Nanobio Technology Enabled Point-of-Care Devices

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Lecture - 40
Lab Demonstration - 3

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Dear all, as you know the different kind of devices, I am going to show you in this Lab course. So, I taught you many devices in this class. So, now let us see the actual how they looks like. See at the very beginning at this class I showed you the glucometer. See this is the glucometer and this is the glucometer chip. So, as you know in this glucometer here, we will drop the sample, then we will insert this one in the glucometer and we will see the reading here.

Why it is not showing now? Because we will drop the blood sample so, just for demonstrations it is looks like this. This device this is just a portable electronic device. It is it can measure the data. So, in this course in the lab class my students already showed you how to design this kind of portable device. So, this is just commercialized this version. And now let us come how we can device similar kind of thing from the lab.

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See, this is commercially available 3 electrode system painted shape you know. So, this center side is the working electrode, this semicircle black one is a counter electrode and a small this semicircle is the reference electrode. So, this is commercially available. Similar kind of electrode we can design for different kind of disease diagnosis. So, not only like the glucose different kind of other analyte can be detected that I taught it in the class.

So, let us show you this all the electrode how we can use in the lab setting for fabrications of the different material. So, last class we showed you the synthesis of nanoparticle. So, we can dropcast here. So, this material suppose you synthesize some gold nanoparticles or something else that you can drop here.

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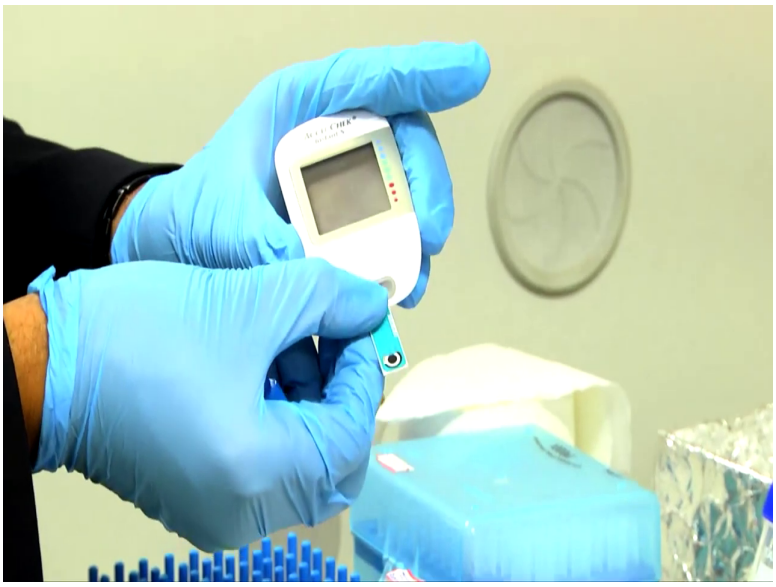

Then we can insert instead of this chip we can insert this chip here same technology instead of glucose you can detect some another analyte. You understand the technology here right very simple basic things. Now, if it is a cancer detection chip it can detect cancer this is the main basic principle, ok.

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Another commercially available very recently this is very important CoviSelf commercially available that can detect COVID. You can see I think I taught you the colorimetric sensors also, right.

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So, we bought this one from the market and as I told you similar kind of things you can also design. See here I taught you already this kind of colorimetric sensor like pregnancy test kit I taught you. By you can drop the sample here. Then this is the microfluidic paper based device. So, it will flow through this paper then see there is a two channel one is the test another is the control.

So, if you have the COVID virus then it will show the test zone color and control zone also. And if you do not have then control zone will show the color test shown, no. So, that principle I taught right. So, you see this is the actually device. So, it is available in the market as I taught you the basic principle you also can design now not only the for COVID, which it is already in the market you can think about something other also device, right.

And you can bring in the market you can start your startup right I told I told you already. So, let us think from the lab itself now you are ready for this making a new startup for your own, ok.

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Something else like I taught you many times like indium, tin oxide coated glass electrode there we can use many sensors. See this is the actually very simple glass chip. See it is very cost effective very simple you can see here right and its very robust see, I am just touching this surface also its very robust this chip.

After cleaning that I taught you know while you will deposit the material that time you should not touch like this. But now I can touch also after cleaning the surface then you can drop your

all the material here then you can use for the sensor development. Many times, I use the indium tin oxide coated glass right this is actually the chip this is very simple and robust chip.

