Biological Inorganic Chemistry
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Lecture -15
Electrochemical Methods

Hello, good morning everybody. So, we will continue now in module-03, where we are talking about the physical methods. The entire module was designed in that way that where we can talk about the different physical methods. What we are using for your, this identification of the biological site, metal ion based biological sites. So, the last one the lecture 15 is devoted now for electrochemical methods. How we can utilize the different electrochemical methods, particularly one particular method we will just continue, where we can consider these things.

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How we design electrochemical cell we all know from our school days as well as the college days. Every day I say whatever you have learnt so far is useful for you to understand that to carry forward. So, not only the electrochemical cell is a historical perspective also a little bit you know how it has been designed. So, the electrochemical potential also related to that, then the redox behavior. So, whenever we talk about the metal ion in biological site, definitely we always try to focus our attention on electron transfer behavior. Such as if you have the ferrous ion, it should go to the ferric.

If you have the copper 2 plus ion in the biology, it should go to the copper 1 plus ion. So, these are the typical properties how we can monitor, how we can study also. So, two particular thing, because you have the dynamic voltammetry, which you also call as the cyclic voltammetry and that technique is very useful, and can be very useful for understanding that site.

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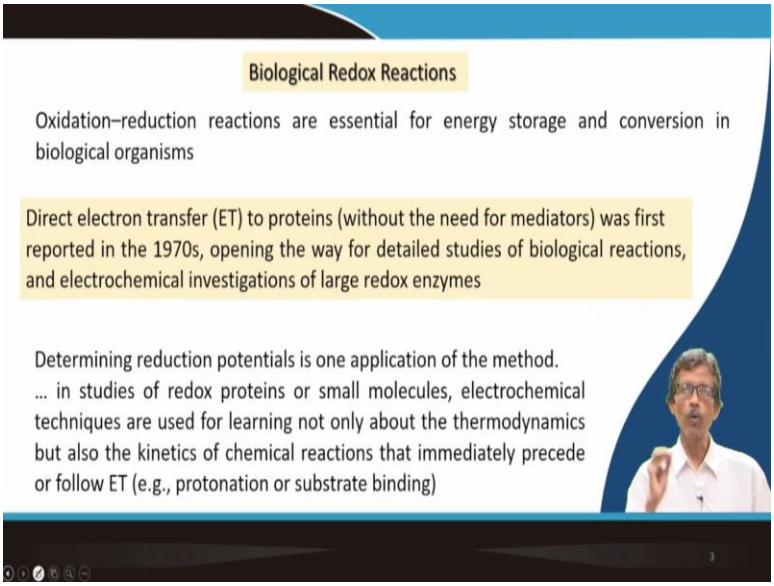

So, what we find that you have the redox reactions we all know; as I tell you every day that you have the ferrous iron or ferric iron in the, sorry in the test tube. So, that particular one we use that for using the redox titration. So, redox titration can also be helpful in finding out the unknown concentration of Fe2 plus in solution; or Fe2 plus in some stock solution given to you. So, biologically also that redox process, that means the electron transfer process can be utilized to know the responses for the presence of iron in the system. And if there is multiple centers of iron is there that we have seen also, we have discussed it also for pyridoxine type of thing.

For two iron ferredoxin 2Fe2S type or 4-iron, 4-sulphur type ferredoxin also can have. All these possibilities that means we can have also the multiple number of electron transfer reactions we can think of. So, two things we will be considering here is the oxidation reduction reactions, and those oxidation reduction reactions which are essential for us also. Starting from the demand for iron, we all know the bacteria has the demand, the plant can also have the demand for iron, and even for the mammals also. The human being also has a typical demand for iron, or the ferrous or the ferric iron.

Why we require all these things? This is due to the energy storage and its conversion. So, we want to store we know that we can synthesize large amount of ATP molecules by the burning process of your food material, such as glucose. Say, glucose burning will give you the corresponding synthesis of ATP through the phosphorylation reaction. So, if we can store it, we know that is the energy currency of our system. So, not only the storage, but also the conversion for making some useful molecules also, in all biological organisms. So, electron transfer is the most fundamental part in all these reactions, where we can see the corresponding direct electron transfer to proteins.

So, ET we can consider without the need of any mediator, because this we will see afterwards, when we find that cyclic voltammetry. We use some electrodes and the working electrodes are there and everything whatever we are measuring, it is on the electrode surface. So, there is no such mediator directly, but sometimes we find that the mediators are required. You have the oxidizing agent or you have the reducing agent; but it cannot transfer directly to that particular protein or enzyme the electron; or it can accept that electron from that particular protein or metalloenzyme. So, what you need? You need some mediators.

So, these mediators are taking electron or giving electron to the electrode or the chemical reagent, which is your oxidizing agent or reducing agent. Then it can transfer to the substrate which is your metalloenzyme or metalloprotein. So, it was a 50 years old story, when people studied all these things the detailed studies of the biological reactions also; and electrochemical investigations of large redox enzymes. If you have a very big redox enzyme and if we see there are multiple number of redox sites. That means, the site which is active in oxidation reduction reactions.

