrode potential is the fixed, right.

So, you cannot means you cannot understand you are know which potential this reaction should occur, right. You have suppose that as I am always saying we have you are applying 0.3 volt; with respect to what, right. You need some reference electrode. With respect to that reference electrode you are applying some potential. So, your reference electrode potential should be constant, it should not be change timely, right.

So, we have to use such kind of like a material or such kind of a electrode as a reference whose potential will not change time to time. There is silver silver chloride a very good reference electrode silver silver chloride. So, this one we are using as a reference electrode. Also, the other reference electrodes are available like hydrogen electrode, calomel electrode, mainly in this biosensor application I will give one example just like a silver silver chloride.

This potential almost fixed always. So, if we apply like 0.3 volt means with respect to this silver silver chloride, ok. So, in this working electrode so, we are modifying your biosensor surface and this is the reference electrode. This electrode potential always fixed. So, if we apply like as I said 0.3 with respect to this, we are applying this potential different, actually this is the potential difference, right and counter electrode.

So, what is the role of the counter electrode? Counter is the help in actually, transfer this electron actually; we are getting the current means all the electrode flow through this counter electrode. So, it is that is why this material this should be highly conductive generally we are using platinum that is why. I mean very highly conductive material we are using at the counter electrode. So, that electron can flow easily through this electrode, ok.

So, this is the role of 3 electrode. So, working electrode, where we are modifying the surface with our biological stuff, and second thing is the reference electrode, with respect to this we are applying some potential on the working electrode and counter electrode helping actually this electron flow, ok.

Then, we are connecting these systems with a potentiostat. What is potentiostat? So, this potentiostat will help you to measure this like if you want to apply some potential you want to get like current or charge, or maybe you wanted to see current change with respect to different different potential. Like, you can see in the display see in this computer you can see. So, that everything you can control in the potentiostat.

So, there is a different software, you can play around here, like that is your cyclic-voltammetry, chrono-amperometry, chrono-coulometry or the impedance spectrometry spectroscopy everything you can anyone you can select in the potentiostat, then you can run. And you can see the changes in on the display, ok.

So, this is the very basic set up for biosensor like all the different technique that you have to start to get the signal where you could get the background data this thing. So, this is the very basic things you should know, that is why I just showing you today.

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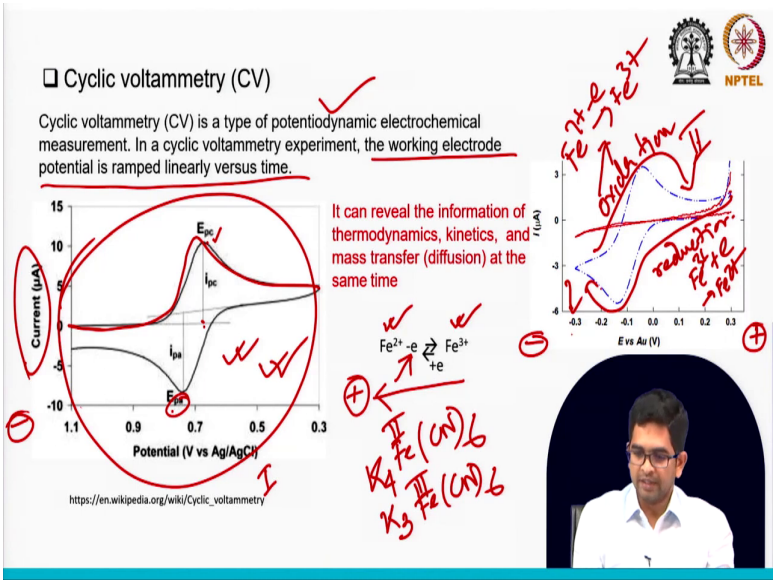

Now, let us come to the cyclic-voltammetry, ok. So, what is the cyclic-voltammetry? You can see this image, why we are saying the cyclic. So, cyclic-means cyclic-will be the type of potentiodynamic electrochemical measurement. Why we saying potentiodynamic?

Because we are getting different parameter, different thermodynamic parameters, different kinetic parameter of your sensor surface different things going on you know in the sensor surface when the target concentration changing, when the some chemical reacting on the sensor surface a different thing happens.

All the thermodynamic parameter, kinetic parameter, mass transfer everything we can deter we can I mean we can predict based on this data. This is very very informative data or cyclic-voltammetry.

