Nanobio Technology Enabled Point-of-Care Devices

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Lecture - 16
Enhanced Electrocatalytic Activity for Biosensors

Dear students. So, today, I will teach you again the Enhanced Electrocatalytic Activity of the electrode surface, and then how those enhanced activities we can use for biosensor development. So, last class, I think you already you understood how we can make a highly catalytic active surface or enhanced surface by using different treatment, that is called tuning. That, we can use for thermal treatment, electrochemical treatment, chemical treatment. I taught you last class just chemical treatment.

Now, I will just come to example, how we can use this chemical treatment for example, sodium borohydride treatment for development or for design a biosensor. Let us come.

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So, today mainly I will cover sodium boride treatments applications for biosensing applications, for biosensor development.

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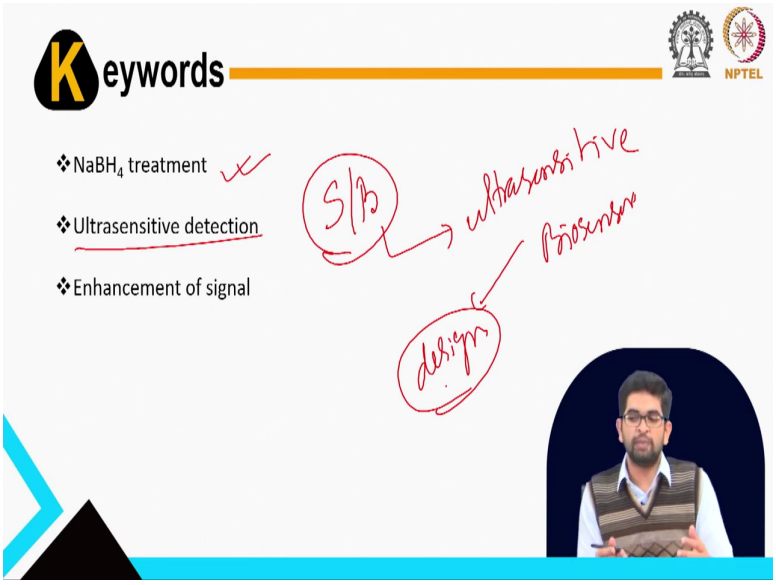

So, main keywords for this if you want to search, also if you can check the sodium boride hydride treatment for biosensors, for ultrasensitive biosensor development by using different chemical treatment. That also can be a good keyword for this topic, and enhancement of the signal. Because this treatment definitely is helping to improve the signal to background ratio and you may get a ultrasensitive biosensor, right.

So, let us design now, design a ultrasensitive biosensor by using this method. So, why we are learning this chemical treatment? Why we are learning this enhancement? Let us use this applications for a biosensor development, ok.

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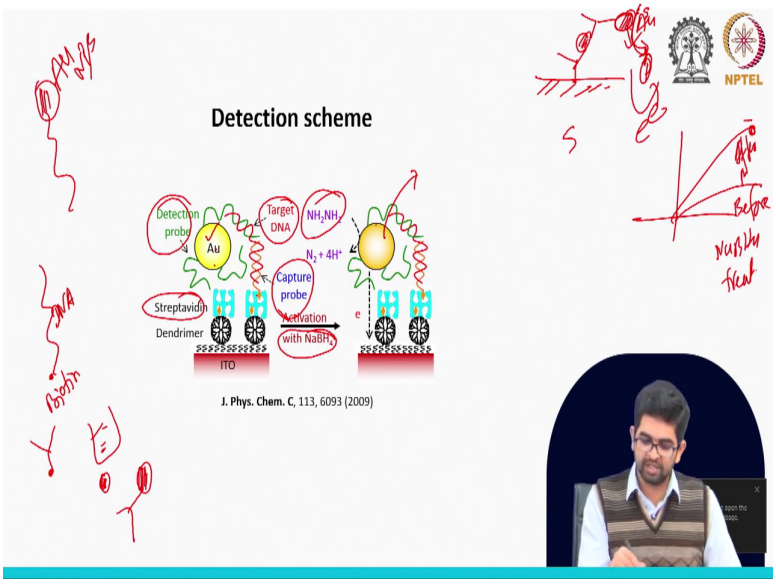

Let us see here. A detection scheme, what detection scheme this one? This is a just a DNA detections. Means similar like the antigen antibody I told you, and instead of using a antibody on the sensor surface we are using capture DNA. This is called capture probe. Similar way, your capture DNA, DNA that can be modified with the biotin, biotin modifier. Something like the similar like the your antibody modified conjugated with the biotin, biotin, like biotin with antibody similar like this.