Like one example, I can give it to you here is that your cytochrome c-oxidase; where you can have multiple number of metal ions, and which are of different types also. You can have iron site, you can have the copper site. If all of them are involved in electron transfer reaction, we will have a very complicated situation. So, how we get all these informations and all these studies? We determine the reduction potentials; which is the very basic application of this particular electrochemical method of study. And while studying all these things, it can be your redox active protein or it can be your small molecule also.

Say, metal ion itself, which is AcO metal ion or the metal ion inserted in the metal ion complex. So, these electrochemical techniques are basically giving us some information about the thermodynamics of the electron transfer. That means, that which particular electrode potential electron transfer can take place from one site to the other. That means, it at where at which particular potential it can donate electron or it can accept the electron. But at the same time whether the method is also useful to find out the kinetic parameter; for any chemical reaction we know that is a thermodynamic thing is there, and the kinetic the rate of the reaction is also there.

But sometime it can so happen that it is the life is also a little bit complicated, that the kinetics of chemical reaction that immediately precede or follow electron transfer. So, if you have the electron transfer followed by something else is not that the two species A and B; they are only inter-converting by electron transfer only. Because in the biological system the system is very much complicated, such as that if you can have the protonation. So, if you have one species and after electron transfer say ferric ion is there say for example. And after electron transfer that it is accepting one electron is reduced to the ferrous ion.

And that ferrous ion can have some susceptibility, maybe on the direct metal ion site, or maybe at the periphery at the ligand site, or at the protein site; because the protein matrix is very big one. So, at some point you can have also the protonation, then the situation will be complicated; because your original thing is now getting protonated. Similarly, for enzyme kinetics we will find that you can have the substrate binding following electron transfer. So, all these things can happen. So, you will know a little bit about these particular complications, and then we can sort it out; try to sort it out with respect to the electron transfer.

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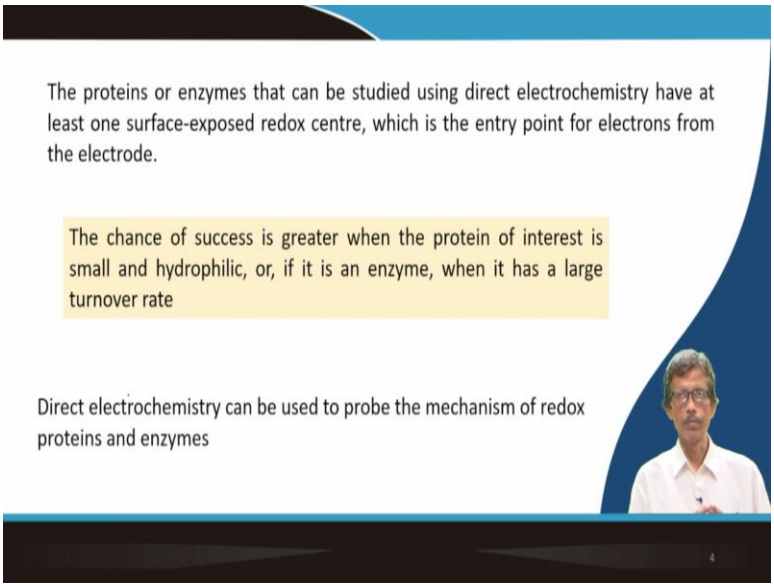

So, both these two categories of biomolecules containing metal ions such as your protein or enzyme, which we can study using direct electrochemistry, where, at least we can have some surface exposed redox center. Because you have the full cavity of your protein envelope, and within that interior position you have the metal ion. So, until and unless your metal ion is a little bit exposed to your oxidizing agent or reducing agent; electron transfer cannot take place. We know there are different types of electron transfer reactions. We call some as the outer sphere electron transfer reaction, sometimes we call it as the inner sphere electron transfer reactions.

That means the coordination of sphere, the outer sphere or the inner sphere are involved for that particular electron transfer reaction. So, definitely we have the surface exposed redox center and which can be your entry point for electrons from the electrode, or the reagent or the mediator or some other oxidizing or reducing agents. So, these are the things; so make your life simple. Here we are focusing our attention only on the electrode in the electrochemical cell.

So, electrode if you switch on the potential using your potential stat and galvano stat. The way we do it for your potentiometric measurements also that electrode can itself be oxidizing agent. And the same electrode can also function as a reducing agent, depending upon the potential which you give to the electrode.

So, the chance of success for these particular measurements, when the protein of interest is small and hydrophilic in nature; that means it can accept proton, or it can accept, or it can have some

good affinity for the water molecule. Or, if it is in the metalloenzyme, then it is a large turnover rate also we can find for these catalytic reactions, which is followed or preceded by electron transfer. So we will have the direct electrochemistry using biomolecules; we call these as the bio electrochemistry also.

The electrochemistry applied to the biological problems to understand the electron transfer reactions, which is happening within the protein molecule or the biomolecule, so, can be used to probe the mechanism of redox proteins and enzymes. So, if we have some molecules, which is driven by oxidation and reduction reactions, so definitely we can consider them as the redox proteins or the redox enzymes. And we can monitor those redox proteins and enzymes by studying simple these electrochemical measurements.