So, whenever you want to understand like the property of a transducer or maybe your sensor surface is modified with the different biological stop or not, you can run a cyclic-voltammetry. You will get lots of information. That I will come one by one or even next few classes also I will show you how you can use this technique to characterize your sensor surface, right. This is a very important tool to for characterizations also of your sensor surface.

See, why we are saying this cyclic-voltammetry? Because we are actually scanning within a large potential window, like one is the oxidation scan and next back is the reduction scan. So, we are making a full cyclic-scan in a potential window. So, this where this potential window actually we are applying on a working electrode so, the working electrode potential is ramped linearly versus time and we will measure the current.

Suppose, you have a redox mediator, some redox sample, like potassium ferrocyanide ferosine as I said always I will potassium ferro means K 4 Fe CN 6. So, here I run two-state, right that I am mentioning like Fe 2 plus y like this and Fe 3 means K 3 Fe CN 6, here I run 3, right I mentioning Fe 3.

So, it will releasing electron, right oxidation. So, cyclic-voltammetry case, you can see in left side we are mentioning as a negative and right side we are mentioning as a positive. So, when you run the cyclic-voltammetry, we will scan the different potential. So, maybe we will start from like; so, you can start from the left side from here, then we can go for the oxidation and then you can go for the reduction.

But one thing you can see some paper they are showing, this the pc means the E pc, means this is the potential for the cathodic. Cathodic means the negative scan, pa means anodic means oxidation means positive scan. But here that is the opposite.

Means some region especially in the US there, they are showing like this way means, cathodic is the up, anodic is the down. But in some other regions like in the basically Korea, Japan and that side, so we are actually, but or in a you consider the international way mainly we prefer

anodic is the up, cathodic is the down. So, cathodic is the negative scan, anodic is the positive scan.

So, that is why I just this one just for example, one way we can represent. Here you can see the right hand side another representations, say left hand side is the say this potential window like minus 0.3, minus 0.2, then it is slowly positive, right positive. So, and up this up, this one is the we are saying the anodic scan and down is the cathodic scan. But here is the opposite.

But two things is the they are right, there is no issue, but you have to explain this way. So, like so here negative to positive you are scanning. So, this one means the oxidation that you have to understand. And when positive to negative, then here reduction happens.

Reduction, reduction means like for example, this one Fe 3 plus is forming Fe 2 plus. So, this scan Fe 3 plus electron it form Fe 2 plus, right and this scan here Fe 2 plus it can release one electron and it will form Fe 3 plus, correct. So, this is oxidation scan and from here this is the reduction scan and full cyclic. And so, let us remove now this one let us I want to show you again the different different point that we can get by using a cyclic-voltammetry.

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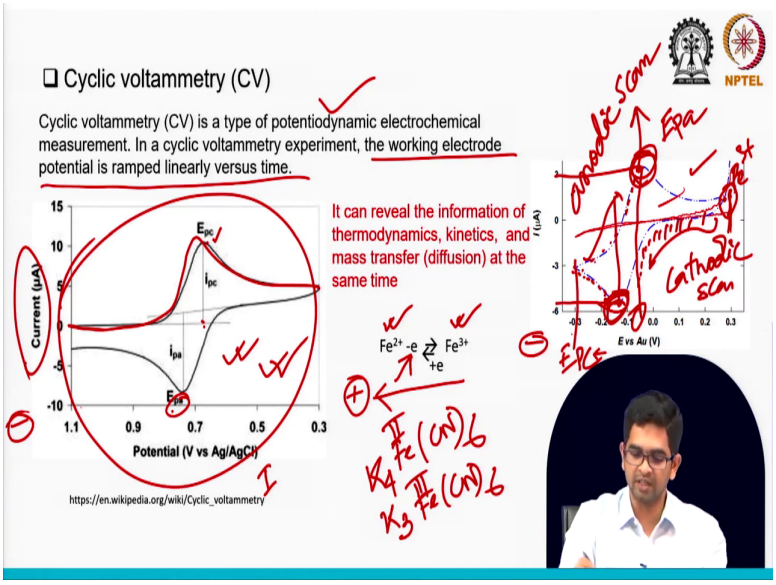

So, let us use let us use these things. So, here you can see when Fe 2 plus actually oxidizing and form Fe 3 plus, so here you can see the highest current, right. And here also see the highest current. So, here the oxidation case we are getting high strength and reduction scan we are getting high strength.