So, your surface is already apt inverse streptavidin coated. It can be avidin or streptavidin coated. And just dendrimer is helping to bind this kind of; this is they all are the link curve basically and they are helping to bind this streptavidin or avidin on the surface and your capture probe already biotinated, right. That is why your capture probe already on the surface, ok.

Now, see you are adding your target solution DNA solution. So, you this is the target DNA, suppose like the antigen, in a solution if you have some protein like target as I was saying you know. So, here as for target you are using DNA. Nothing, there is no difference just; before we use antibody antigen interactions, here we are using just DNA interactions capture probe target DNA.

Now, on this target DNA as for example, we use last time secondary antibody here we use a secondary that is called detection probe this is called for the DNA case this is called detection probe and that is also conjugated with gold nano particle, right. Last time like the secondary antibody can be conjugated with gold nanoparticle, and here detection probe also can be conjugated with gold nanoparticles, ok. So, your biosensors already developed on the sensor surface, clear.

Now, these gold nanoparticles can be used for the signal amplifications. How? We can apply that sodium borohydride treatment method that I taught you last class. See, if we activate this gold nanoparticle with sodium borohydride then its catalytic activity will be improved. Say, suppose for example, last time I told you know that you have the primary antibody and you have target antigen, secondary antibody that secondary antibody conjugated the gold nanoparticle. This is just your like labelled antibody.

So, if this label is highly active, then definitely it will produce some electrochemical active product and they can easily they can oxygen on the surface. And if this process kinetically very fast with the label substrate and product formation, it is kinetically very fast. So, definitely your reactions will be very fast you will get the high electro transfer rate and you will get the high signal, right.

That is why we need to improve the electrocatalytic activity of this label gold nanoparticle. You may say why I should be improved, right. That is why. Here we use hydrogen just for example, a substrate. So, hydrogen oxidation on your biosensor surface. So, at the beginning when your gold nanoparticle was not activated by sodium borohydride, that time activity was

very low and you may you may get the low signal this is before sodium borohydride treatment, before NaBH 4 treatment.

And after the treatment, after treatment you are getting here. So, it means this signal means your hydrogen oxidation signal will be improved, ok.

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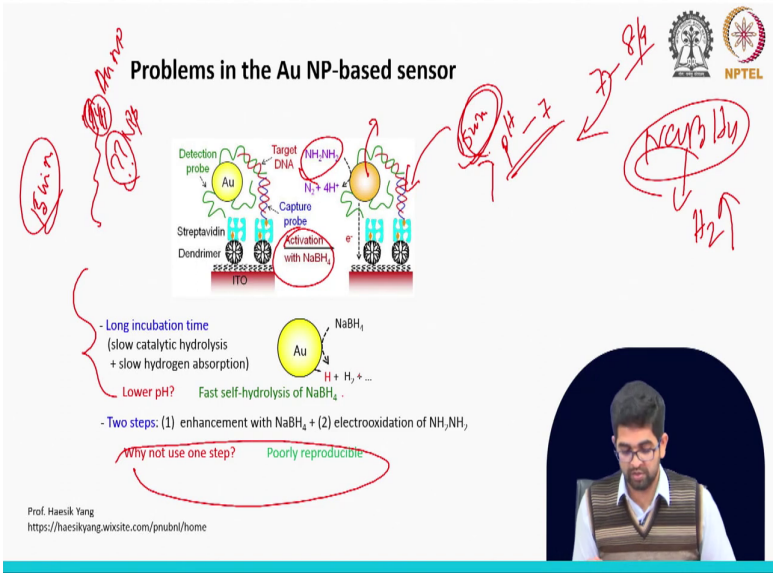

Now, I will show you the problem of the gold nanoparticle based sensor. So, it may ask me to say just you told the gold nanoparticle sodium borohydride is very good, then now you are asking this problem. Definitely, we need to further improve the electrocatalytic activity of the sensor surface.

