Nanobio Technology Enabled Point-of-Care Devices
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Lecture - 38
Lab Demonstration - 1

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Hello everyone. So, today we are in the lab. So, I taught you all the courses for the Nanobio Technology Enabled Point of Care. You know the technology; I taught you all the theoretical backgrounds. Now, this is the good time to show you practically how you can handle the basic technology in the lab. So, I will show you today all the electrochemical characterization technique, how to develop the sensor chip and then finally device. That is our main theme like lab to market.

So, now we are in the lab. So, let us develop step by step from the very basic things like you are here today. I will show you like let us fabricate your sensor, how we will start from the

biological molecules, how we will synthesize all the nano particles and let us use them for diagnosis. So, this is the right time for you today.

So, my all teaching assistants are here today. So, they will demonstrate you all the basic technology like cyclic voltammetry, chronocoulometry, chrono-amperometry and the electrochemical impedance that can be useful for sensor development. So, let us start our lab today, ok. Let us introduce my TA. So, here Sristi.

Hello.

And Raghav.

Hello.

So, who are going to show you the very basic technology for the electrochemical characterization. Here, Mukthi and (Refer Time: 01:26)

Hello.

Hi.

They are going to show you the synthesis of the nano particles and their applications for your electrochemical study. And here, Jai.

Hello.

He is going to show you the how to develop the portable potentiostat and the app development for the smart phone based device.

The three electrode system is a very popularly used electrochemical system for electroanalysis purpose. It consists of a working electrode, a reference electrode and a counter electrode.

Working electrode consists of different materials such as metals and semiconductors and carbon, of which the metal electrodes have a lower cathodic over potential and the problem of hydrogen evolution persists.

On the other hand, a glassy carbon has a very wide potential range and it is applicable for a wide range of electrochemical applications giving wide potential window for operation and inert electrode by nature and useful for variety of purposes. On the other hand, we have a counter electrode which has a higher surface area over the working electrode and it is also inert in nature and it facilitates the current movement and the closing of circuit for the three electrode system arrangement.

The reference electrode is a ideally non polarizable electrode where a negligible current flows and it helps in maintaining the potential between the working electrode and the reference electrode. And this set of together is widely known as a three electrode system.

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So, we have till now known that there are three electrode systems we are dealing with. So, this is the working electrode. We have three types of disk working electrodes here. This one is a glassy carbon. We see the glassic type shining here. That is why it is called the glassy and then this one is the gold electrode followed by the third one it is a platinum electrode. So, these are the three working electrodes.

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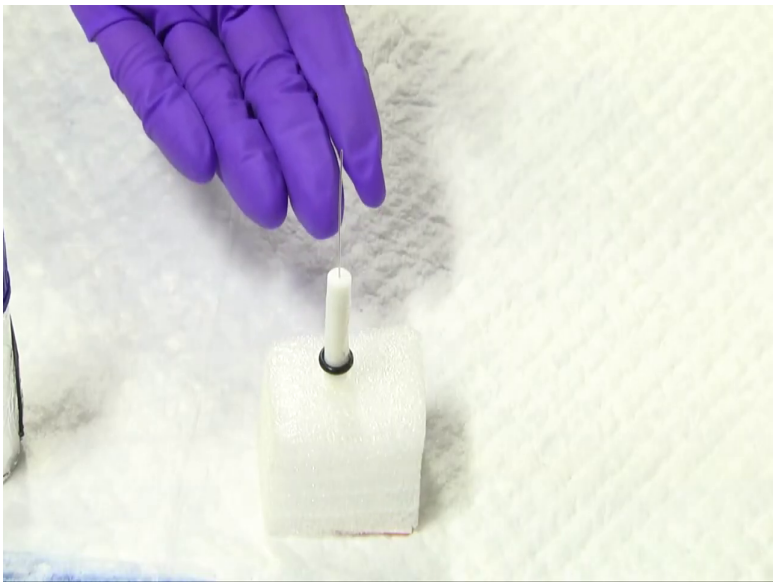

Now, this one is the platinum counter electrode. The thin wire is the platinum.

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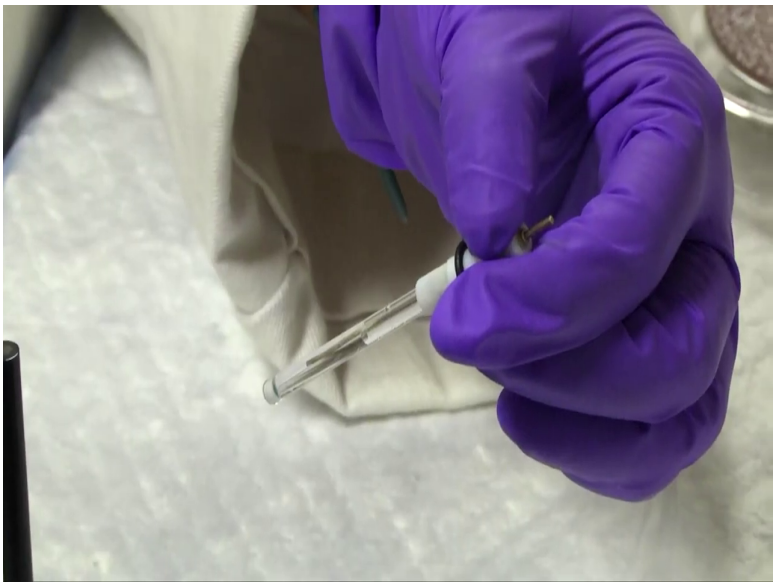

Now, for the reference purpose, we use the Ag AgCl reference electrode which is being dipped in three molar KCl. Now, we will be demonstrating how to clean the working electrodes.