So, this one is called oxidation space. So, this is the peak potential. Which potential is the peak potential? So, in this region, right so, this potential is the peak potential. We can say it is called peak of anodic because this is the oxidation that is anodic scan.

This scan is also called anodic scan, ok and this scan also called cathodic scan. And this peak potential we are saying E pc, right. So, E pc means this is the potential of peak potential, but cathodic peak potential, ok. So, here we are getting the highest current.

And then why? Because Fe 2 plus is oxidizing oxidizing oxidizing from the Fe 3 plus slowly, and almost they are oxidizing. Then again, almost all the Fe 2 plus is oxidized and still again you are applying the oxidation voltages and again this current will drop. But in this then we stop the scan or oxidation scan or anodic scan, we stop here. Like suppose around 0.3 stop.

Then, again you want to go back. So, you want to start the cathodic scan, means reduction you want to start, right. So, cathodic scan means reduction. So, here from here Fe 3 plus will form the Fe 2 plus see. So, as you have the lots of Fe 3 plus here because almost all the Fe 2 plus is oxidized. So, lots of Fe 3 plus we have. So, it will get the electron rate.

So, reduction current increase see, there is a increase, increase, increase. Then here maximum reductions can happen you will get the peak potentials. So, then again maximum all the reductions happen and then this reduction again will decrease and then again you stop at the minus 0.3. You are stopping.

So, this whole means cyclic-scan actually we are starting from here, we are stopping here, again reduction starting from here and stopping here, ok. So, this is the parameter solving. I just summarizing again, like what is the cyclic-voltammetry and kind of information we can get from the cyclic-voltammetry, right.

So, cyclic-voltammetry looks like this. We can oxidize like your substrate. You can oxidize in different electron mediator on a sensor surface, you will get lots of information. So, if your substrate or if your if you have a chemical on a sensor surface that is that can go for like this redox reactions, like Fe 2, Fe 3 this redox kind of thing. Then, you will get this kind of cyclic-voltammetry say.

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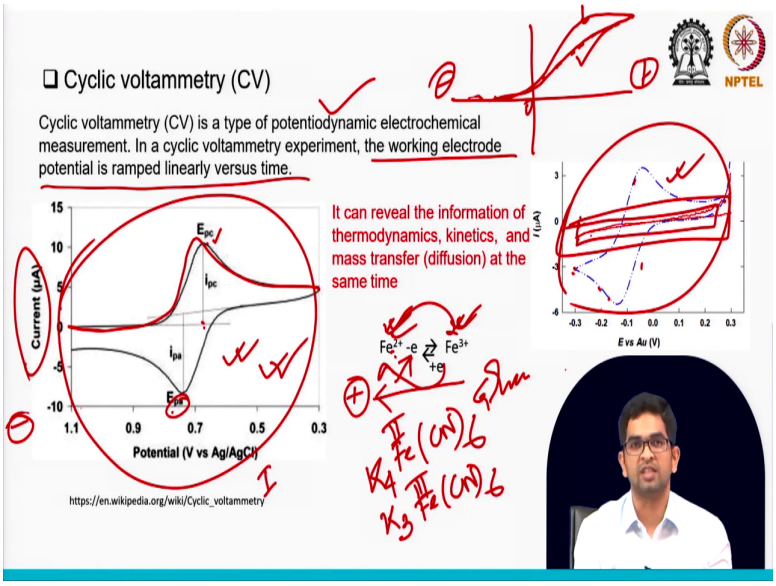

But suppose you have a one substrate, like glucose, this is not the redox redox couple, right glucose. Glucose just a substrate. So, in this case you may get like this like glucose, like here negative, here positive like this is the 0. If you oxidize the glucose then you can get like this, then again this like this. Why? You may get like this say because glucose can only oxidize like cannot, you cannot reduce back to glucose again.

But it means you do not have this kind of like it is kind of mediator kind of things like Fe 2 plus 1 Fe 3, Fe 3 can go fully again go back to Fe 2 not possible for the glucose case. That is you will get the only oxidation or reduction will be very low. You will get the reduction camera go very low because it will not go back to again Fe 2, I mean glucose ok.

So, this is the another step of the cyclic-voltammetry. This is very good like the oxidation reduction step for the cyclic-voltammetry, and if you do not have this kind of mediator like

oxidation mediator, oxidation reduction this couple if you do not have, just you want to run a cyclic-voltammetry in a buffer solution, right.