Yes, when we are using the gold nanoparticle, there is some drawback. Let us show you the drawbacks using the gold nanoparticle. Then, we can further improve the catalytic activity or sensitivity of the biosensor by replacing gold nanoparticles with other nanoparticles.

So, that this kind of research actually going on, that we are replacing one nanomaterial to another nanomaterials. That is why you will have some lots of scope to work again, like gold nanoparticle there is some problem. We replace another nanoparticle, I will show you right now. Then, maybe you can think about some other nanomaterial. That is why, we have lots of scope.

See, when you use these activations with the sodium borohydride, just I showed you in the last slide, and hydrogen oxidations, and in this case the problem is we need a long incubation time. How long? You need at least 15 minute incubation. So, you have to dip your sodium borohydride in this sensor surface for 15 minute and your pH of the solution should be less than 7. Your pH should be less than 7. Then only you will get higher activity electro catalytic activity of this gold nanomaterial. This is a true problem.

Because if we use high pH like pH 7 or more than 7, 8, or 9, in this case one thing you can see, this activation rate is very slow for the gold case. So, this is the one pH factor, but if you decrease the pH for the gold case. Another problem, sodium borohydride may decompose very fast by like by evolving the hydrogen gas. So, solid concentration can be changed with time.

This is not a good factor if you decrease the pH. So, gold like the low pH, but in the low pH sodium borohydride can be decomposed fast. And another problem for the gold case, it needs long time reaction, at least 15 minute or more than or 30 minute. So, this is the two problem. We can solve by using another nanomaterial. That is why we need some another nanomaterial.

So, the your detection probe, your DNA detection probe which is already conjugated with gold nanoparticles that we can replace some another nanoparticles. So, the question mark is

which nanoparticles we can now use, right. So, let us this questions now we have, why not use one step I means instead enhances sodium borohydride and this sodium borohydride reactions, actually can be slower rate for the gold.

And another problem is the self-hydrolysis, that is why the concentration of sodium borohydride with time can be decreased and you may not get very reproducible data.

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So, this problem can be solved by using another nanomaterial that is called palladium. While just you said why I suddenly switch the palladium because palladium has this kind of properties. So, it can adsorb the hydrogen very fast. When the in the gold case you is just 15 minute, and in this case 1 to 2 second only is enough, for this hydrogen adsorption. Because this hydrogen adsorptions only the cause for surface reconstruction, I think remember, the surface reconstruction.

That is why we are getting the highly catalytic electric catalytic surface. Now, if this hydrogen adsorbed very fast on your surface, then no need to go for the long time incubation. So, for the gold case where you use the 15 minute, now you can go 1 just 1 to 2 second if we use palladium nanoparticles. And this very fast hydrolysis or the sodium borohydride that also we can stop by using higher pH.

So, in the palladium nanoparticle case, we will use the pH more than 7 or around 7. So, in this case, we can avoid the decompositions and electrocatalytic activity also will be increased very fast.

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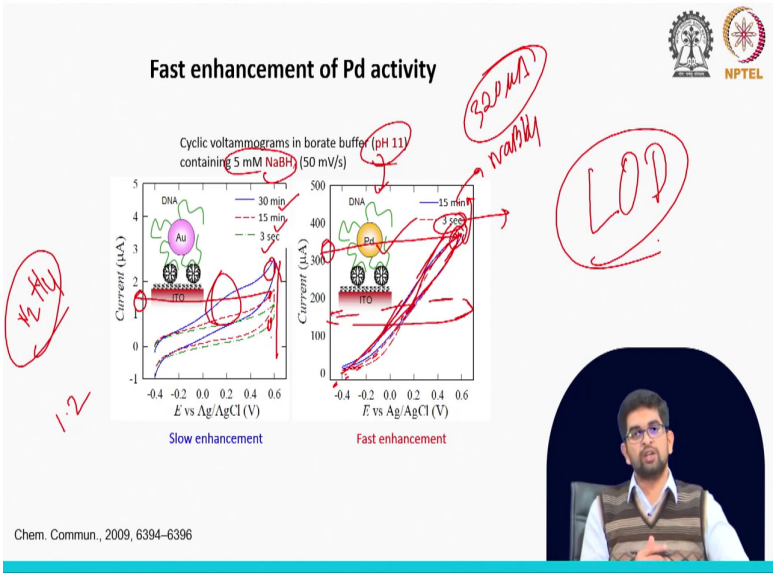

So, let us comes on example. See here. So, when we measure a cyclic voltammogram suppose this is just we measure just after treatment of the sodium borohydride. For example, a 50 millimolar sodium borohydride of gold nanoparticles. And we checked this is just a oxidation

of hydrogen. Suppose, we are measuring the hydrogen oxidations into H 4, hydrogen oxidation we are measuring.