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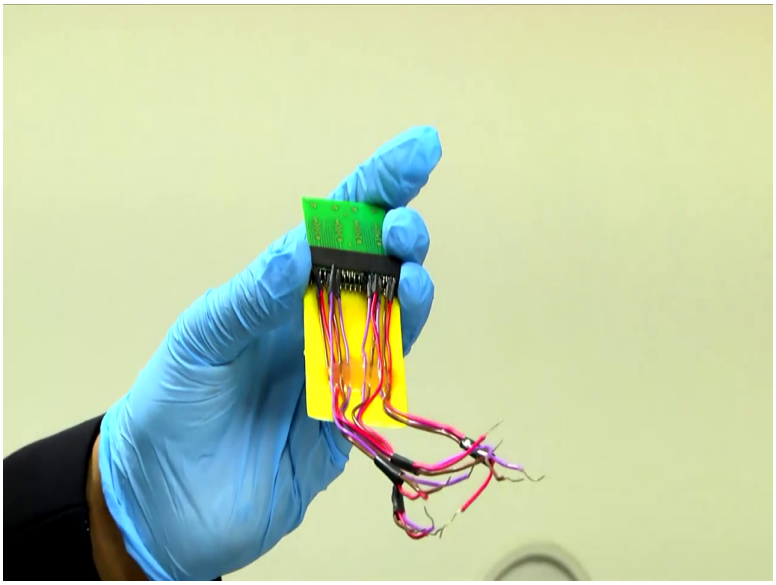

And printed circuit board many times I told you know PCB based biosensor see this is the this kind of chip already available right in the electronics if you open any electronics any kind of instrument. If you can see this kind of board this is the printed circuit board. See here 1, 2, 3, 4 here multiplex detection you can go. So, here one detection here another here another here another so, 4th channel are there.

See here is the connections here is the another like circuit connector here you can connect it. And then this shown actually we can connect to your portable device something like this say portable device it can be connected. See this is the very handy simple right that why I told

mobile phone base. It is right now it is separate, but same thing you can insert not like here to your mobile phone.

Then inside the mobile when instead of using this one then your mobile phone will be your display, right you can see the data on your mobile phone right, it is clear. Now, let us again come to the very basic lab setup where I will show you to develop the signal amplifications. See I taught you signal amplification strategy like electrochemical chemical-chemical redox cycling for getting high signal to background ratio.

Why we need high signal to background ratio? To detect the disease very early stage; so, let us see the for example, electrochemical chemical-chemical redox cycling how it works. Now, I will show you one example of ECC redox cycling and you can understand how you can amplify the signal that technology you can now use the chip and get the new device and you can think about your own startup, ok students now I am showing you a very basic electrochemical chemical redox cycling how to take.

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So, today I will show you the ECC redox cycling with ruthenium, hydroquinone and T sep these three combination based. So, let us see how the signal amplifying based on this ECC redox cycling.

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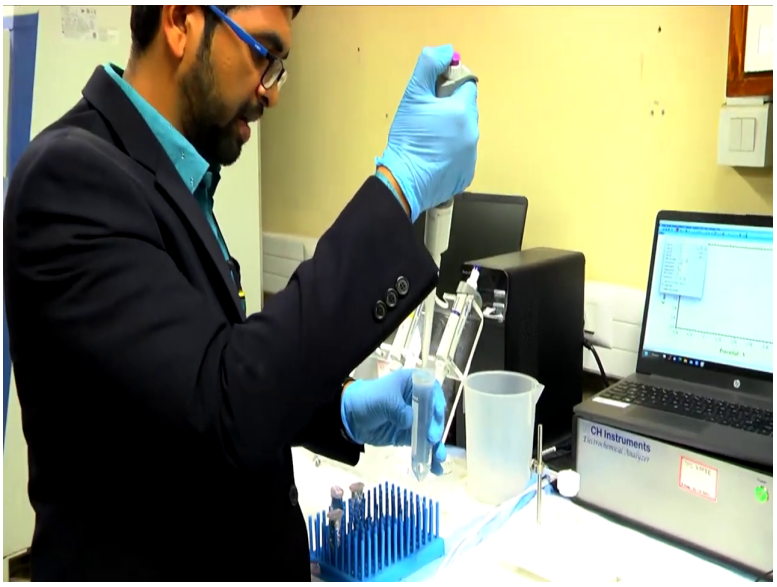

