Bio-electrochemistry
Prof. Mainak Das
Department of Biological Sciences & Bioengineering & Design Programme
Indian Institute of Technology, Kanpur

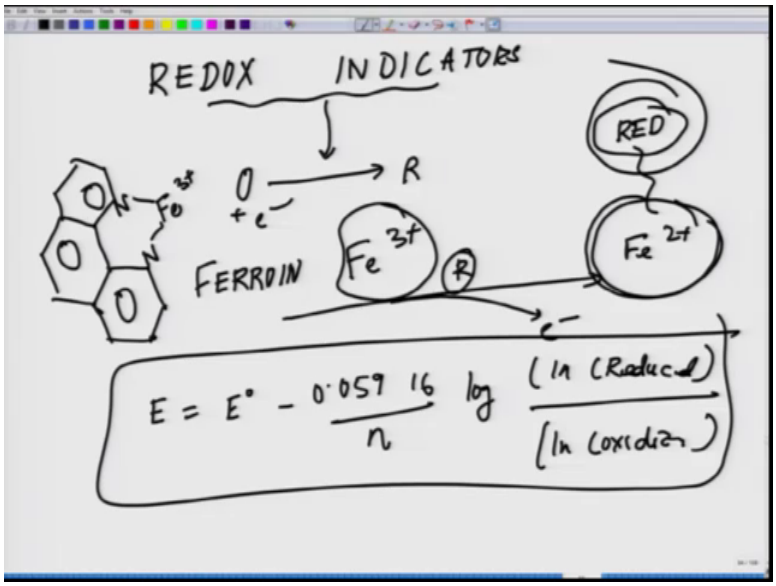
Lecture – 19
Redox Indicators Amperometry - Glucose, O2 Sensors

Welcome back to the lecture series on Bio-electrochemistry. So, we are on to the nineteenth lecture, which is our fourth lecture of the fourth week.

So, as of now we have talked about ion selective electrodes and change in potentials, we have talked about metal electrodes, I have given 2 examples one of the glass electrode, where the P H measurement is being done then we talked about the ion selective compounds like Vilano Mycin, which could be used for detection of potassium ions and there are several such ion selective molecules available in nature, which could be exploited to develop ion selective membranes.

Which eventually could be coupled with electro chemistry set up to know the unknown concentration and these are some of the very potential areas of biosensors, what I wanted to highlight get give you an glimpse. Today also we will next to classes also will be our essentially last 2 classes will also go on those application lines of analysis of biological molecules using electro chemistry technique.

(Refer Slide Time: 01:28)



Today it is our lecture 19 this is week 4, lecture 4 W 4 L 4. So, one of the example what I wanted to give it is part of the redox iteration is Redox indicators. What are redox indicators? Redox indicators are compounds that change color, when it goes from it is oxidized to it is reduced state oxidized to the reduced state this redox indicator will be changing in color. The indicator of ferroin changes from pale blue to almost colorless pale blue or almost colorless to red.

So, oxidized ferroin is which is play pale blue in oxidized state as soon as it gets reduced. So, it changes to red. So, in other word what is happening in ferroin is iron, which was in F e 3 plus state a is getting losing an electron become F e 2 plus and because of that reduction reaction which is happening here this is red in color?

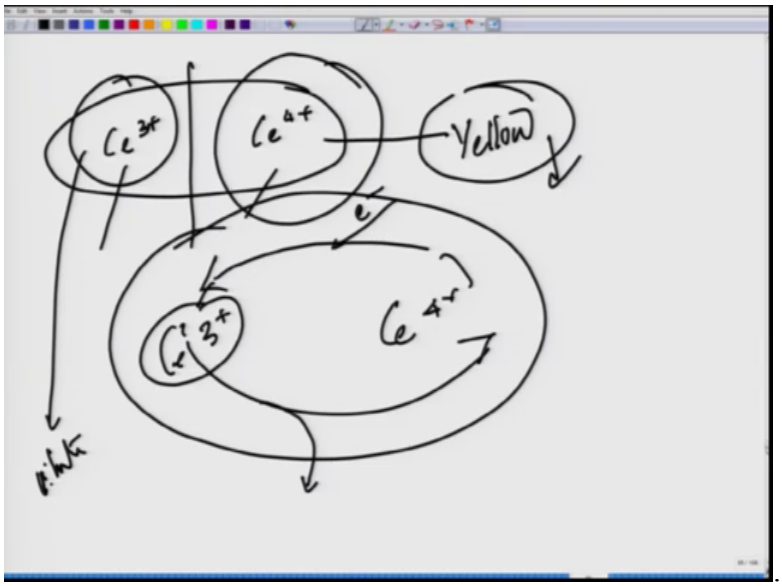
Whereas F e 3 plus which was pale blue or almost you know and this molecule is essentially, when you talk about the ferroin ferroins are something like this here. So, you have a nitrogen out here you have a nitrogen out here you have iron 3 plus this is what ferroin molecule looks like and as soon as it becomes F e 2 plus it becomes red.