See, at the we are slowly we are optimizing different different time, and oxidation of gold with sodium borohydride treatment, ok. And we use the pH at the 11, high pH, we use more than 7 as I mentioned because at the high pH that the decomposition of sodium borohydride will be slow.

See, we tried 3 second we tried 15 second, we tried 30 minute at the high pH. So, 3 second 15 minute, they are still not matching group. We tried after 15 in 30 minute, it is improved, but not that much. So, in the gold case, if we decrease the pH series like less than 7, then it can be improve very fast. But in the high pH as I mentioned, it will not be improved very fast. See after 30 minute also, it is not improved much. See now, we use the palladium.

Just your DNA that conjugated with the palladium nanoparticles. So, again now we started the optimization different different time. See this is the red one, this curve, just after 3 second, just 3 second we measure. We got very high catalytic activity. See here in this case how much current we are getting just 1.2 microamps. And here after 3 second we are getting like 320 microamps, right.

So, this much high current we are getting. See, where is just 1.2 and here the 320 microampere. So, this much high amount of current we are getting using palladium nanoparticle just after 3 second. Now, we are checking after 3 second and again 15 minute incubation. See, after 15 minute sodium borohydride treatment, see they are showing almost the singular current.

So, it means we do not need long, it is almost saturated after 3 second itself. Means just deep, and sodium borohydride treatment will be very effective for palladium nanoparticles, right. That is why it is dependent on nanoparticles to nanoparticles.

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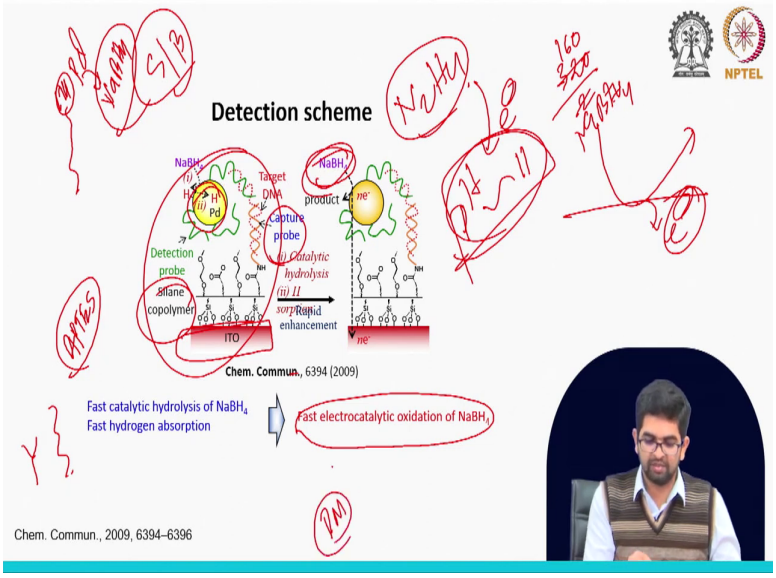

So, this behaviour now we can use for a biosensor development. So, as I showed you that gold nanoparticle case just like 1.2 microampere current increase, but palladium case 320 microampere current increase is how like 320 by suppose 2 micro amps. So, how much? 160 times, right. So, this much current actually improved.

So, definitely, so if we conjugate your detection probe with the palladium nanoparticle and improve the electro catalytic activity by sodium borohydride, then think how much signal to background ratio you can improve, right. So, you can see here, we modified a I till now, now we are designing a biosensor by using this palladium nanoparticles. Yeah, ITO electrode that we modified is silane copolymer. This is just a co-polymer.

So, another way we can modify the sensor surface something like APTES you can search. This is kind of the name of this polymer, and we can modify like primary antibody or capture

DNA. This kind of capture like who are helping to wind the target, those molecules we can modify. So, for the DNA case capture probe, we modified on the sensor surface by using a silane co-polymer.