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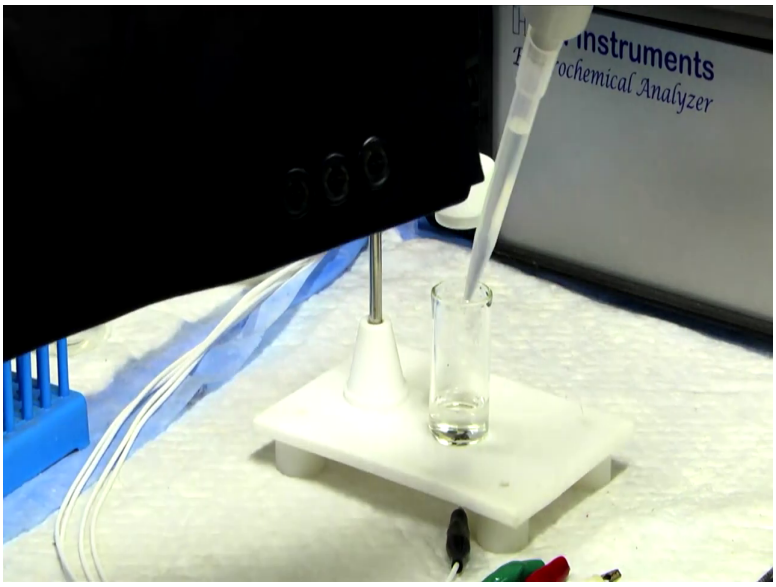

So, I will take first 1 millimolar ruthenium hexamine say this is just a buffer. So, here I am taking just PBS phosphate buffer saline and I will dilute this ruthenium salt already prepared see here I covered the ruthenium salt with aluminum foil because this is light sensitive.

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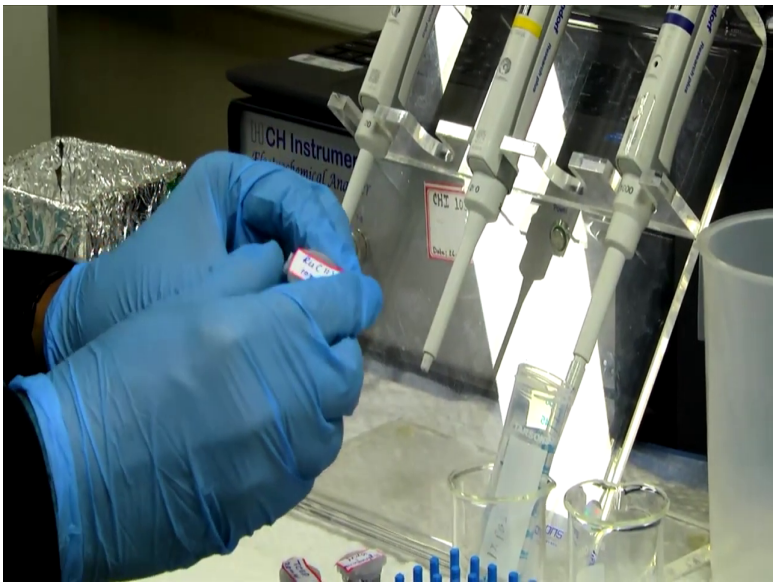

So, here we prepare 10 millimolar ruthenium salt. What is the ruthenium salt? This is ruthenium hexamine here ruthenium oxidation state is 2. So, it is reduced from ruthenium. So, it is 10 millimolar. So, I will take 1 millimolar here for getting a cyclic voltammogram so, here 100 micro liter. So, I will take from here 100 micro liter of no. So, at first let us take the 1 ml.

So, I will take 1 ml ruthenium hexamine 2 salt. So, I am now mixing properly. So, see here I took 9 ml phosphate buffer saline buffer and 1 ml ruthenium 2 salt that is 10 millimolar. So, here you can understand now total volume is a 10 ml it means a ruthenium 2 salt concentration is now become 10 times less means 1 millimolar, ok. Now, let us check the cyclic voltammogram in this solutions.