Electrode cleaning is a conventional procedure that is widely utilized especially for analysis in disk electrode applications. It involves mechanical polishing, gentle washing using ultrasonication and DI water and electrochemical pre treatment. So, the following will be demonstrating it by performing the mechanical polishing, ultrasonication in the enhanced water and acid treatment following which will be demonstrating the electrochemical experiments.

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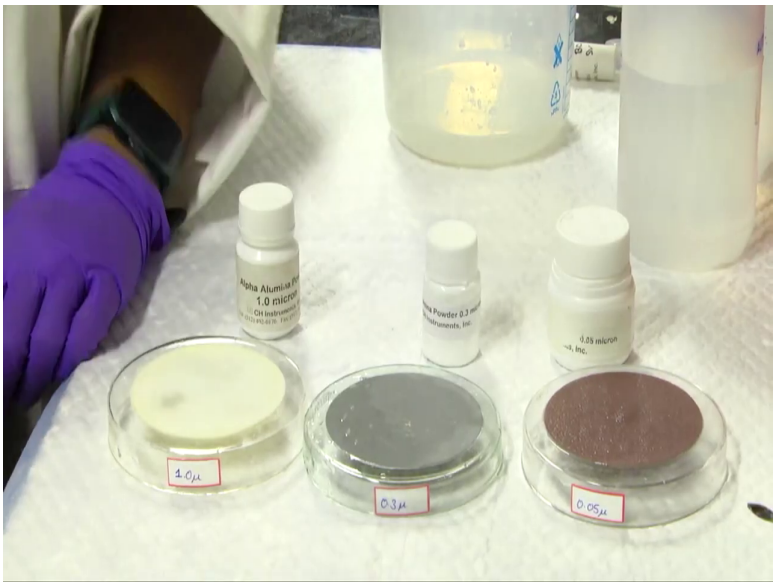

So, now we will be doing the mechanical cleaning of the electrodes. These are the three micro pads of different micron sizes namely as 1, 0.3 and 0.05 microns. So, then followed by these are the alumina slurry powders of the same sizes 1, 0.3 and 0.05 microns. So, for the cleaning demonstration, I have chosen the GCE.

Now, what we will be doing is that first we will take the 1 micron powder since it is a 1 micron pad and we will take spatula and just take a small quantity of the alumina slurry powder. Then we will take small quantity of type 1, deionized water then we need to make a slurry out of it.

We must keep in mind that we should not press the electrode tightly onto the surface. It should be very gentle. Now, the slurry is being made. We need to take the electrode as whole

like this tightly, but press the electrode very gently onto the surface of the micropad and then we will make slowly 8. As you can see slowly, we are making 8.

So, while making 8 according to the different kind of shapes that we have tested widely, it is being concluded that making 8 shape is the mostly best form of cleaning the electrodes.

Now, another thing that we need to keep in our mind is while cleaning we also have to rotate it by 90 degree to ensure that every portion of the disk electrode is being cleaned. Like this then slowly rotate, then this, then slowly rotate then this so, doing this several times. Now, once this process has been done.

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We need to wash it with the type 1 water. Same procedure is to be followed with the 0.3 and 0.05 micropads using the respective alumina powders. Now, we will be shifting towards the

cleaning of the electrodes with the ultrasonication methods. Here, we will be demonstrating the cleaning with the type 1 water that is the DI water. So, now, we will be moving forward for the ultrasonification cleaning method where we will be demonstrating with the DI water that is type 1 water.

So, now, we are going to do the ultrasonification. This is the electrode that we have earlier clean with the mechanical cleaning and now, this is the glass while where we have filled it with the DI type 1 water and this is the temporary setup. So, that it can float in the bath sonicator.

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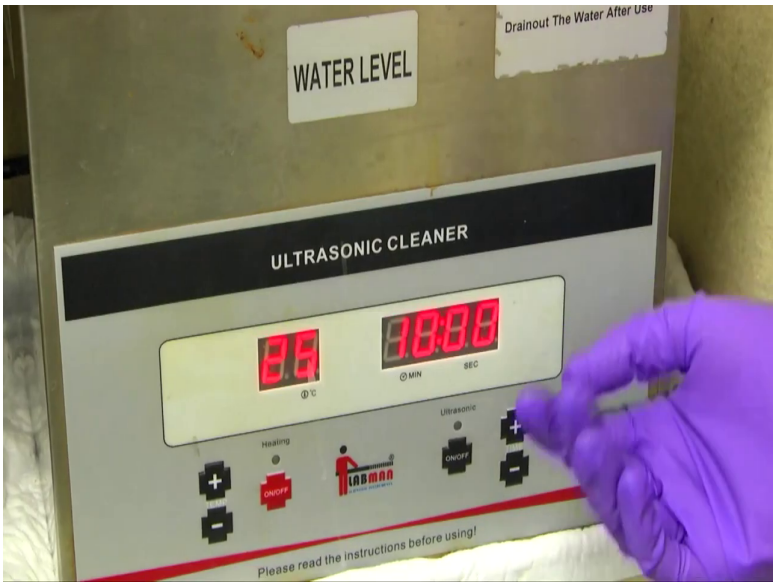

So, this is a ultrasonication machine where here we have the timer setting. Here the temperature of the bath sonicator, the water here is type 2 water.

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Now, we have adjusted timer to 10 minutes and temperature of the water is 26. The setup was such that the while floats onto the water. Now, we will start and the sonification will complete in the 10 minutes. So, now, the ultrasonification has been completed for 10 minutes and.

Hm.

We will take out the electrodes from the ultrasonification machine. So, now, we have completed the ultrasonication of the electrodes. And now, we will move on to the electrochemical method of cleaning the electrodes using the CV in 1 molar H 2 SO 4.