See, then we drop the target DNA. Target DNA and they already attached on the capture probe. Now, we add the detection probe. These detection probe now conjugated with palladium nanoparticles. See, now this catalytic hydrolysis hydrogen adsorptions will happen very fast because of this sodium borohydride treatment and we use here palladium nanoparticle that is why this fast phenomena we can observe.

Now, let us observe, now let us check your electro catalytic activity you can use here. See, in this scheme, we use sodium borohydride itself say substrate. Like last time we use hydrogen like into H 4 as the substrate oxidation, right, on the sensor surface. Here, what is the new modification we did; when this is a kind of the design of the sensor, right. So, you can select different chemical like which chemical you can add for the substrate.

Last time say hydrogen you add as a substrate, here we use sodium borohydride for activations. Again, we use sodium borohydride for as a substrate, whether it will show this chemical like, how the electron transfer on the surface. So, basically, sodium borohydride is actually oxidizing on your surface and you are getting this output by measuring this electron transfer.

See, so, in this case as sodium borohydride current oxidation current, we are measuring, it will show very fast oxidation of the sodium borohydride and fast catalytic hydrolysis of the you know BH 4 and fast hydrogen adsorption. Everything will happen because we use the palladium nanoparticle. And one important thing, here we use pH around nearby 11, means high pH. So, hydrolysis of sodium borohydride also very low. So, we can avoid the reproducible problem also.

Last time we cannot use sodium borohydride the substrate, because last time we use the low pH. At that low pH sodium borohydride can be hydrolysis concentration time by time also decrease. So, it can effect on reproducibility. But in this case, as we use the pH 11 high pH, at

that high pH it is very stable and we will get the reproducible data. And at the same time very high electro catalytic nanomaterial surface, ok.

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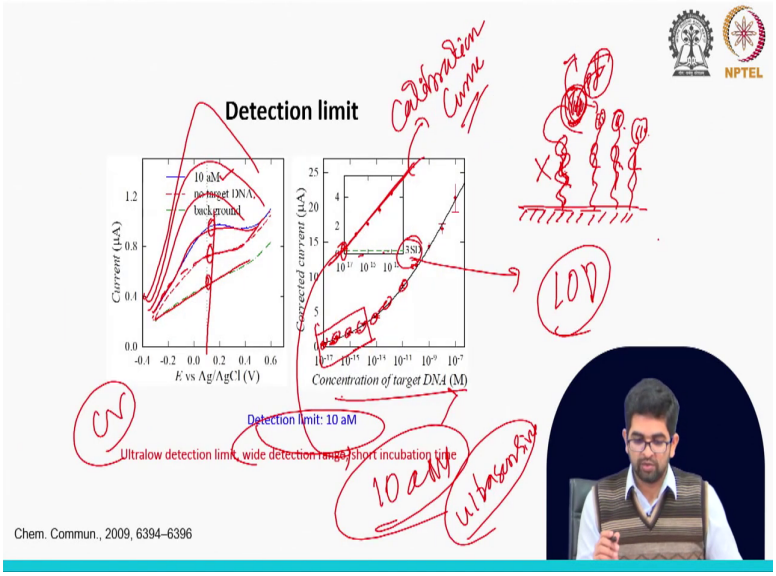

Now, let us come here the detection limit. So, our biosensor already done, right. So, your this is just a schematic. This is your sensor surface. You have capture probe, and then you have your target already bound on the sensor surface. Now, you have the detection probe that already conjugated with palladium nanoparticles.

Now, see slowly you are increasing the target concentrations. So, I am telling about the calibration; this is calibration curve, right you know, calibration curve. See, now if we increase the concentration of target DNA, we are increasing the concentration of target DNA. When is like very small number of the target, then number of palladium also on your sensor surface is very low.

Now, when you increase the target concentrations like you have the many capture probe, so many target will come and then many second like your detection probe will bind. So, many palladium nanoparticles will come on the sensor surface. So, basically, more number of palladium nanoparticles means you will get higher and higher current.

See, even this very very if you have very low number of the target concentrations and if you have the low number of the palladium nanoparticles surface because of the highly active, still you can detect the same current. Because very fast catalytic activity is showing, right.

If you see now, here one calibration curve we measure like target with target DNA, we measure 10 up to auto molar, then we can see the difference. See, this is the background current. Background means just with the capture means just a sensor surface. Then, no target DNA.