In this case you may get like this see, this kind of cyclic-voltammetry you may get like there is no mediator, no like Fe 2 plus Fe 3 plus, then you will get simply like this. One kind of a straight line or only maybe you can get this kind of a output. This is because you do not have any oxidation species or reduction species.

Only this is called a double layer current that current only you can see in your cyclic-voltammetry, ok. So, I will now come next few slides then I will show you how we can use for the biosensors the measurement for the cyclic-voltammetry. So, before that I can show you the another technique that is called chrono-amperometry.

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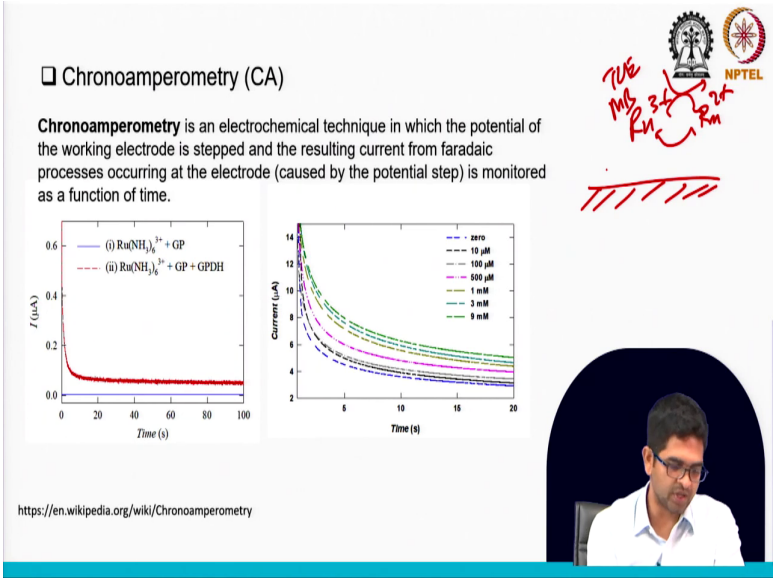

What is chrono-amperometry? So, chrono-amperometry, it is also a electrochemical technique, but this technique case will apply certain potential suppose, from the actually that potential we will decide from the cyclic-voltammetry. Suppose, in your biosensors surface you have different chemicals no; suppose ruthenium hexamine, right ruthenium 3 plus and ruthenium 2 plus this couple, right they are making a redox cyclic-reaction that I told you.

If this kind of redox couple you have, but some reactions, so you can make first one cyclic-voltammetry, right. So, it is about, here and you are using like T-safe or methane blue, this kind of chemical suppose you are using. So, this whole chemical mixture you can take a cyclic-voltammetry first. You can take a cyclic-voltammetry. How?

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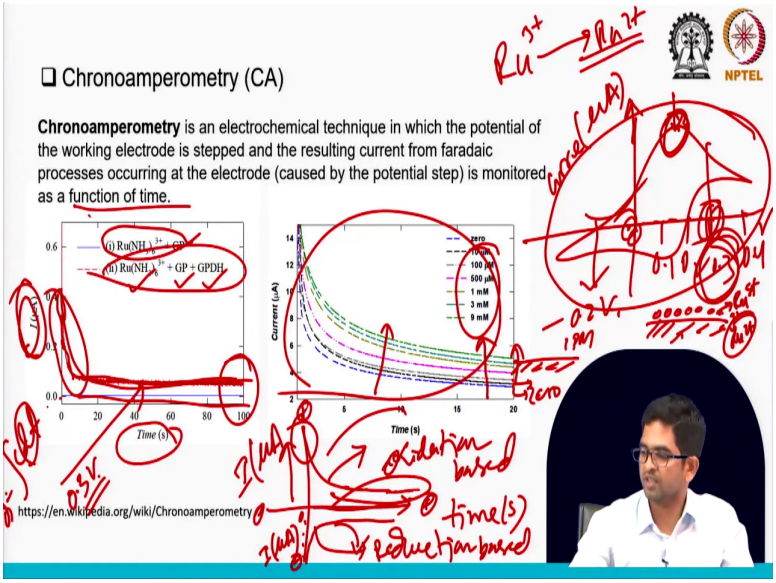

Means you just take a sensor surface and that chemical and then take cyclic-voltammetry suppose it looks like this, ok. So, now what is chrono-amperometry I told? It is a

electrochemical technique in which the potential of the working electrode is stepped and the resulting current from the faradaic process.