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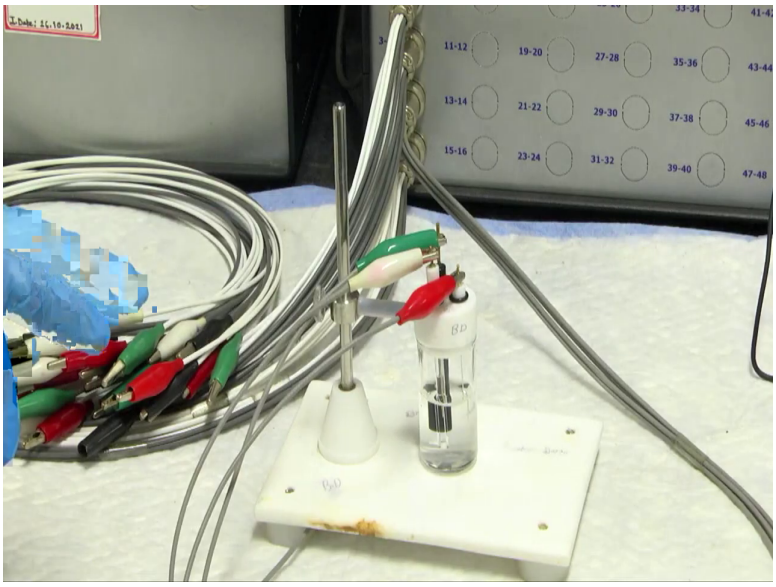

This is the 3 electrochemical system with the electrochemical stand. As you can see, you have a working electrode, reference electrode and the counter electrode within this arrangement forming an equilateral triangle like arrangement. It should be ensured that the electrodes does not touch the walls of the container. So, this solution consists of 1 molar H 2 SO 4 and we will be performing the acid pretreatment for the working electrode in order to clean the surface.

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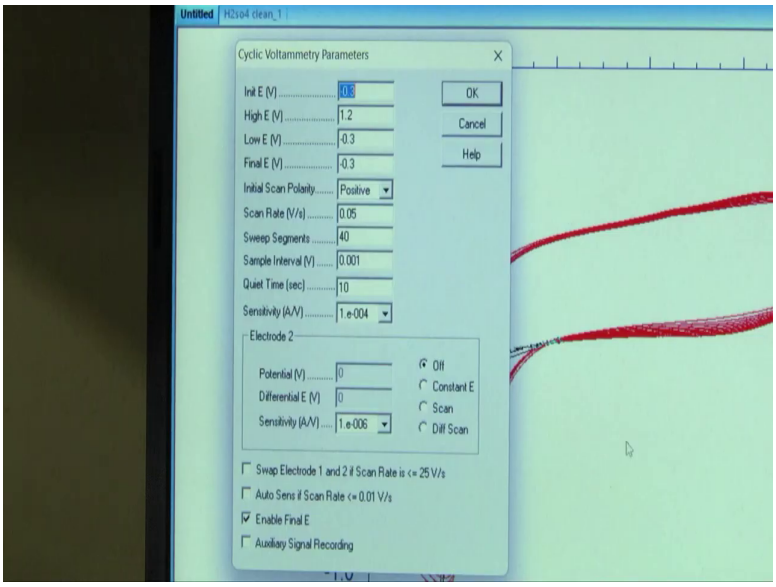

So, this is the electrochemical pretreatment using cyclic voltammetry procedure. And these are the parameters as you can see where we had set the potential window from minus 0.3 to plus 1.2. And we shall be performing the electrochemical cycling for almost 20 cycles or 40 segments at a scan rate of 15 milli volts per second.

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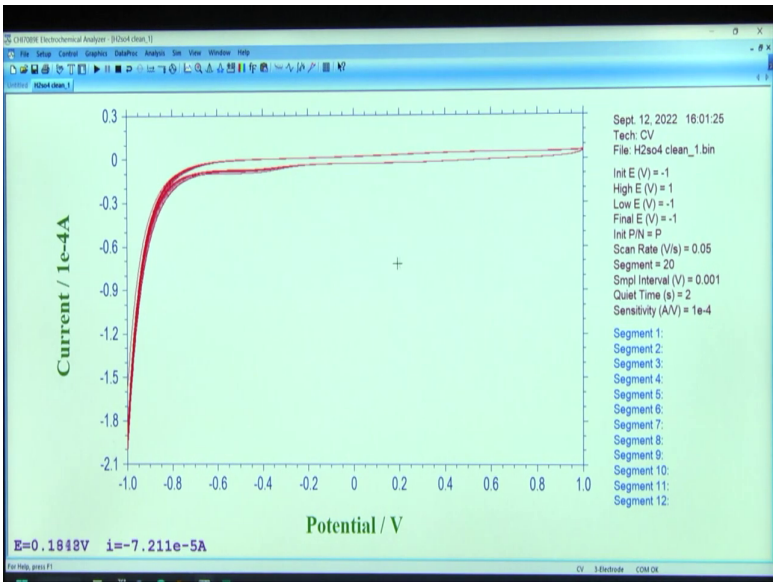

After performing the electrochemical cleaning in 1 molar H 2 SO 4 for almost 40 cycles, you can see this is the spectrum that will be receiving, indicating that there are no significant peak seen here, indicating the electrode is clean. After performing the electrochemical cleaning in 1 molar H 2 SO 4 for 40 cycles, this is the resultant CV spectrum. You do not see any significant peaks in the spectrum indicating the electrode is clean.

Electro analysis experiments mainly consists of voltammetry, amperometry, coulometry and impedance spectroscopy experiments of which the voltammetry is further divided into many other types, in which cyclical voltammetry is a very popular technique, which is used mainly for revealing information on the mechanistic sense as well as on the sensing purpose.

So, in cyclical voltammetry, the idea is you will be subjecting the working electrode to a voltage ramp and the corresponding changes in the current is recorded. From the spectrum,

we will be able to obtain many values of scientific importance such as diffusion coefficients, effective surface area, the number of electrons transferred, the charge transferred during reaction and many others.