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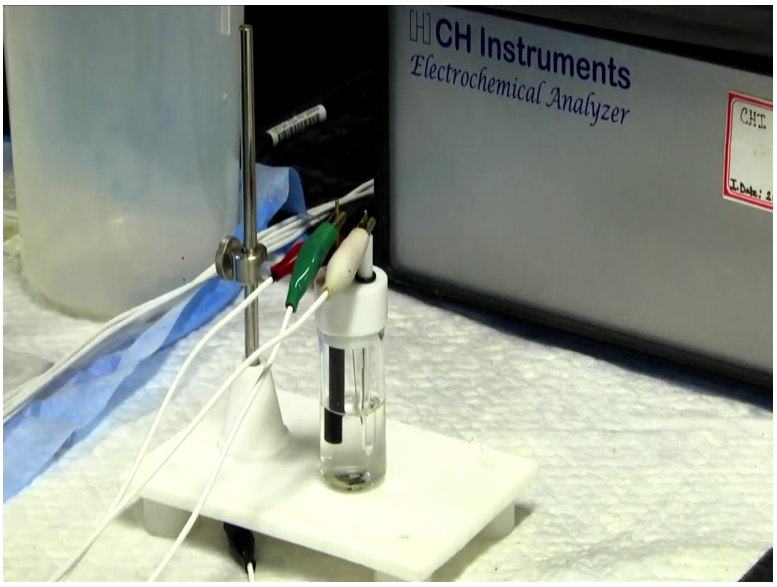

So, where I will take? I will take on a simple glassy carbon electrode that is already cleaned that we showed you last class how to clean the surface. Then after cleaning so, this is the working electrode I am going to dip into the solutions here silver-silver chloride reference electrode.

So, we will measure the potential difference based on silver-silver chloride here platinum counter electrode, ok. So, your 3 electrode set up is ready to take the cyclic voltammogram ruthenium hexamine. See I am now we have to dip inside this solution. So, 3 electrodes should be dip properly.

Now, let us connect. So, like this way we are actually handling the electrochemical workstations inside a lab this is the very basic set up of 3 electrode system. So, this one for the working electrode, this red one is for counter electrode I am connecting and this white one

for reference electrode. So, it means say they all electrodes are dipped and they are connected each other. So, now, we are ready to take the cyclic voltammogram.

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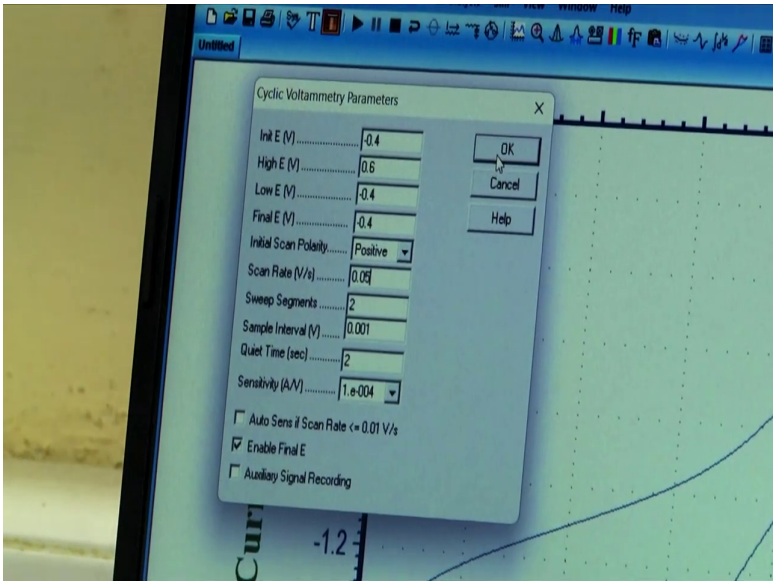

So, here is the ruthenium hexamine say it means the oxidation state is the 2. So, I have to start from the left side negative to right side positive means it would first oxidize 2 to 3 and then 3 to 2. So, that is why I fix the parameter initial parameter is minus 0.4 then I fix 0.6 as a positive because I know the ruthenium hexamine oxidation split with so, in between. So, accordingly we have to fix the parameter, ok.