So, this is a very interesting situation. So, if you look at this reaction. So, what is happening oxidation, oxidized molecule plus electron become getting reduced.

Now, if you see the left and the right hand side you can actually write an equation on this E is equal to E 0 minus 0.0 5 9 1 6 upon n which is the number of electron log of in the reduced state and an oxidized concentration. Based on that you can actually calculate what is to predict the potential range over with the indicator color will change and that is what the Nernst equation the basic equation what I told you.

And you could have many many such situations, where Nernst equation could be used and I am just giving you some of the examples, which will help you to realize that there are several ways you can do it. Similarly you can do it using cerium, which has been done several times C 4 plus and C 3 plus.

(Refer Slide Time: 04:44)



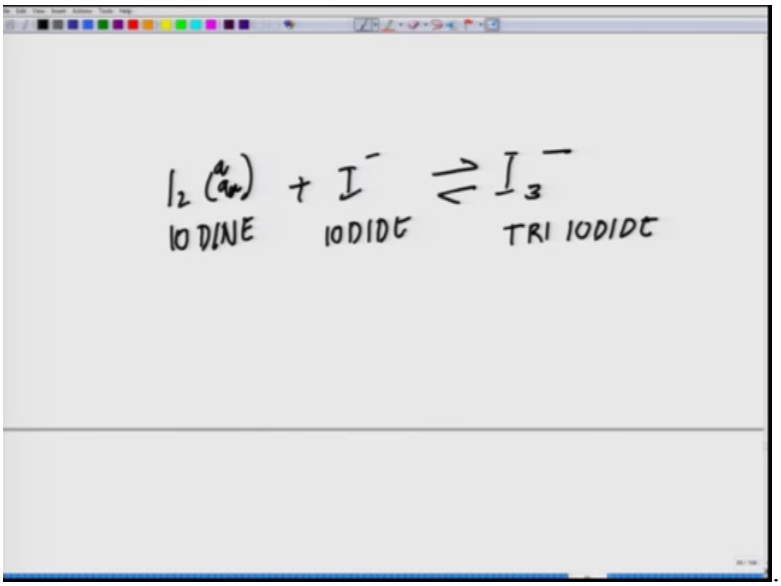
So, cerium those of you do not know cerium if we look at the lanthanide series. The second atom in lanthanide series is cerium and cerium in it is Nano state remains in 2 different oxidation state C e 3 and C e 4.

And it can auto catalytically shift from C e 3 plus to C e 4 plus is this there is an autocatalytic shifting, which happens in the narrow state in the Nano state. Now cerium has another very interesting properties cerium 4 this compound is yellow in color and this one is white or colorless kind of situation. So, this is a drab whitish color.

Now, say for example, if C 4 accepts an electron and gets reduced, then it will become C e 3 and similarly if C e 3 loses an electron, it will become C e 4. So, you can actually use this assembly cerium to see the change in color to figure out how much electron is being transmitted. And if you could connect it to a potent using a voltmeter, you can actually calculate this an oxidation of C e 4 plus. So, C e 4 plus plus electron making C e 3 plus.

And if you look at there are different potentials which could be measured using it. Similarly, oxidations with potassium dichromate, and then you have another one where you have a lot of sensors which are being using iodine.

(Refer Slide Time: 06:31)



Which is iodine aqueous if you look at it iodine aqueous iodine plus iodide I 3 minus which is tri iodide.