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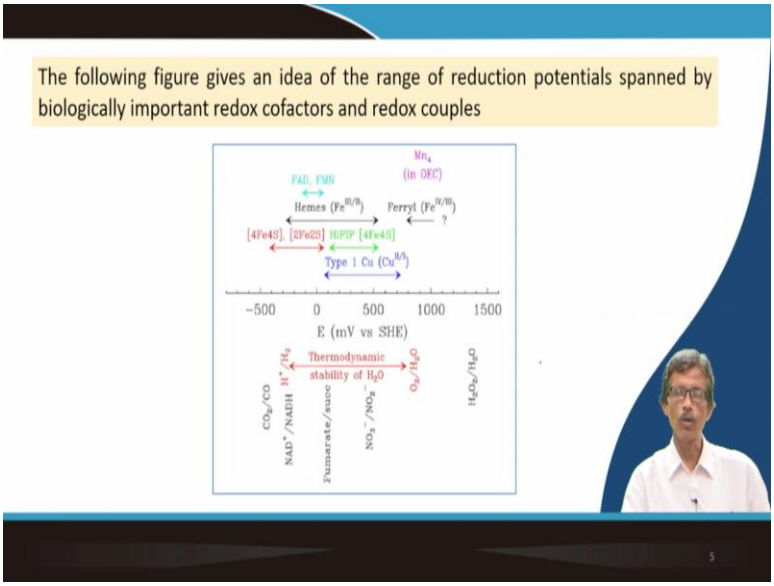

So, we have some idea now that the following figure gives us a range of reduction potentials, is the window basically spanned by biologically important redox, cofactors and redox couples. So, little bit we should have some idea about this particular table. Maybe I gave you this particular table, while my introduction, introductory classes, introductory lectures, I talk about all these things.

So, if you have a scale of minus 500 to 1500 millivolt versus standard hydrogen electrode. So, which is the standard reference electrode standard hydrogen electrode, we cannot use it, but we always say for this particular potential, while we report the potential values. Otherwise, we can

have the other reference electrodes, like saturated calomel electrode, the mercury-mercury chloride electrode, mercury-mercury sulphate electrode, and all these things.

If your window is spanning say beginning from say minus 300 to 1500 millivolt; we see the position of all these groups. Basically, the what we have the within this particular window; we have the stability of the water molecule. So, you have the thermodynamic stability for hydrogen production and the water production. Then we (()) (12:50) that the top the hemes; the heme group having two oxidation states of iron, the ferrous and the ferric.

We can talk about within a potential of a little bit less than about 0, and up to only 500 millivolt; that means, 0.5 volt. But, if we go for the manganese-4 center, the tetra nuclear manganese center all we know which is available in photosystem-2, PS-2; where, we have the water oxidation center. So, at the bottom you see that you have the water oxidation producing O2 molecules, so these are high potential proteins. So, this will definitely be high potential protein, because you need a higher magnitude of potential for the oxidation of water molecules, even in the presence of these photosystem.

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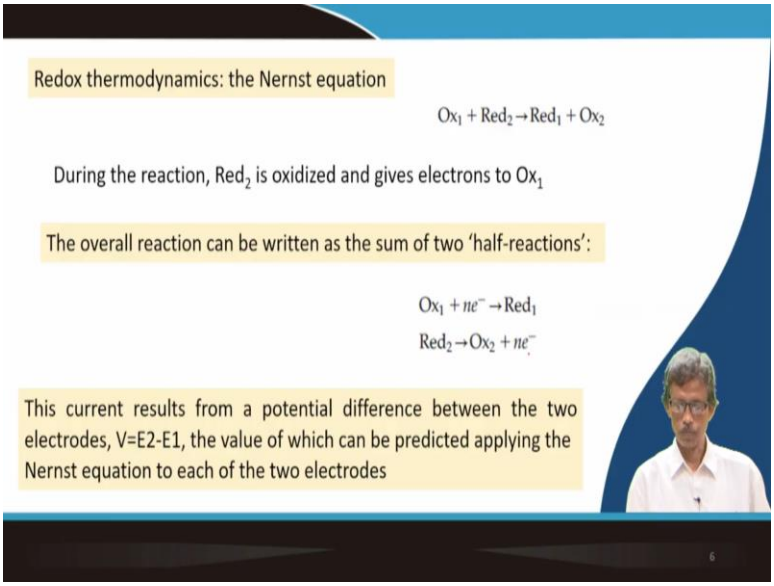

So, what we find that you just bring the Nernst equation all we know you have studied earlier; so, it is some kind of recapitulation for your studies. So, redox thermodynamics if we just consider following the Nernst equation. We know one is oxidized form, another is the reduced form; if we have two species. The oxidized form in one and the reduced form of two; then after

the electron transfer, the oxidized form is converted to the reduced form, and the reduced form is convert into the oxidized form. So, if you have A-B like this; so, A oxidized and A reduced; and B oxidized and B reduced forms are there, which can give you this particular reaction.