When you do not have any target DNA, then, you are getting this much this much current. This is the cyclic voltammogram. And here actually we are showing only one oxidation curve. There is no reduction curve, only oxidation curve of the sodium borohydride, ok.

So, just background, just background means you can say that with the capture probe, then we checked with no target DNA and with 10 automolar very low concentration of target DNA. And we can see the difference of the current, ok. So, if you see in this potential, we can differentiate the target concentrations.

Now, if you further improve like 10 automolar to 100, then if you see to the femtomolar, then to the picomolar, then if you see then you will get very high current difference you can see. So, if you see here the calibration curve, see the current there is a change of the current.

And now you place at the very low concentrations, in the inset you can see all the low concentrations we put 3 times standard deviations that we measure that we obtained because to calculate the limit of detections, right. You can remember, what is the limit of detections? Limit of detections means how the lowest amount of the target you can detect, ok. So, lowest

amount of target if you like 1, 2, 3, 4 this like 4, 5 points if you put then they looks like a straight line.

Although, all the if you all the points if you put the not all the straight line, but if you put this few line, few points at the very low concentration, you will get the straight line. Now, 3 times standard deviation we see here. And now, if you draw a straight line with this few concentrations and this theta standard deviation line, then intercepting this one. So, this limit of detection is almost is 10 automolar. So, it is very ultrasensitive this biosensor, ultrasensitive.

So, why is possible to that much very low detection limit? Because of this your palladium activity. If you not add the sodium borohydride, if you go back here see. So, if you without sodium borohydride if you go up to this stage, so then maybe you may get some detectional limit maybe in the picomolar range, because it may show the current if you go back here say without sodium borohydride treatment, maybe if you see the current may be very low in this range. But after the activations you are getting the very high range. So, this happened as we add the sodium borohydride.

So, this chemical treatment we use for the ultrasensitive biosensor to get very low limit of detections. So, these things you have to remember. Not only the design of the sensor surface, also some treatment also can improve the further of your sensitivity of the electrochemical biosensors, ok.

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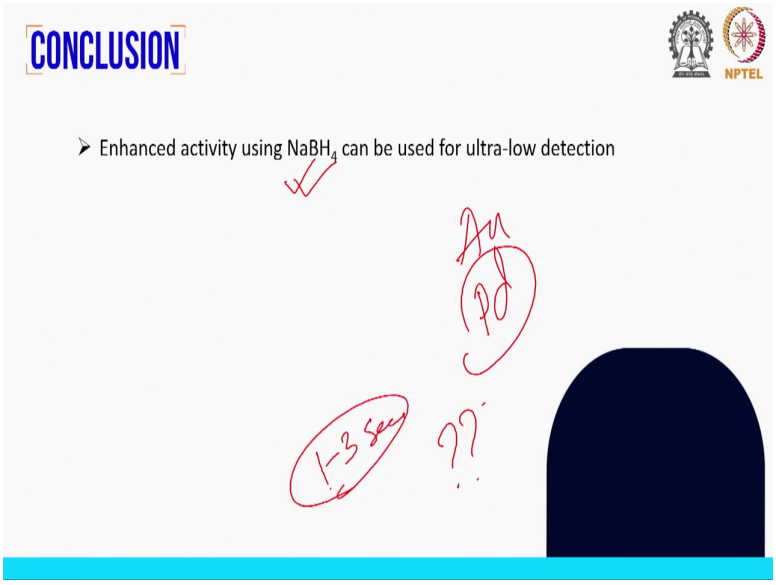

So, these things are actually we learned today, that how we can enhance the activity of the electrode surface and this kind of enhancement can be done by various simple chemical that is called the sodium borohydride. And this tuning also can be done, not only the gold, also we can use some other nanomaterial like palladium. So, and also you have another scope, just thing, maybe other nanomaterials also can be used. And this treatment also very simple.

And we need just 1 to 3, like few second only, and enhancement can be done. So, this all the tuning method I taught for biosensing design. Next class, again I will teach you some other enhancement method and then apply those enhancement or those tuning, those pre-treatment as I told, this method like chemical electrochemical treatment, thermal treatment this is kind of pre-treatment method. Those treatment also can be can help for further enhancement.

And I will show you different different design of biosensors based on all these pre-treatment design method, ok.

Thank you for today.