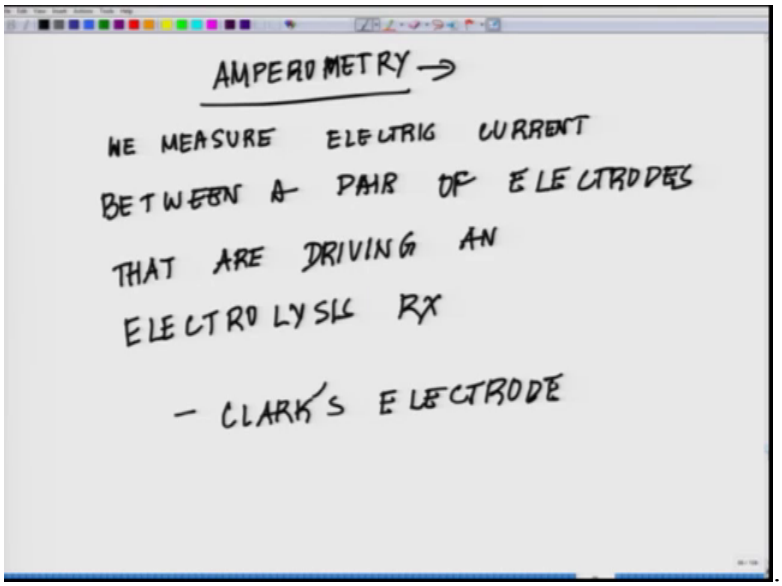
So, this is another indicator where iodine has been used as an indicator, when a reducing analyte is triturated with iodine to produce I minus the method is called iodometry in iodometry and oxidizing analyte is added to excess I minus to produce iodine, which is then titrated with standard thiosulphate solution.

So, molecular iodine is only slightly soluble in water, but it is solubility is enhanced by complexation with iodine. So, another process which is being used for redox titration is iodometry, you can use iodometry.

So, there are several ways you can utilize your basic knowledge of chemistry for different kind of sensory apparatus. Now from here we will move on to a much more interesting platform, where we will talk about as of now what we are doing we are writing everything in terms of potential difference.

Now, we will talk about something where we will talk about a technique which is termed as amperometry, ampero metry and what is amperometry?

(Refer Slide Time: 08:03)



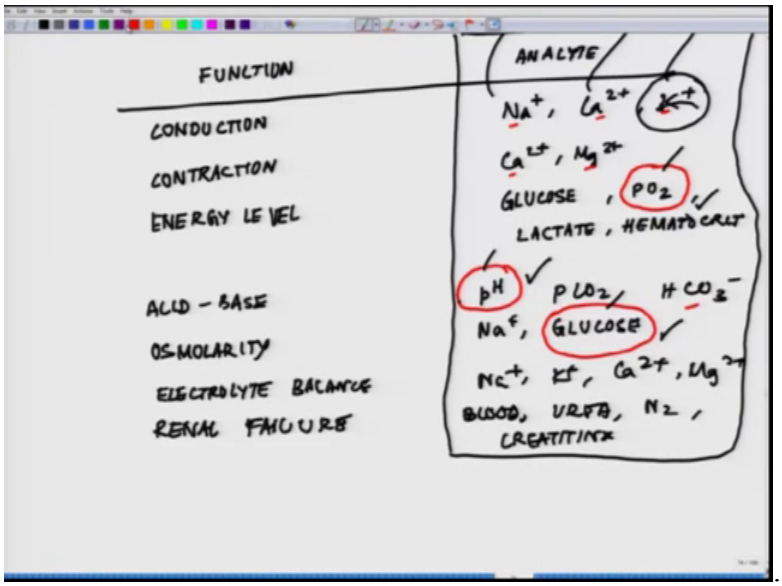
An amperometry we major electric current between pair of electrode that are driving an electrolysis reaction.

In our amperometry we measure electric current a major electric current between a pair of electrodes, between a pair of electrodes that are driving and that are driving and electrolysis reaction.

So, now we are talking about the current measurement and interestingly. The measurement of dissolved oxygen is being done by an amperometric technique, which is called the Clarks electrode. So, now, we if you remember when we talked about in the second class.

Now, let us go back what we promised each other what we are going to do now look at it.