So, during this particular reaction that reduced form-2, the Red2 the reduced form-2 is oxidized basically, to give the gives the electrons to oxidized species 1. So, these are the two that means, the Red2 and Ox2 one will react for electron transfer reaction. And the overall reaction if there is a half reaction; because if we consider the change for the Ox1 to Ox2, and Red2 to Red1; so, you have 2 half cell reactions. One is that Ox1 is getting reduced to Red1, and Red2 is oxidized to Ox2; so, you have 2 half cell reactions. And the current results from the potential difference between these two electrodes, we can measure.

So, if you have two half cells, E1 and E2 like your two energy levels. The corresponding potential in V, which can be calculated or which can be measured also, can be predicted applying the Nernst equation of to each of these half electrodes.

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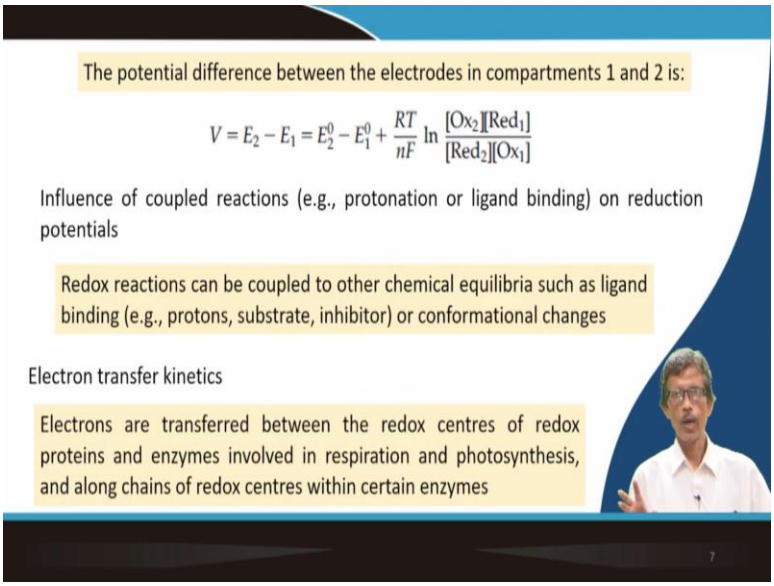

So, half cell reactions are therefore important for particular type of reaction. And if we only focus our attention or concentrate our attention on the electron transfer of one particular species is not that. When you are oxidized during our redox titrations, we all know the permanganate we use for the estimation of iron dichromate also we use. So, these are the two partners; iron in the

ferrous state is the, it is the species which can be oxidized by permanganate or dichromate. So, one will be oxidized and the oxidizing agent will be reduced.

So, you have if you have two compartments 1 and 2; so, ultimately how V you can calculate it out through this Nernst equation we all know that E2 minus E1 is equal to E2 naught minus E1 naught, plus RT by nF ln of the four species, concentration of all these four species. So, the measured quantity of V is dependent on the relative concentration of all these four species in solution.

So, definitely you have a equilibrium, so, you have a corresponding redox equilibrium. Once the equilibrium is reached in terms of your potentiometric measurements also; one it is reached to that particular equilibrium, we can measure or we can monitor the corresponding potential value. So, this relationship and also these are all concentrated dependents. So, if you find that we all know that the this E0 values are concentration dependent, E0 values are pH dependent also.

So, when we couple these two reactions together and along with the other coupling reactions; so influence of coupled reactions such as just we told you. I told you that the protonation or ligand binding following electron transfer. So, it will have some profound effect on the reduction potential values. So, these redox reactions can also be coupled to other chemical equilibria, such as ligand binding as per protons, substrate or inhibitor. Or you can have the conformational change which is very important and which is very crucial also.

Even if we find that the typical metal ion complex, we know that the corresponding geometries also; we know two isomers also in coordination chemistry. We know that the facial isomer Suppose, you have a NO bidentate ligand; and your nickel center or ion center giving octahedral complex; that is it is forming a tris chelate. So, that tris chelate you can have two forms; one is your corresponding facial form and another is your meridional form. Now, if we try to oxidize that particular species or that particular metal ion complex; NiL3 basically. So, if the nickel is in the plus 2 and all these L giving you a 1 negative charge; the whole charge on the complex will be NiL3 1 minus.

So, if we now see that if you have some preference for the corresponding isomeric form; that means whether it is present in the meridional form or whether it is present in the facial form; whether we can have a different E0 values. So, isomeric form similarly, the conformational

changes during electron transfer for the metalloenzyme or the metalloprotein; will find that the different E0 values or E values can be found. Where, you can have particular environmental effect on electron transfer. Because electron transfer is always coupled with the other factors, where you have the protonation, where you have the substrate binding or sometime the inhibitor.

Basically inhibitor is trying to bind the metal ion center. And it may invite the electron transfer or it may facilitate the electron transfer during that particular state. So, already I told you that when you talk about the redox thermodynamics, we know about the E0 values and the V value. Here we have seen that the V value. So, the corresponding potential value which is related to the thermodynamic equilibrium or the chemical equilibrium related to the electron transfer. But whether we can have some informations about the kinetics? How fast and how slow the reaction is for your electron transfer reaction?