As far as chronoamperometry procedure is concerned, it is a technique that facilitates measurement of the current signal in a continuous time scale. This technique is very much commercializable and holds immense applications. To cite an example, the glucose biosensor is one such one. In this technique, we apply a particular potential or a potential window and obtain the current changes for the corresponding real time measurements.

Chronocoulometry is an extension of chronoamperometry technique in which instead of the current values, our idea is to harness the information from the charge characteristics. This further helps in utilizing a electrochemical surface area and surface reactions and many other such measurements.

Apart from all these, we have a spectroscopy called electrochemical impedance spectroscopy, which has immense insights on the mechanistic characteristics of our electrode arrangement. In this technique, we apply a small alternating current impulse and we perturb the system in a very minute manner and try to understand the changes in a linearized fashion.

From this technique, we shall be able to comment on the solution resistance, on the charge transfer resistance, Warburg impedance and mass transfer characteristics which holds again lot of mechanistic information as well as holds key for electroanalysis applications such as immunosensor and many other affinity sensor measurements.

Ok, let us start with very basic electrochemical technology that is cyclic volumetric.

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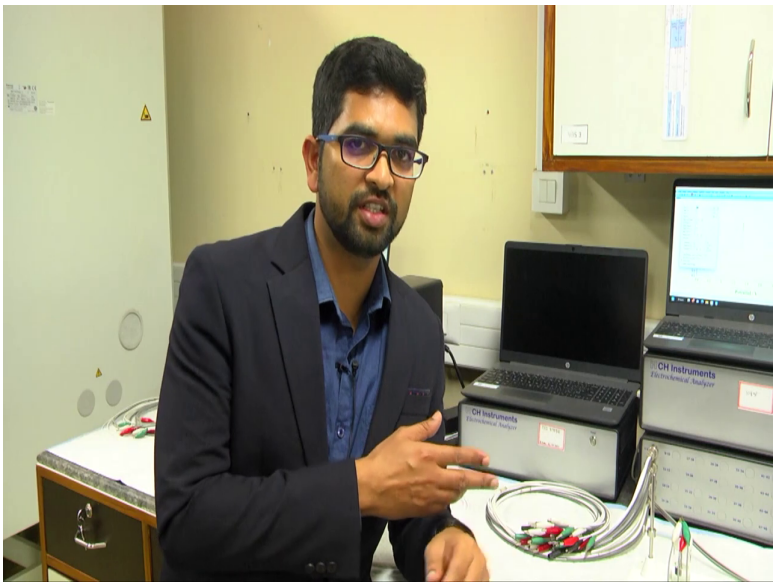

So, now, I am going to show you here the how to take the cyclic volumetry. That you can see here, we start first potassium ferrocyanide. As I mentioned during the teaching, potassium ferrocyanide, its oxidation state is 2. So, first we will start from the left side means it would start first that it means negative mean oxidations and then reductions and then we will complete the whole cyclic process. Let us say, ok.

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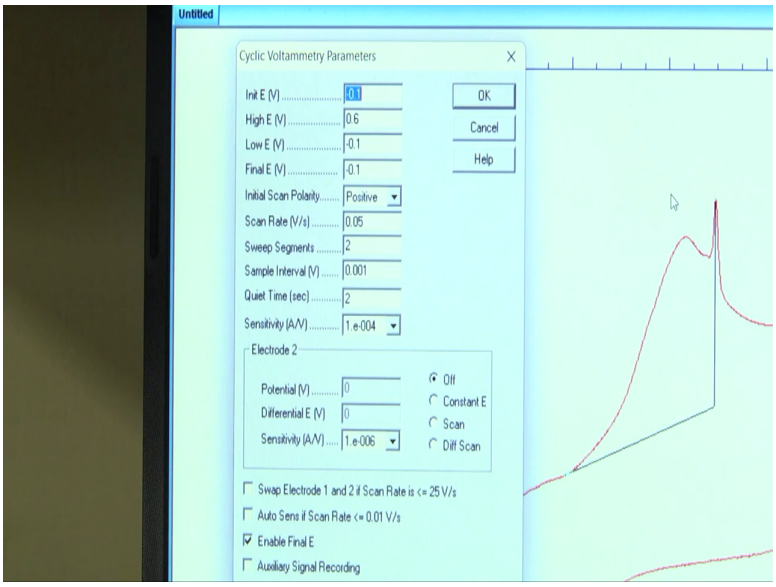

You can see the parameters here for the cyclic voltammetry, we will start from the initial potential minus 0.1 and the final potential 0.6. As I told you, the solution is the potassium ferrocyanide is a is the iron 2 oxidation see that is why first it will oxidize then it will reduce. That is why we set the parameter like this. So, let us start the technique then you can see the shape of the cyclic voltammogram.

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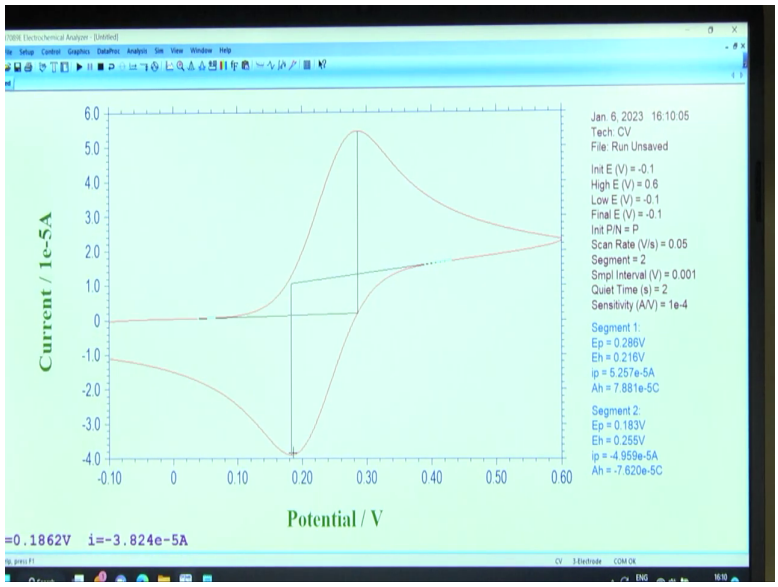

So, let us start the technique, ok. You see the iron now slowly from negative side is oxidizing that is why this current is increasing, increasing, increasing and then the oxidation is completed and then I put here up to 0.6 potential. After from there, see now current again the reduction current starting. And you see the reductions peak potential and here complete the reductions and as we put the potential window up to minus 0.1, then it will stop there.