(Refer Slide Time: 09:53)

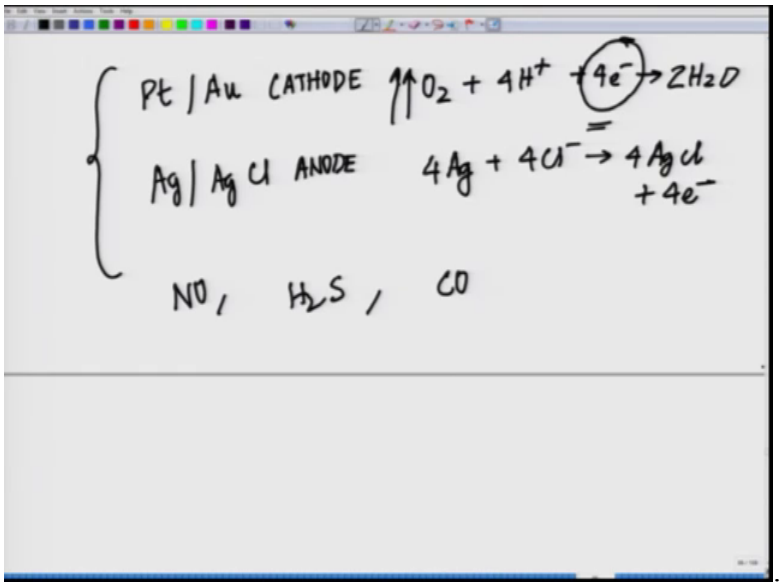


So, we talked about we have already shown how potassium could be measured, we talked about how P H could be measured now we are into oxygen and it will be followed by the glucose.

We are talking about the Clarks electrode now. So, the car Clark's electrode if you talk about it is widely used in medicine and biology to measure dissolved oxygen by amperometry, it was discovered or it was fabricated by Leland Clark.

Who invited the electrode also invented glucose monitor, what we are going to talk next and the heart lung machine. Now here also we will exclusively talk about the cathode and the anode reaction. So, basically it consists of a glass body of the micro electrode with a very fine point.

(Refer Slide Time: 10:48)



So, do not get worried about it let us look at the reaction what is happening at platinum A u which is the gold cathode.

The reaction is this oxygen plus 4 proton plus 4 electron leading to the formation of 2 H 2 O. Similarly at A g A g C l anode we have 4 A g plus 4 C l minus 4 A g C l plus 4 electron.

A Clark's electrode is calibrated by placing it in a solution of known oxygen concentration and a graph of current versus oxygen is constructed. So, basically based on the oxygen concentration your current is going to change the electrode also contains a silver guard electrode extending most of the way to the bottom the guard electrode is kept at negative potentials.

So, that any oxygen diffusing in from the top of the electrode is reduced and does not interfere with the measurement of oxygen diffusing through the silicon membrane at the bottom. And similar electrode has been detected or have been formulated for detection of N O which is another biological component hydrogen sulfide and carbon monoxide.

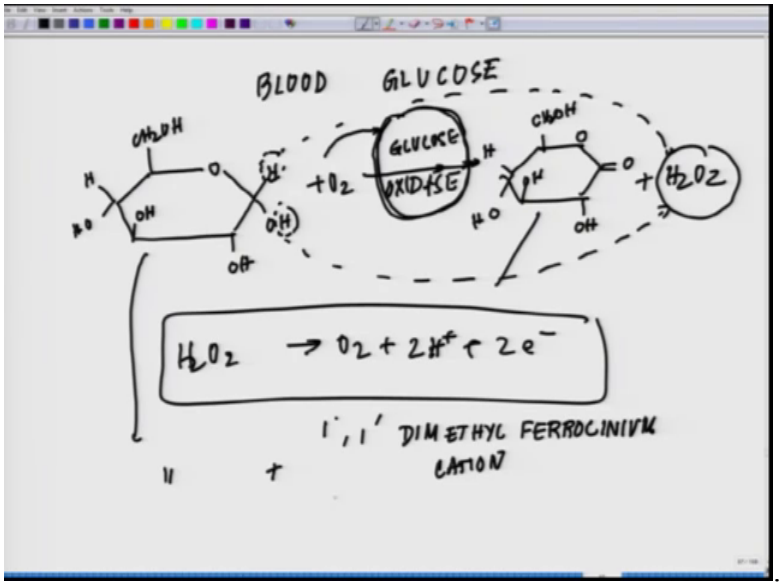
So, what it is essentially doing is we are measuring the current using the amperometric technique by virtue of which one can figure out, what is the concentration of oxygen which is dissolved in the analyte. And you could see where you are talking about the electron if this oxygen concentration.

Now, if you at the cathode where you are actually if this this goes up automatically your current is going to go up. So, this is the fundamental basic of where we are doing current measurement instead of voltage measurement. So, in that same line we will talk about a next analyte which is electronic nose. Electronic nose are basically in the old days the chemists have prided themselves on their ability to identify compound by odor.