So, when you transfer the electrons through the redox centers are redox proteins or enzymes, which are also involved in our life also in respiration to photosynthesis and along chains of redox centers with certain enzymes, so, you have the cytochromes electron transfer chains we know. At one end you have the food material say glucose you have and another end you have the oxygen, the di-oxygen. And this di-oxygen is utilized for bonding your glucose molecule; so you have many mediators. So, all are cytochrome basically, cytochromes, FAD, NADPH and all these are there redox mediators are always involved. But, this oxygen is not directly attached to your glucose molecule to burn it.

So, we know we have to have some separation between this oxygen and the glucose. So, along the chains, so, that way you have the chain of redox centers within certain enzymes or within certain metalloproteins. And it can have that particular influence that it can stepwise go for the electron transfer reaction; that means many number of mediators will be involved there.

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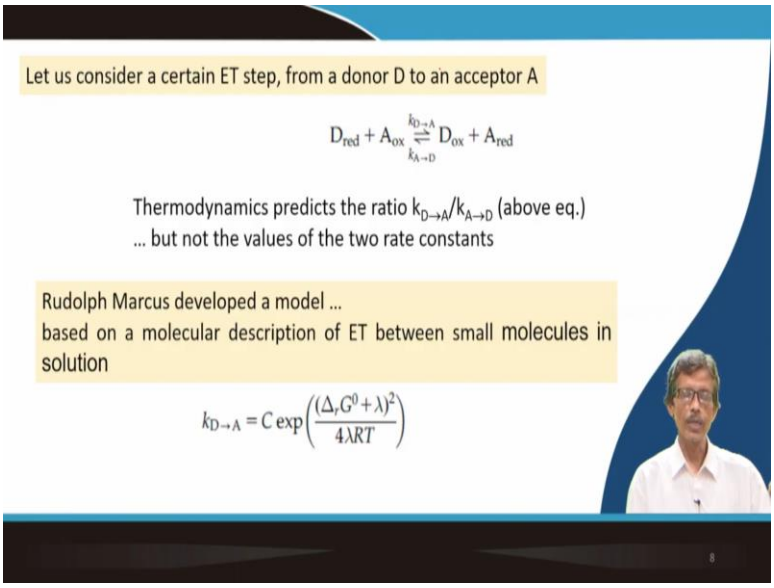

So, if we consider that we can we can have electron transfer step from a donor, which is D to an acceptor A. Then you have donor in the reduced form and acceptor in the oxidized form; then we bring the kinetic part the small k. Always try to remember the capital Ks are always the equilibrium constant values, and the small ks are your rate constant values. But, when you concentrate your attention to the thermodynamics of the process, we can only find the ratio, because we are reaching equilibrium process. So, the ratio of K D-A by k A-D; from the above equation we can find out all the thermodynamics can predict. But not the values of the individual rate constant.

So, what we can do for that electron transfer reactions. So, Henry Taube got this particular Nobel Prize for electron transfer reactions, particularly the metal ion complexes in 1983. So, following that, the people have also tried to understand about the electron transfer reactions. It is the Rudolph Marcus, which is the theory for that also he got the Nobel Prize in 1992. So, he developed a particular type of model, so where we can consider as a molecular description of electron transfer between small molecules in solution. So, it is a very standard process or standard technique; but he gives some equation, where you see only one of the parameter. That means, the kinetic parameter K D-A for the forward reactions.

The way we have written over here the D red plus A ox going towards right hand side, giving you D ox and A red. So, so, the free energy term, the lambda value, RT values and C; the pre

exponential term all these things are there. So, something we can monitor if you just look at, and we can find out theoretical justification or theoretical analysis of all these reactions.

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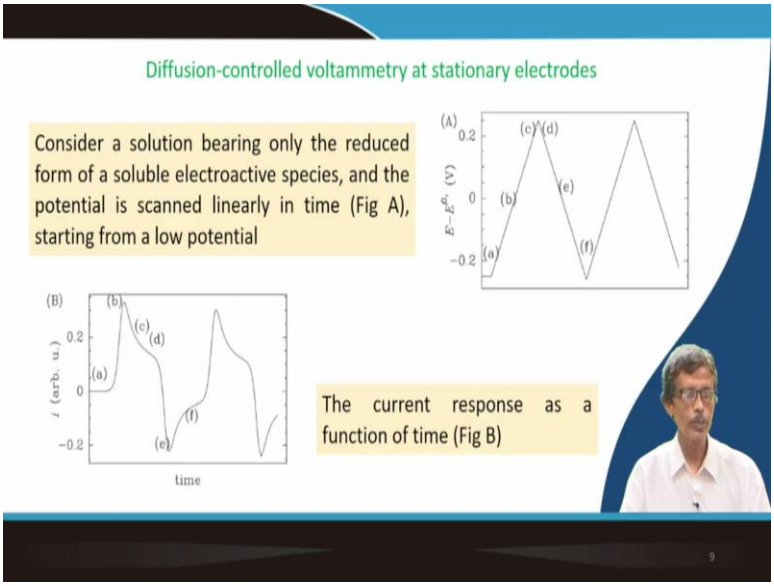

We can find that we can several parameters we can extract out of these equations. So, these Marcus parameters will also control the rate of the reaction and also the exchange reactions between these two species. Now, we see what we can know about the corresponding voltammetry at stationary electrodes; so, you will have the diffusion. So, the diffusion control thing is there; so, how the diffusion will also tell us something about these particular measurements. So, you have the solution and you have several electrodes; how many electrodes you need. So, always ask immediately yourself that whether you require two electrodes, the two compartments we are talking about, or you require more?