So, here you can see that this is the oxidations peak current here. Because and this is called the this potential is the oxidations peak potential that I taught you already in the during the theory class. And see here this is the negative side this one is the reductions; this is the reduction peak potential and this current is the reduction peak current.

And as I taught you if you make the (Refer Time: 15:59) this is the like E P A C means anodic peak potential, this is the cathodic peak potential. If you made the addition then

divided by 2 then this will be your formal potential, right. This is the all the story for the cyclic voltammogram here.

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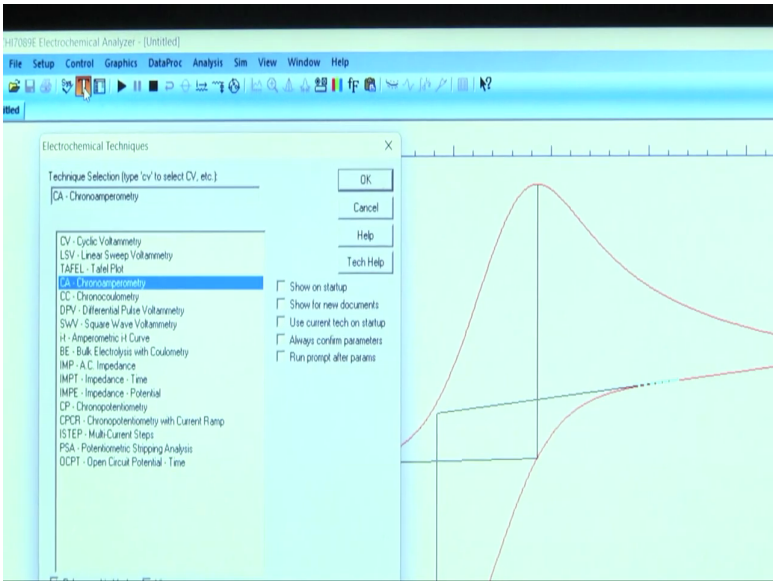

Following cyclical voltammetry, we will be now performing chronoamperometry procedure.

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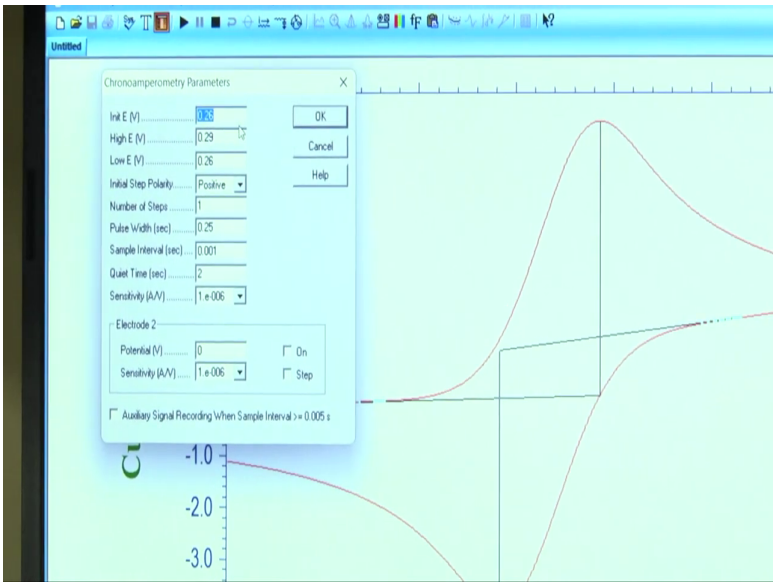

In which we will be setting the initial potential as 0.26, the high potential will be 0.29, the low will be 0.26 and we are going to perform it for one step with a pulse width of 0.25 seconds. So, these are the parameters as you can see, we are said the initial potential as 0.26 volts, the high as 0.29 volts, the low as again 0.26 volts. And we shall be performing it for one step for a time duration of 30 seconds so that the reaction runs for 30 seconds.

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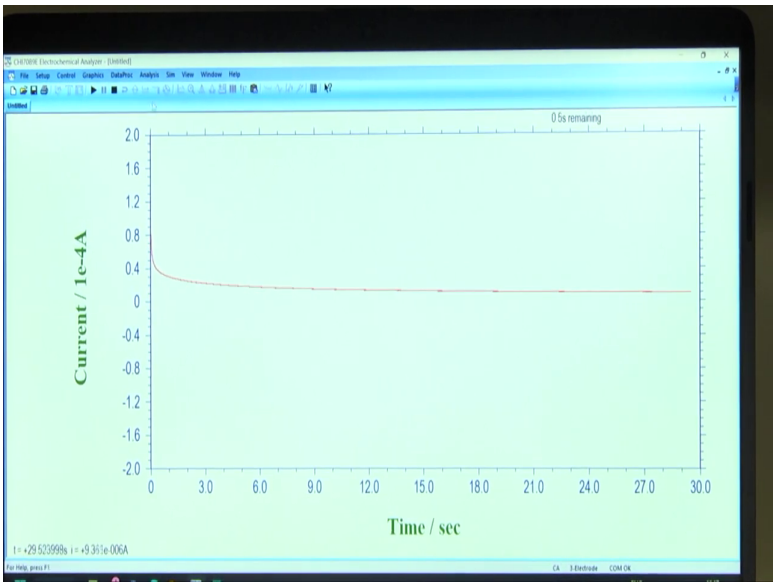

So, the here you can see as the time progresses the current value saturates indicating the formation of a diffusion layer which dictates the current signals that we are getting.