So, essentially what we are talking about is there are vapors which bound just like we talk about oxygen; you could have re several volatiles which bound to a selective membrane. And binding of the volatile could be detected in terms of the change in electronic electrical conductivity.

There are electric nose which people are working across the world especially in the different sector, where they use amperometric technique from here we will move on to the glucose blood glucose monitor.

(Refer Slide Time: 14:06)



So, again the important part is for you to understand the reaction. So, what is happening? So, it is not the design of it. So, most of the people who are using blood glucose monitor diabetic patients. So, many people with diabetes must monitor their blood glucose sugar levels several times a day in order to you know control their disease and decide when to take the insulin injection.

So, again the our reference is A g A g C l. So, you have a glucose molecule what you are trying to measure is the glucose concentration. Here you have the glucose the O H H O H C H 2 O H O you have hydrogen you have hydroxyl you have the O H and you have the O H there.

Just purposefully labeling this H plus oxygen in the presence of glucose oxidase, it forms gluconolactone which is compounds like this, which is gluconolactone and C H 2 O H rest all remains the same H O H O H O H.

Plus H 2 O 2 and that is why I label those. So, which is hydrogen peroxide and this is gluconolactone in the absence of the enzyme the oxidation of glucose is negligible. So, basically glucose is getting oxidized the earlier glucose monitor measured the H 2 O 2.

Which is being liberated here they measured the H 2 O 2 by oxidation at a single working electrode, which is held at positive 0.6 volt versus A g A g C l this was the reaction which was H 2 O 2 oxygen plus 2 H plus plus 2 electron.

So, reaction at the working electrode one the current is proportional to the concentration of H 2 O 2, which in turn is proportional to the glucose concentration. So, essentially current is proportional to the concentration of hydrogen peroxide. So, early glucose monitor measured H 2 O 2.

And by oxidation as single working electrode, which in turn is proportional to the glucose concentration in the blood. So, one of the major problem, which it faced was these kind of monitors their response is dependent on the concentration of oxygen. One of the problem with this early glucose monitor is that their response dependent on the concentration of oxygen in the enzyme layer.

So, this has to come close to enzyme layer and here is the enzyme glucose oxidase. In the presence of glucose oxidase this is only going to happen, if the oxygen concentration is low the monitor responds as though glucose concentration is very low a good way to reduce oxygen dependence is to incorporate.

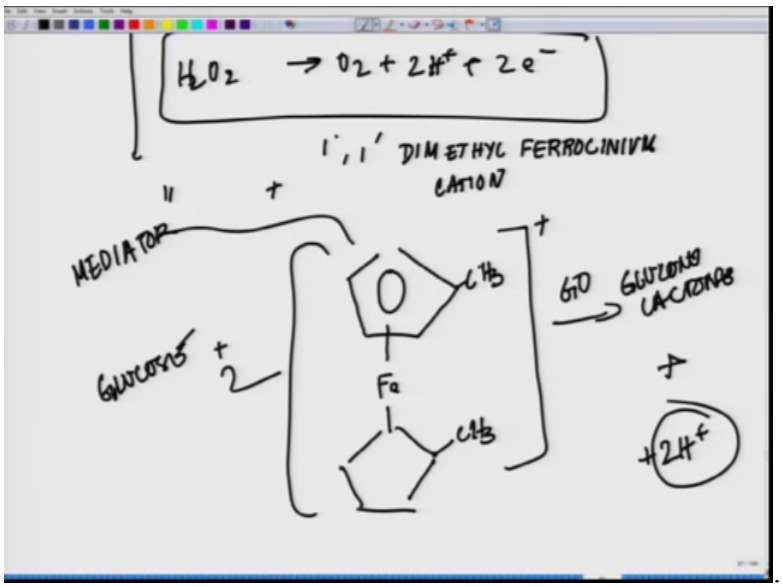
So, how you can do this is in a different way, because this enzyme we will act when depending on the response glucose might is that their response depending on the concentration of the oxygen in the enzyme layer.

So, this oxygen has to be on top of this enzyme layer, but now if the oxygen concentration is low the system will show as if there is low glucose. So, you are totally dependent on oxygen for this reaction to happen. Now one way is that oxygen is nothing, but an electron donor.