So, faradaic process means this redox process. This current will measure by applying a fixed potential, so its and with the function of the time. So, you can see I just showing example in the left hand side. So, with respect to time we are here we are applying a fixed potential. Suppose, 0.3 volt we are applying. So, we will get the in the output in the form of current with respect to time.

In the cyclic-voltammetry case, so this current at the very beginning sorry in the chrono-amperometry, chrono-amperometry the very beginning it will be very high, then slowly it will be stabilized with respect to time. So, why at the very beginning it is so high that I will tell you now.

First let us understand how we can start this chrono-amperometry. So, first you have to measure a cyclic-voltammetry then fixed a potential. So, in a chrono-amperometry case, it can be oxidation based chrono-amperometry or it can be reduction based chrono-amperometry.

So, if you want to start a oxidation based chrono-amperometry, then you have to apply a potential more than; so, suppose this is the your in the cyclic-voltammetry and this is the oxidation peak potential, right. So, you have to apply more than this suppose here. So, here this much potential you have to apply, not here. If you apply here, then in this potential reductions will be much I mean favourable than the oxidation.

So, as I am always saying you know you have to apply some potential, but which potential that you can decide just by measuring a cyclic-voltammetry, ok. So, you can so from here; so, you have to suppose this is 0, this is 0.1, 0.2, 0.3, 0.4, this is the volt, this is x axis and this is the current suppose micro amps, micro ampere.

So, in this case 0.3 volt you have to apply. But if; so, in this case you can see chrono-amperometry it looks like this, but there is another reduction based cyclic-voltammetry. Reduction based cyclic-voltammetry case, so in the x axis again, the

current I micro amps, right. So, this is 0. So, this is negative, this is positive, this is positive, this is negative.

So, again this one also I current microamps bar. This is the negative, negative, negative power and this one time, x axis is the time second. So, if you apply negative potential then you can see like this. So, in a negative side, this is the reduction based chrono-amperometry; and if you get like this is the oxidation based chrono- amperometry.

So, if you apply 0.3 then you will get something like this, but suppose you are applying here minus 0.2 volt, right. So, if you apply this one then you get something like this. This is reduction based and this one oxidation based, ok. This (Refer Time: 24:56) may be very important, when you want to apply some potential and how your output it looks like. So, that is how to understand, ok.

So, now as I said that at the very beginning your current is really very high, then slowly it will be saturated and something like this will look like. So, why at the very beginning it is very high? See, suppose you are using here ruthenium, hexamine, right ruthenium 3 plus. So, it will be reduced to ruthenium 2 plus, ruthenium 2 plus and that 2 plus actually you are oxidizing on the surface, right because you are applying something 0.3 volt.

So, this potential you are applying. So, it is very high potential, ok. So, that is why at the very beginning your sensor surface you have many ruthenium 2 plus; at the very close to the surface. So, this is if you this is your sensor surface there is so many ruthenium 2 plus. That is at the very beginning you will get a very high current.

Now, you when you apply some potential this ruthenium 2 plus form the ruthenium 3 plus, then again, then slowly by time ruthenium 3 plus and ruthenium 2 plus there will be equilibrium that is why slowly it will decrease, decrease and then it will form like saturated by equilibrium. That is why, ok.

So, this is the shape of the chrono-amperometry the oxidation base, reduction base and you need to apply certain potential, which potential you would apply that everything I discussed,

right. Now, if you want to increase the concentration, suppose you have on the sensor surface, you have some target like 1 picomolar, right. Then, you will get like chrono-amperometry. Suppose, this one 0, like just background this is 1 pico or 10 micro, then 100 micro.

If you increase the concentrations of your analyte just slowly slowly your current also will be increased. So, like this way you will get different different current with respect to time with by changing the concentration of your analyte.

This is the actual shape of the chrono-amperometry and different different current you may get based on your concentration of the analyte. So, this is a very practical example.

After few maybe next class, I will show you again like just draw a biosensor, then change the concentration, how amperometry change, how cyclic-voltammetry change that I will come. You can see here like when you use only the background see you see the very low current, when you use the signal reaction like you can examine GP GPDH like this is a signal reaction, then you get the high current, right. So, like this way you can differentiate it.