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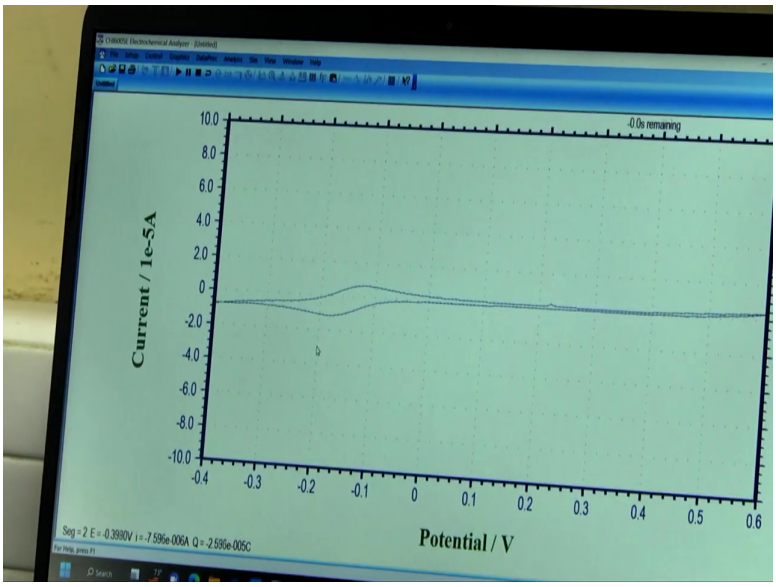

Now, let us run the cyclic voltammetry in this solution. So, at the beginning you can see quite time. So, we put some time then the cyclic voltammetry will start you see. So, it is oxidized here and further just see just you will get the just some lines means it will not show any of any peak here. Why? Because ruthenium property is this oxidation will show only this region around minus 0.1 here you will show the oxidation and then you will see the see the reductions here, ok.

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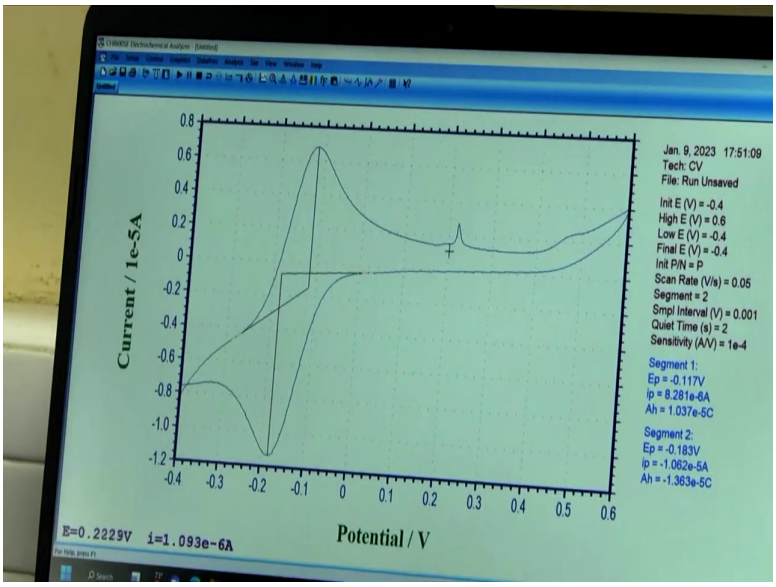

So, see so, this is just for any kind of see this is also good that you got some another small hump this is just for I mean if you have any impurity in your surface or your solutions because this electrochemical sensor is very very sensitive. So, you may get this kind of peak. So, why maybe some kind of impurity if you have there it is good that you can see something, but if there is no impurity your surface is properly clean your solution is very very clean very pure then you will get only this peak and then reductions peak.

And see they are very much reversible this oxidations peak and reductions peak they are very close to each other, ok. So, at the very beginning for the ECC redox cycling that I taught you have to take first the electrochemical signal for the background. So, your ruthenium hexamine your basic background, but you are taking the three combination right ruthenium hexamine hydroquinone and T-sep.

So, what is your background then ruthenium hexamine and T-sep is your background. So, let us take then T-sep plus ruthenium hexamine how much they are contributing on the background. So, let us check the another cyclic voltammetry solution mixture will be ruthenium hexamine 2 and T-sep.