So, what we get this sort of plot and this plot is telling us something that E0 values, E0 minus E0 E minus E naught prime. So, which is nothing but a solution having a reduced form of soluble electroactive species; your species should be electroactive. The way we are talking about the your ferrous and the ferric ions should be electroactive, such that when you put in the inside the cell, the electrochemical cell, it give you the response. And the potential is this is the scanning of the potential with time. So, this lower x-axis is your time axis; so time axis versus the potential axis, which is your figure A, starting from a low potential value.

So, when you start from minus 0.2 volt to plus 0.2 volt and with time; so, is a cyclic one. So, you are going back and forth for this particular scanning; then what should be your current response? We know this potential we scan against time. So, there should be something where we get the corresponding current response as a function of time also. Again your current response will be something like that when you have a, b, c, d, e, f all these positions are there. So, again the i is your current in arbitrary unit against time. So, you have all these positions are marked over here from a to f that gives you all these positions.

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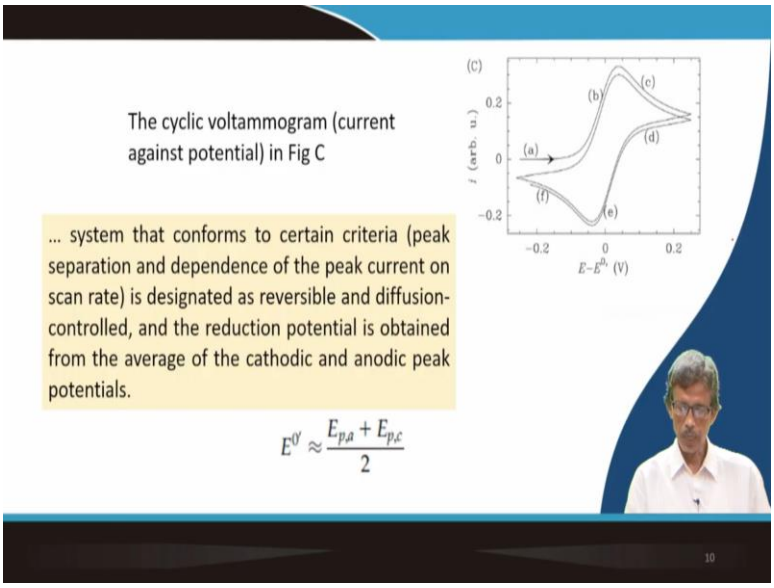

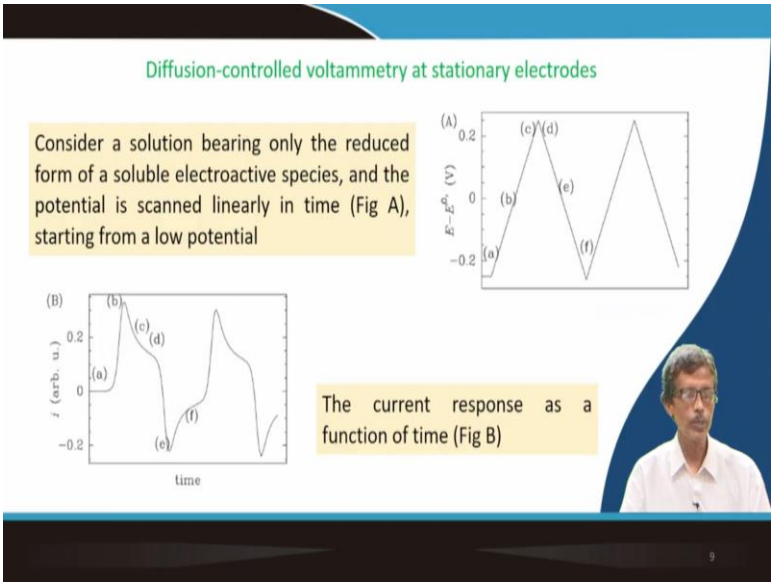

So, what basically from there we get? We get a voltammogram. So, volt is your one is your volt axis; that means the potential axis; now your potential axis is your x-axis, and the ampere is your y-axis. So, that is why it is voltammetry or voltammogram, volt versus amp. So, is a cyclic process; now everything is leveled. So, where we have level, this particular one for your a, b c, d, e, f. So, similarly, we have leveled all these here also, where you were as a. So, the we have seen that a, b c, d, e, f; similarly, for your, this thing also the current response. So, how it looks like when you actually scan it; so, a to b, b to c, c to d, d to e, e to f only.