If some way or other you can substitute oxygen with something else another mediator, so, what people did after that they use the glucose scheme as it is plus they use something called a 1 1 prime dimethyl ferrocene cation di methyl ferrocenium cation.

So, di fee which is an ferrocene as you could see this is a basically a iron which is present there C H 3 C H 3 plus.

(Refer Slide Time: 19:14)



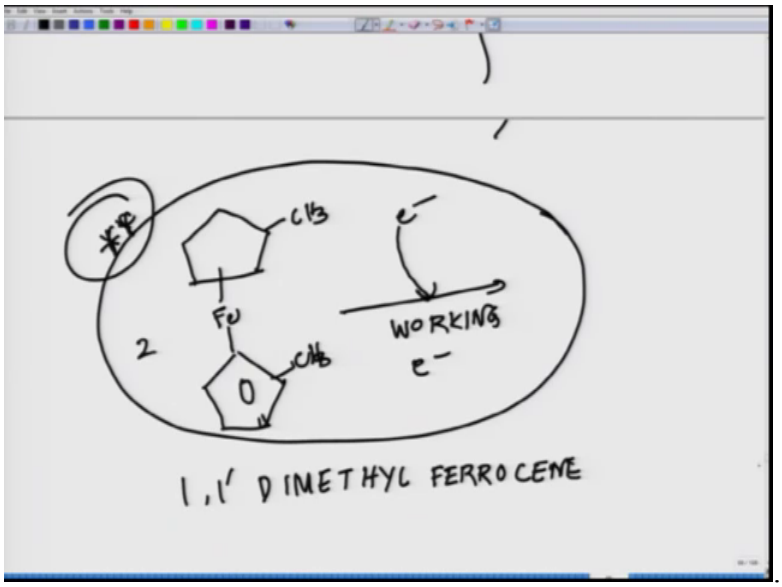
Now, in the presence of glucone glucose oxidase this particular compound becomes C a 3 C H 3 and F e and it.

What is happening here is that glucose plus 2 dimethyl ferrocene cation, which is acting as a mediator in the presence of glucose oxidase which is in here glucono lactone plus, you have 2 of this 1 1 prime dimethyl ferrocene and plus 2 H plus.

So, it forms a 2 protons which are getting generated the mediator consume in the reaction is then regenerated at the working electrode.

So, what is happening is that at the working electrode this ferrocene, which is dimethyl ferrocene 1 1 dimethyl ferrocene, which is produced? So, 1 1 dimethyl ferrocene so what is being liberated out C H 3 F e C H 3 2.

(Refer Slide Time: 21:00)



So, this is 1 1 prime di methyl ferroscene. So, this dimethyl ferrocene is now you give an electron to it at the working electrode. So, you are basically injecting current to it and again make it this compound dimethl dimethyl ferrocene, dimethyl ferrocene cation.

So, the amount of current what you are injecting to make this product, which is getting generated here out here you have that. So, you are making this reaction dimethyl ferrocene. So, the way you are doing it is very simple think of it.

Say of the glucose you have oxygen have glucose oxidase and you are making gluconolactone and in that process you are producing H 2 O 2. Now you are giving H 2 O 2 somewhere to dissociate H 2 O 2 by injecting current.

So, amount of H 2 O 2 is essentially on the working electrode is telling you how much glucose is present it is an indirect measure. So, you are injecting you are keeping it at a certain voltage and measuring the current and based on that you are calculating how much it.

So, if you have 2 you have 4. So, automatically the current is going to tell you how much current you are measuring. Similarly here what you are doing instead of because we realize that glucose oxidase needs a coating of oxygen on top of it to work.

If there is a lower oxygen it will show low glucose which is not true. So, instead of oxygen we picked up a mediator, which is in the form of dimethyl ferrocenium ion cations, which is this one and dimethyl ferrocenium cation become dimethyl ferrocene.

And then dimethyl ferrocene is further on a working electrode transform back to dimethyl ferrocenium ion cation. And the amount of current which it is showing or which has to be injected into it will tell you how much dimethyl ferrocene has formed with respect to how much glucose was present there.

So, it is kind of an indirect measure of calculating the glucose concentration. So, this is how you can use amperometry techniques to understand the concentration of different kind of biologic and analytes, which are present or which regularly one needs to take care in the hospitals.

So, I will close in here and in the next class we will talk about some of the techniques were protein redox chemistry is being studied using different voltammetry techniques.

Thank you.