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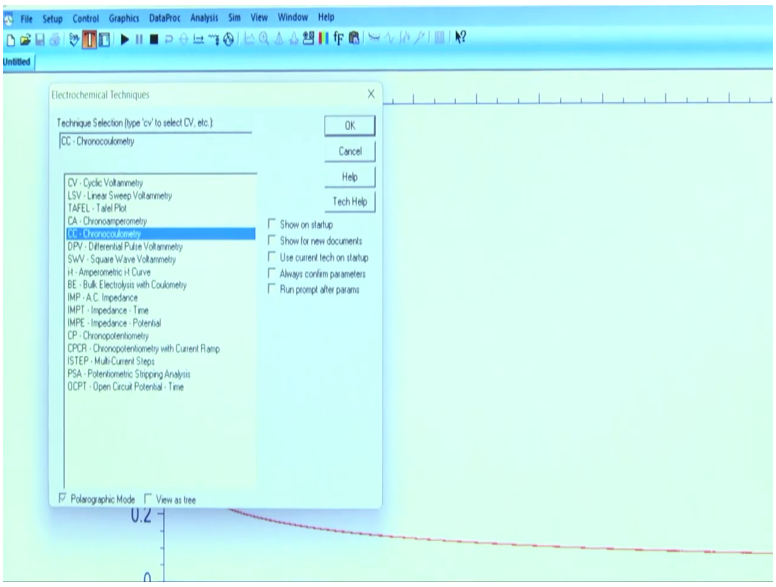

The next technique that we will be demonstrating is chronocoulometry.

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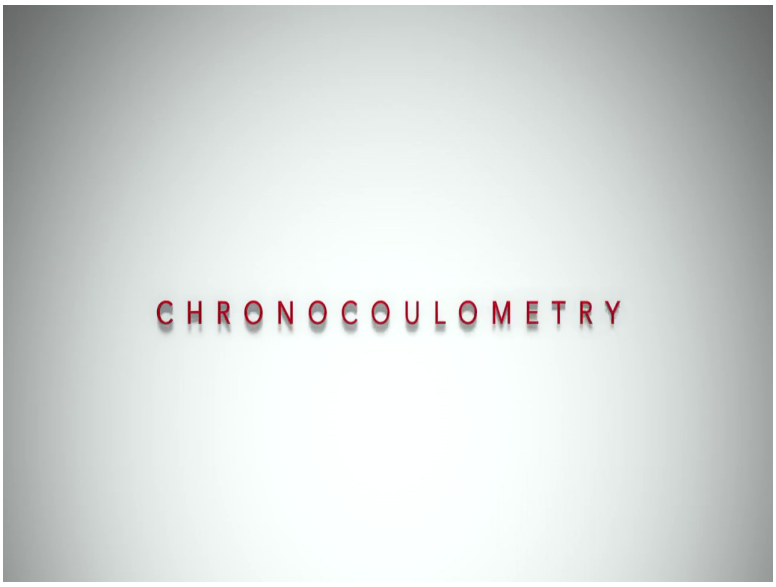

Which is a complementary technique wherein.

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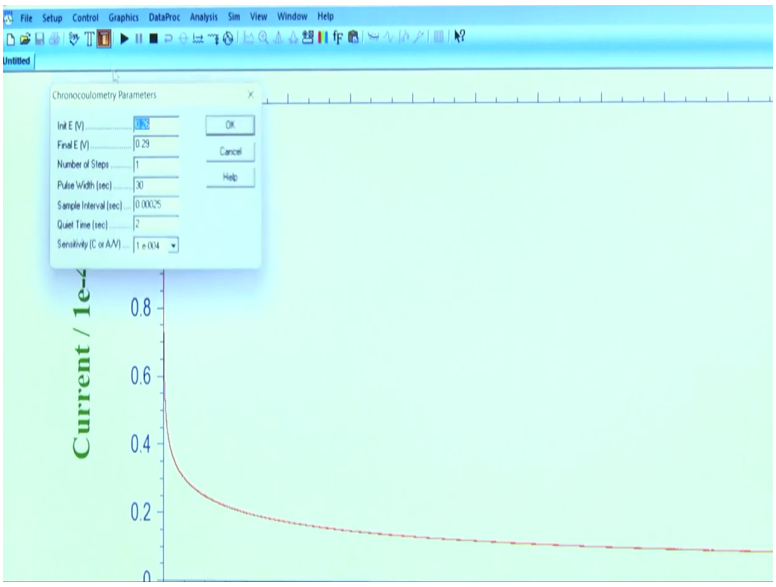

We are going to set the parameters as 0.26 again initial voltage, 0.29 final voltage which will be running for a pulse width of 30 seconds. Now, this technique it is the integration of the chronoamperometry.

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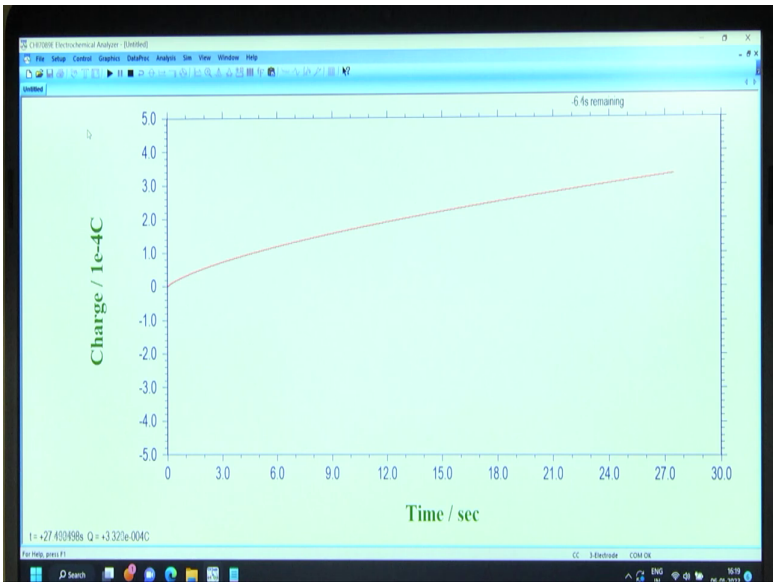

And you will see a steep increase in the charge value over time. And it keeps increasing as the time progresses.