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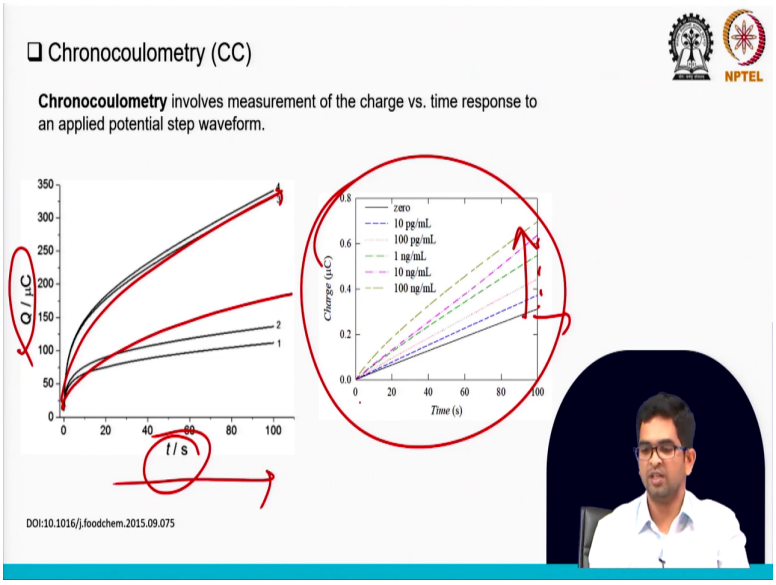

See, another technique the chrono-coulometry or CC. It is similar like chrono-amperometry, here also we will apply a fixed potential. But here we will get the output is the charge, chrono-amperometry is we are getting current with respect to time here charge.

So, actually we are if you go back like chrono-amperometry, if we integrate with respect to time, this current, you will get the charge, right. So, if you integrate say q equals to cdt you know. So, if you integrate this current with respect to time, you will get the charge, right so, chrono-coulometry something like this.

Just you can integrate it. You know basically they are similar chrono-amperometry and chrono-coulometry. Just; that is why you see chrono-coulometry is always increased that

charge because with respect to because time also increase. Now, with respect to time we are integrating the current, we are getting the charge, nothing else.

Here also we are applying this a constant potential and we are getting charge. See, you can see just for example, here like 0, then we are increasing the concentration and you can see the charge also increasing, right. So, like this way, based on this output, we can determine the concentration of the target.

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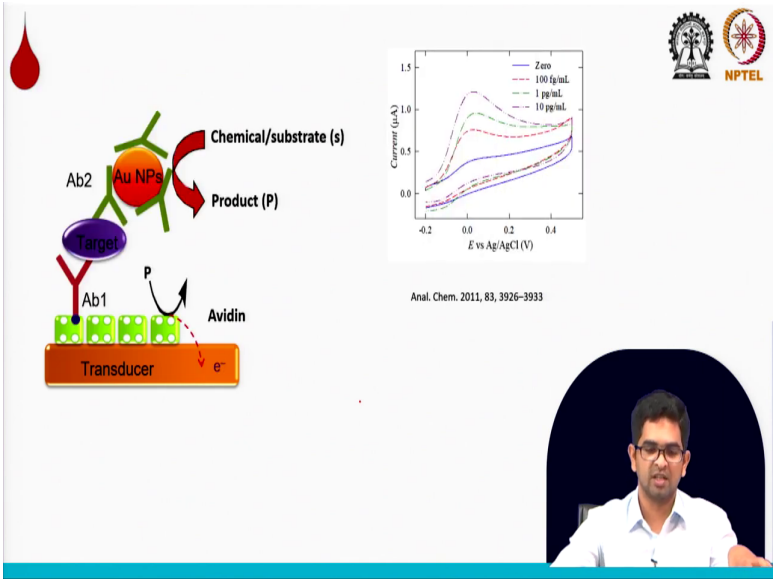

So, ok, this is just the example. Then, in the next class I will show you like this kind of things like develop a sensor by using the cyclic-voltammetry by chrono-coulometry. And use them for detections like how much concentration present, ok.