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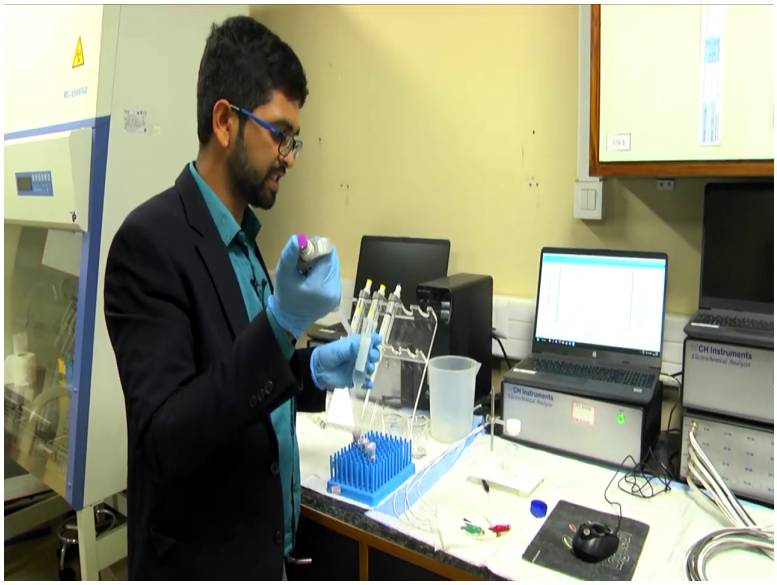

So, now I will take ruthenium hexamine 2 this solution and T-sep. So, as I told you how to make this actual different concentrations of this solution, right. I told you that I made 10 times higher the concentration ruthenium hexamine 10 millimolar T-sep 20 millimolar. So, they, but I am going to take 10 times less.

So, how will dilute the solutions let us learn this one. So, I will take 8 ml buffer that is phosphate buffer saline inside the electrochemical cell. So, here 4 ml and then another 4 ml

phosphate buffer saline ok, then I will take 1 ml ruthenium hexamine this is ruthenium hexamine 1 ml.

So, but always just keep tight remember while you will take from a stock solution just do not forget to shake it properly just properly you should make the homogeneous solution. So, take 1 ml ruthenium hexamine and put in the electrochemical cell as pipetting few times to make the homogeneous.

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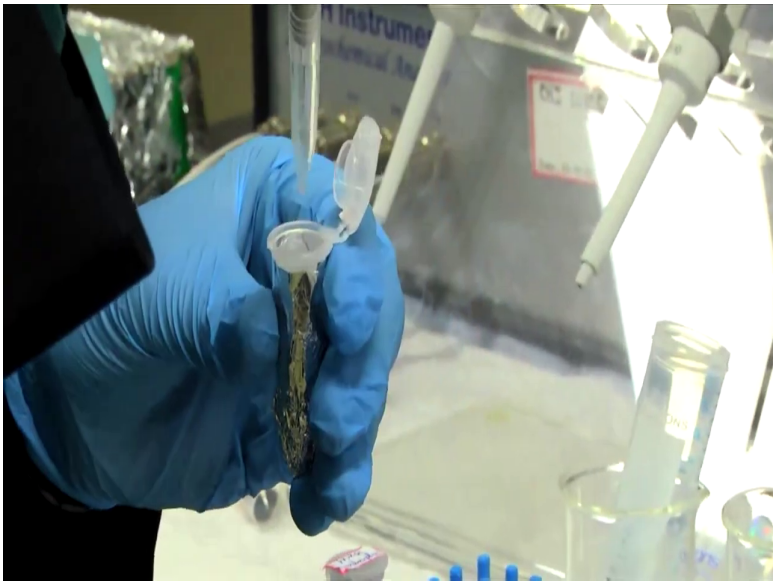

And then I will take T-sep that is a reducing agent unit because this ruthenium hexamine T-sep is responsible for your the background current of your ECC rate of cycling right, you just remember. So, what you will do just shake it properly and take 1 ml from the stock solution and put inside the electrochemical cell, ok.

Now what I will do I will again we will make the homogeneous solution you have to pipette it pipette out for few times. So, that your solution become homogeneous see now the final volume is the 10 ml, 8 ml buffer 1 ml ruthenium hexamine and 1 ml T-sep. So, final concentration now ruthenium hexamine become 1 millimolar and T-sep become the 2 millimolar they become 10 times less.