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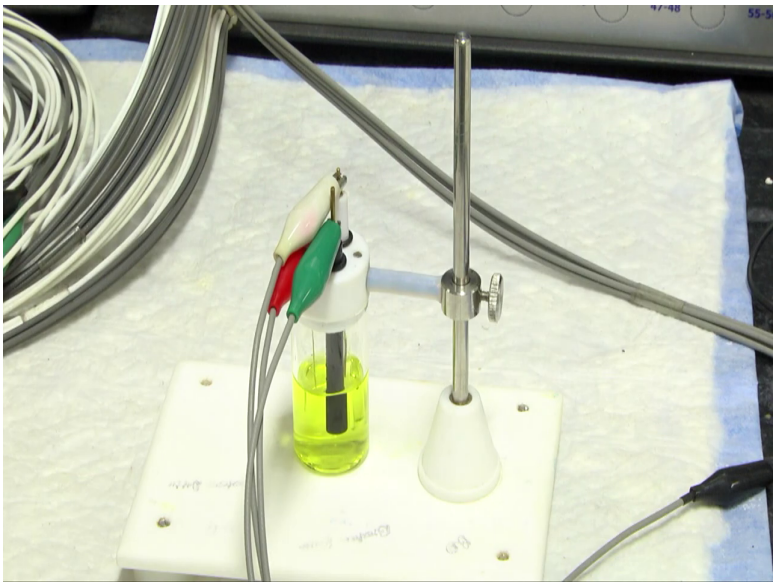

For the technique of electrochemical impedance spectroscopy, we have prepared a mixture of ferriferrocyanide solution which contains equal amounts of Fe 3 plus and Fe 2 plus for the reaction mechanism. For the technique of electrochemical impedance spectroscopy, we have prepared a solution mixture which contains 5 milli molar of potassium ferrocyanide and 5 millimolar of potassium ferricyanide in 0.1 molar KCl mixture.

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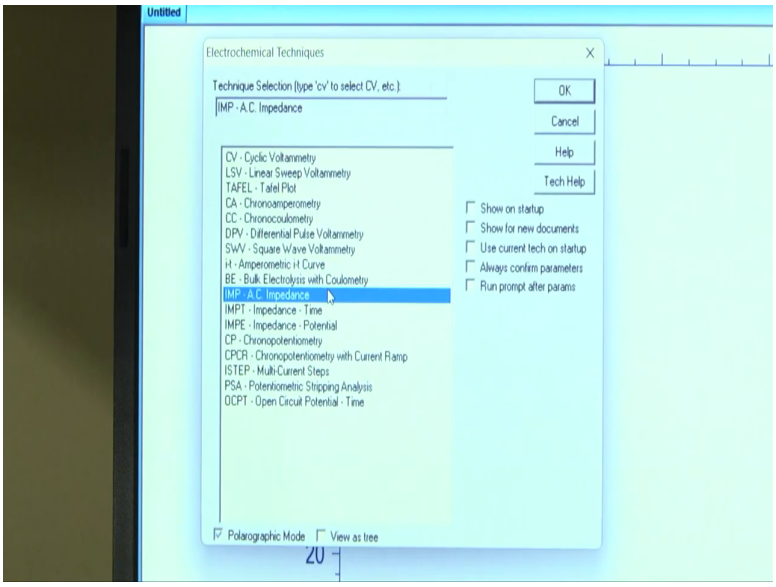

In as far as the electrochemical impedance spectroscopy is concerned, we shall be going for the techniques and performing the experiment under AC impedance spectroscopy.

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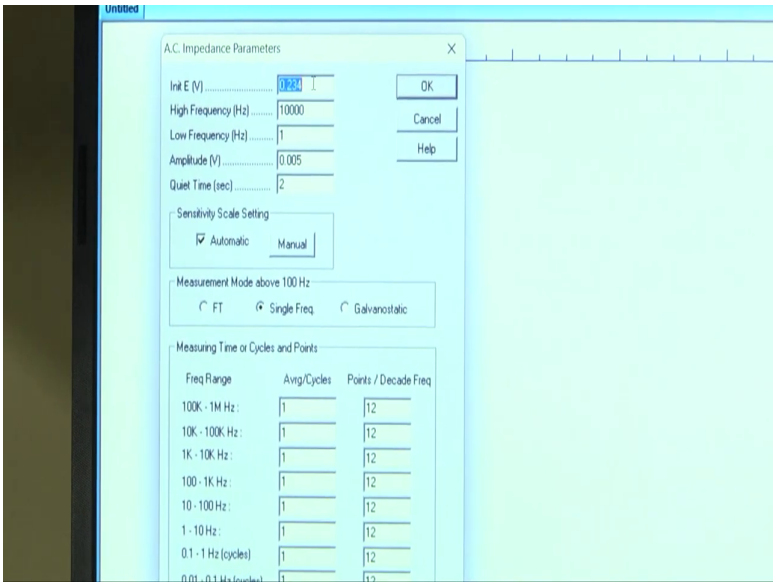

For the parameters we will be giving an initial potential of 0.2345 which corresponds to the E half value from the one that we have obtained from cyclic voltammogram with. Now, the High Frequency is 10 power 4 hertz and the Low Frequency is 1 hertz. We shall be performing the experiments at an amplitude of 5 milli volts for a quiet time of 2 seconds.