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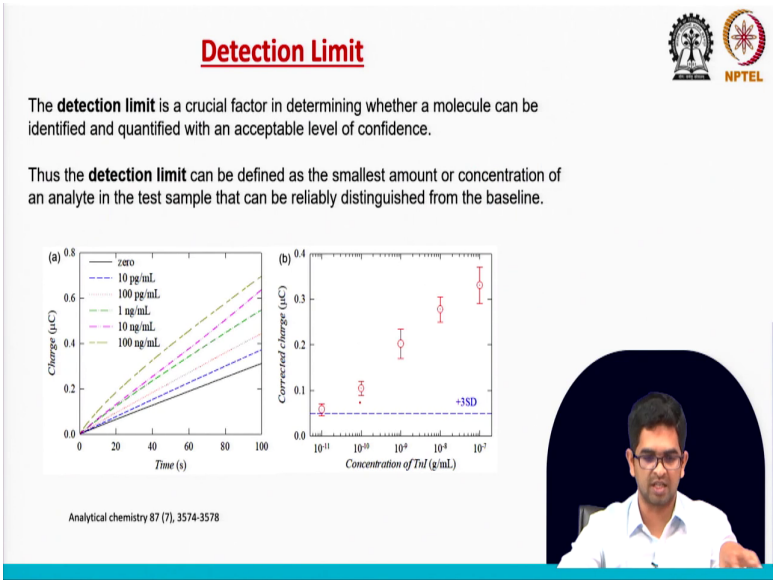

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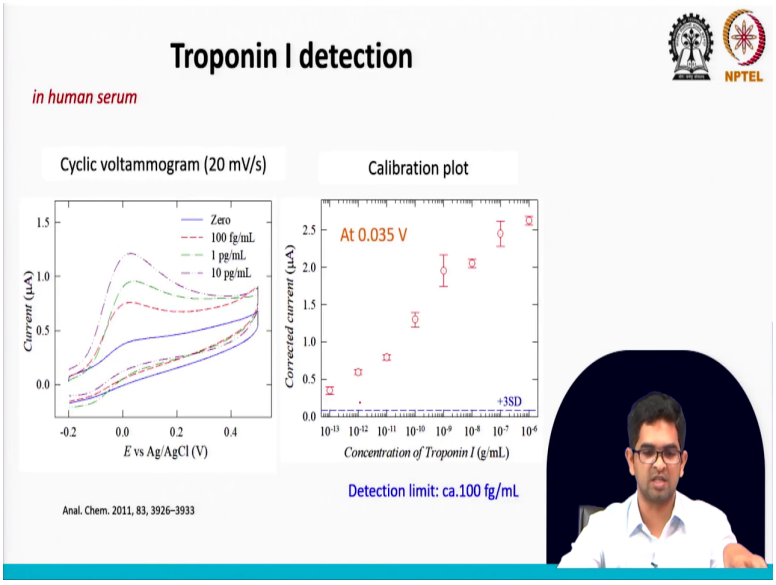

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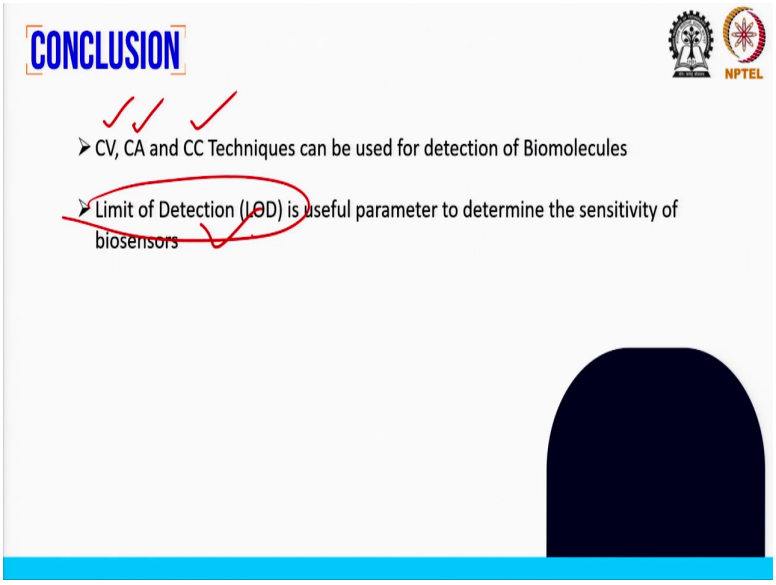

So, in the this class the main conclusions that we can use the CV, CA, CC techniques. And that can be used for the detection of the biomolecules. And next class, we can determine, I will show you again the limit of detections. See, the sensitivity of the sensor is very very important. So, by using the cyclic-voltammetry, amperometry, coulometry, how we can determine the limit of detections that I will teach you in the next class again this is very very important parameter.

Thank you very much.