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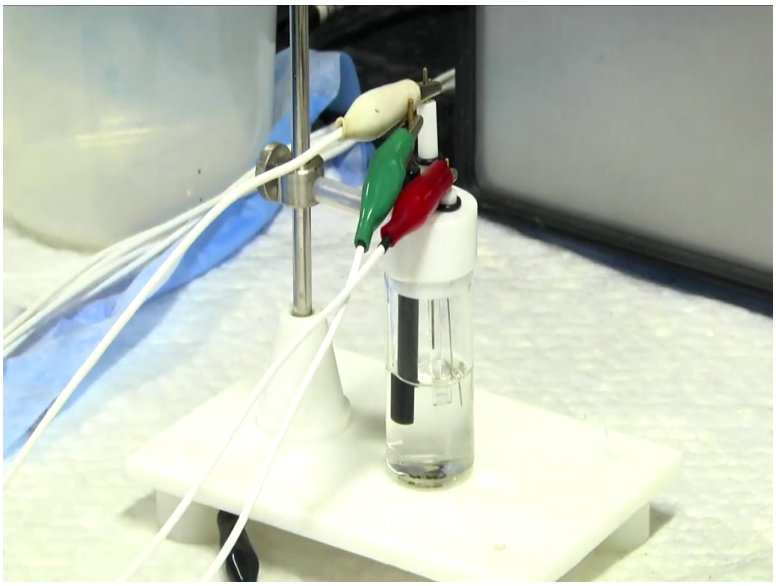

So, again I will put that I already made this 3 electrode setup so, working electrode. So, let us put this 3 electrode system back to this cell. So, let us dip inside this solution. So, now, this solution is responsible for your background current, right. So, in the same potential window you can see here, ok. So, this is ruthenium hexamine CV only.

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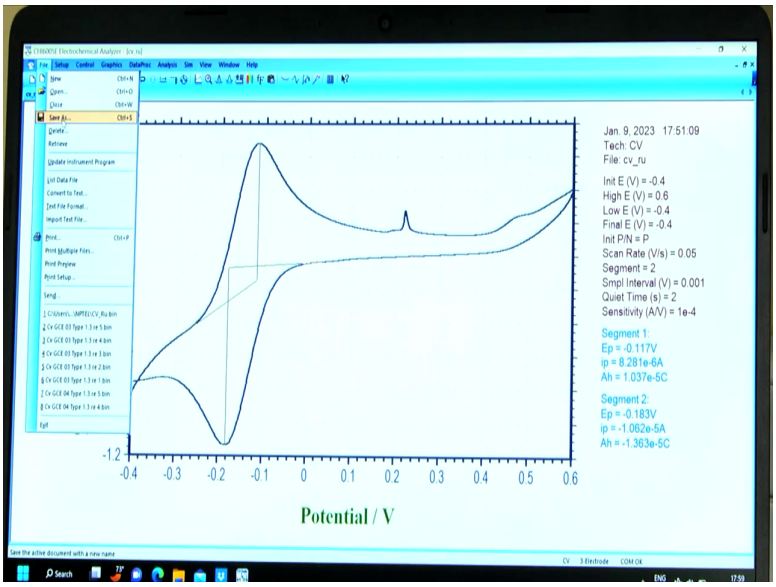

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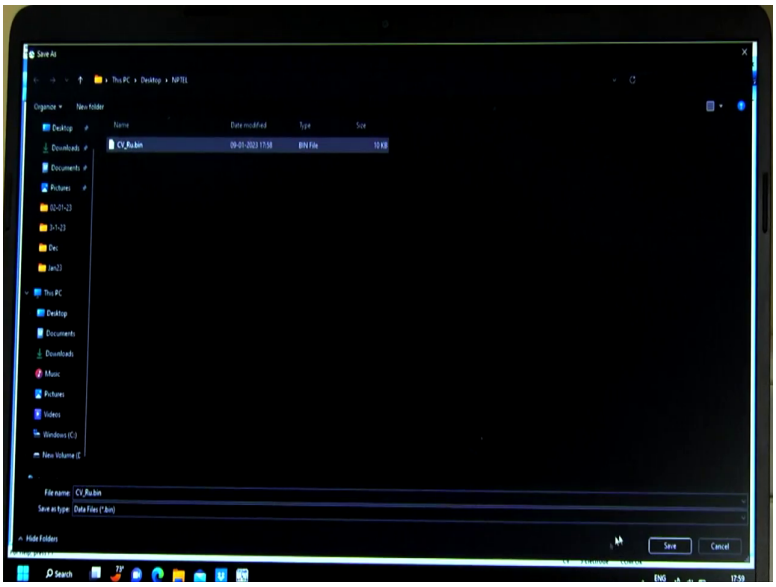