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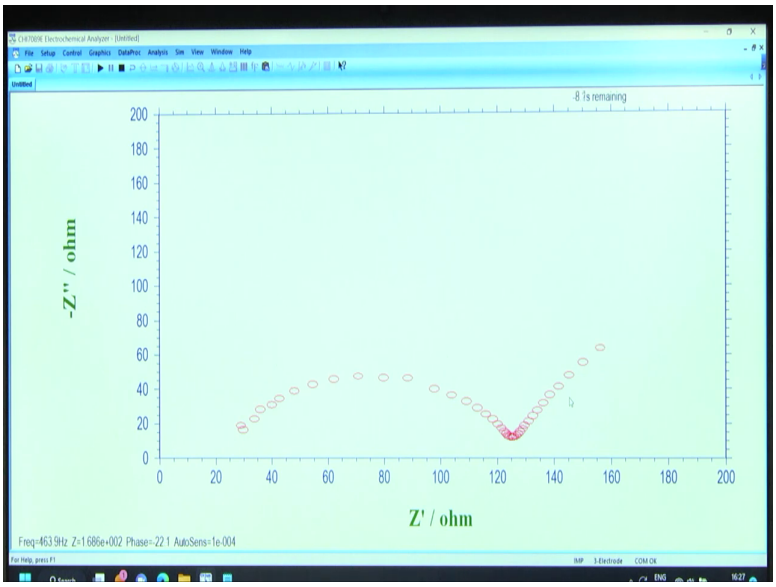

So, as you can see here this graph that you are seeing here is a Nyquist plot where it contains in the y axis the imaginary component and in the x axis it contains the real component.

Now, the mechanism that essentially takes place in an electrochemical cell is an electrical double layer arrangement where we will be having effects of both charge transfer and capacitance because of the capacitance double layer. Now, you get a characteristic semicircular arrangement as you can see where it starts from here and you get a semicircle at the end.

Now, the difference between the x axis values corresponds to the charge transfer resistance associated with the electron transfer across the double layer. Moreover, the value of this x

axis indicates the solution resistance of our electrolyte and the value of the other end indicates that till then we have the charge transfer resistance.

So, we will have to subtract the value of the right most end and the value of the left most end to get the charge transfer resistance. Following this you can see a steep linear increase in the graph along the y axis indicating the effects of Warburg diffusion or mass transfer effects. So, this is the overall Nyquist spectrum, which talks about the electrode arrangement.

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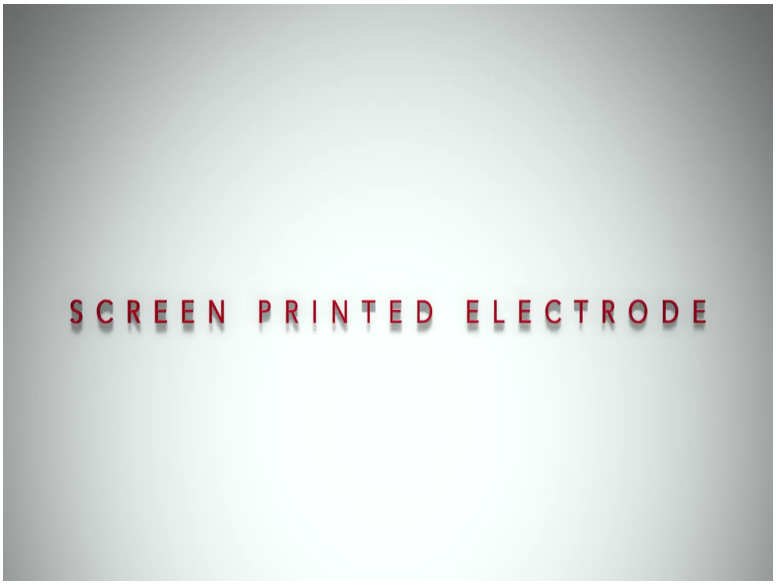

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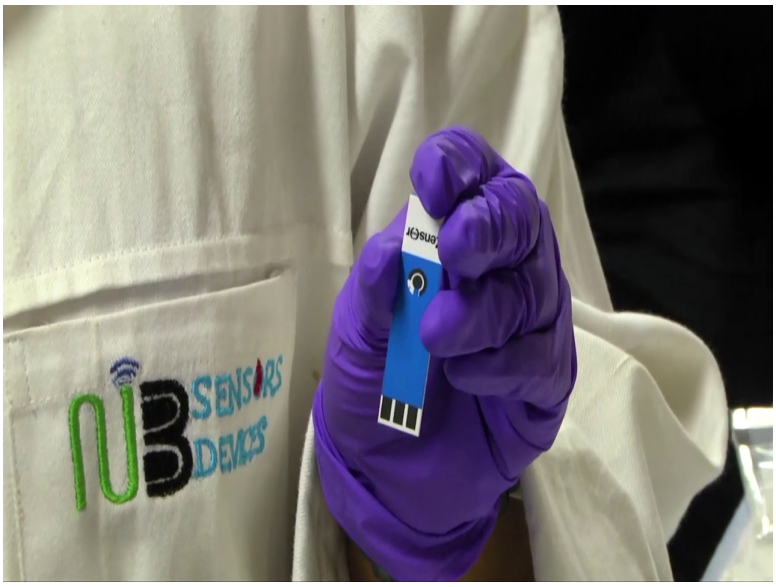

So, this is another kind of electrode which is a screen printed electrode. Here it is consist of all the three electrodes being printed. So, the circular part which is present at the center is the working electrode followed by the larger part which is semicircular in type and this one is known as the counter electrode and the small dotted portion that we are seeing of some gray color is the reference electrode. So, this is the complete setup of the SPE.