So, what we get is basically a cyclic voltammogram, which is the plot against current against potential; and current i in milliampere or microampere. So, is micro amperes is very small current we will get. When you talk in terms of the corresponding measurement for the proteins and the enzymes, we expect that you can have also more lower potential values as well as the current values. So, if your current can go down that means the nano ampere or pico ampere also So, the sensitivity of the instrument if it is big enough, we can measure that also from that particular measurement. So, the system which confirms certain criteria, that means peak to peak separation.

So, this is one peak, one is the top peak, and another is the bottom peak. One can be your cathodic peak reduction peak, and one can be your anodic peak or the reduction peak. So, sorry anode is oxidation, a cathode is for the reduction. So, these separations, so if it is one is there another is there, so you have the delta. And if it is peak to peak separation, you have the delta Ep value. So, this separation is dependent on the peak current on scan rate, and it is designated as a reversible. If certain magnitude of this separation we can only consider, which is 60 millivolt only; and that also 60 millivolt in the standard condition

We sometime we guess also, whether you have a one electron transfer or a double electron transfer. So, average of the cathodic and anodic peak potential value is giving us the E0 prime or E0 naught or E half values, which is the Epa value plus Epc value divided by 2. So, these are the top which is your Epa and Epc value divided by 2. So, that way we get the corresponding E half value for a particular electrode transfer reaction.

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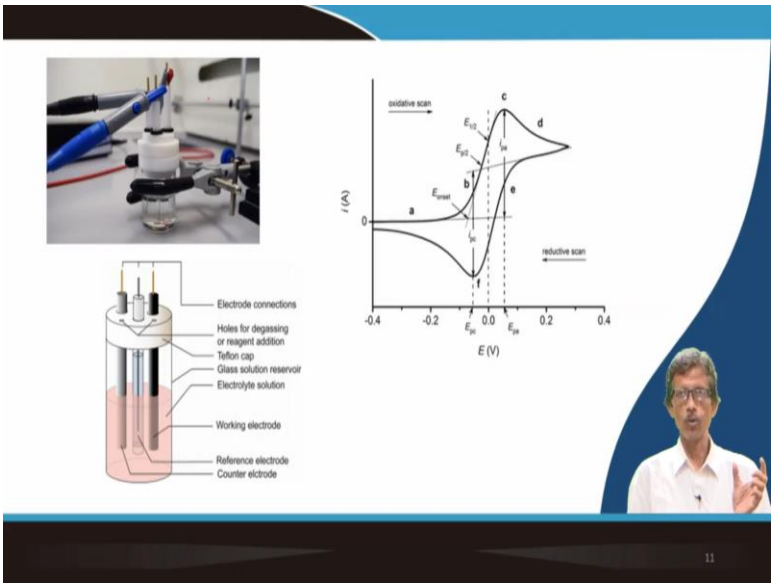

So, this is the setup you just quickly see now, that how well we have reach there. So, it is the particular setup, where you have the cell electrochemical cell. And this particular electrochemical cell is utilized for the connectors. So, you have at the top you have the connectors; you see just is visible is a three; but you see that three electrodes are there at the top, you have three tips are there. So, three tips are for three electrodes; one is your working electrode, another is your counter electrode, and another is your reference electrode. So, is a three electrode system.

So, all these cyclic voltammetric measurements require a three electrode system and what are these. So, this block diagram, so what actually it looks like, and then the block diagram you should know; and you should familiarize yourself with this particular block diagram also. Because the solution the solute is can be a different thing; solute can be your metal ion, solute can be your corresponding protein sample, or solute can be your enzyme sample also. Then you have to add supporting electrolyte, then you have to choose the corresponding medium. That means, whether you are able to do it in water medium, or the aqueous medium, or some other non aqueous medium; or the organic solvent like acetonitrile, dichloromethane, or even DMF or DMSO.

So, you need a dry solvent for that, you need the gas bubbler also, to have the inert atmosphere, and you have the starting bar also at the bottom. So, that you start the solution; so, initially you

mix up the homogeneous mixture you get. And then finally you stop the stirring and record it. So, what we get? So, this is the standard figure for any cyclic (volta), so textbook type of cyclic voltammogram and you see the IPA values, IPC values, EPA values and EPC values. And that is what you know that the E half values.

So, E half is given over there for the oxidative scan; so, the first one if you go up is the oxidative scan, and when you returning back is your old reductive scan. So, if you have iron center in the cytochrome, you can go for the oxidation of that center 2-ferrous-2-ferric; and then you can return back to the original position. So, it is a cyclic process, and if you go for a very fast scan; you will be able to tell me that the system is stable within that particular time period for electron transfer; because many such biological electron transfers are pretty fast.

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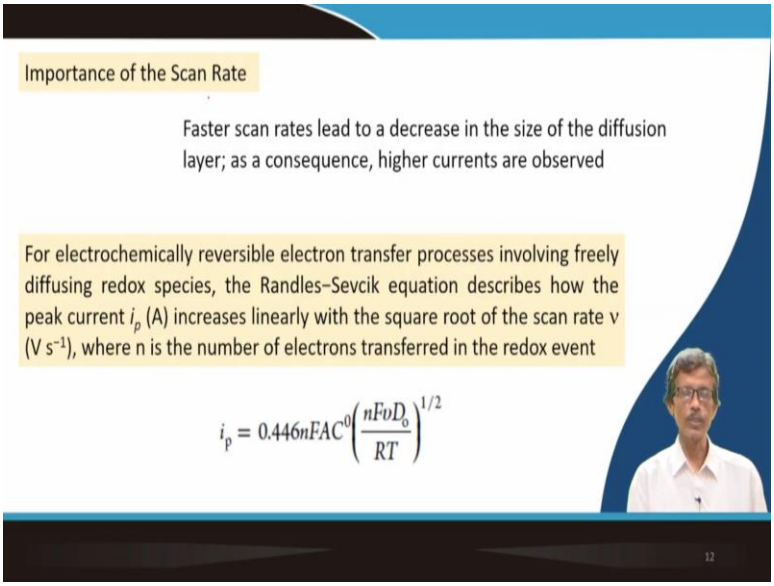

So, rate of those electrons transfer is pretty fast; so, that is why the dependence on the scan rate is important. So faster scan rates lead to decrease in the size of the diffusion layer, for the diffusion control current and all these things. So, Randles and Sevcik equation is describes how the peak current IP in ampere, increases linearly with the square root of the scan rate V in volt per second; so, this is a particular equation. So, that equation tells us that how these are related for the standard condition, if they are deviating, we can consider that something is happening.

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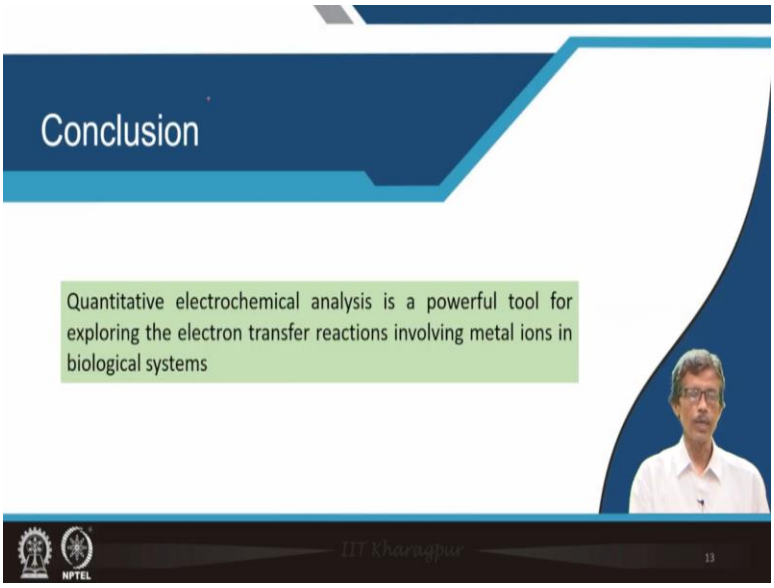

So, we have reached to the concluding side, where we can think of what we have learned in this particular half an hour class that we are trying to have some good idea about the quantitative electrochemical analysis; which definitely the powerful tool for exploring the electron transfer reactions on the metal ions in biological systems; where the metal ions are presents in the biological system.

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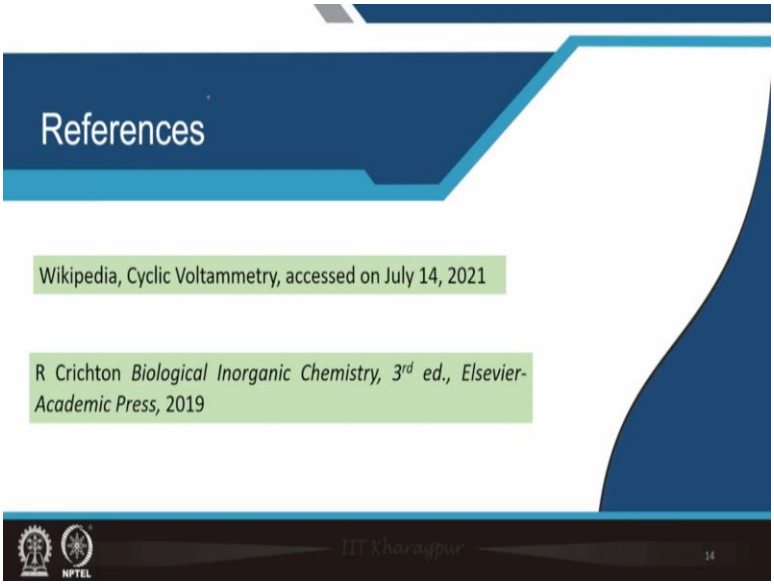

So, like other classes, you have the references, just simply Cyclic Voltammetry in the Wikipedia page, as well as the Crichton’s book is also useful for you. So thank you very much for your kind attention.