So, I save this data in my computer CV ruthenium. So, it is safe already now I will take the background of this ECC redox cycling. So, background means ruthenium hexamine and T-sep.

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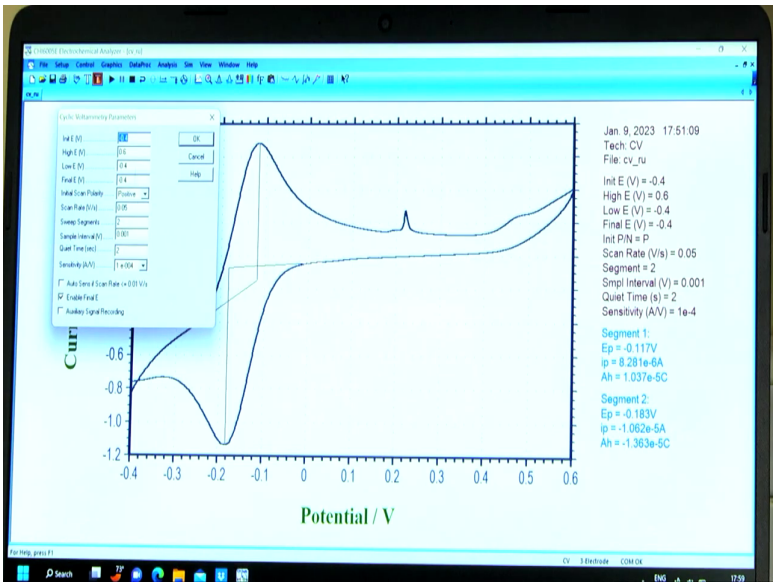

So, this solution is ready and we will use the same parameter we will not change the parameter otherwise you cannot compare you know. So, in the same potential window let us see the cyclic voltammetry in this mixture, ok.

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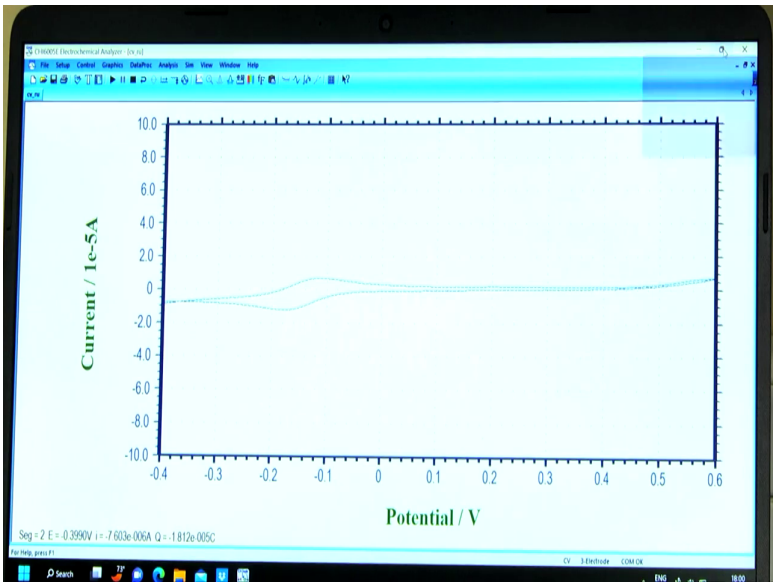

Then we will compare with this CV and only ruthenium hexamine. So, as you know if you want to design a very good ECC redox cycling verses biosensor. So, you are reducing agent should not react with your electron mediator, right. So, that is why I want to compare ruthenium hexamine CV and ruthenium hexamine plus T-sep CV. And you will see there is not much change that I taught let us see practically how much change you will get, ok.

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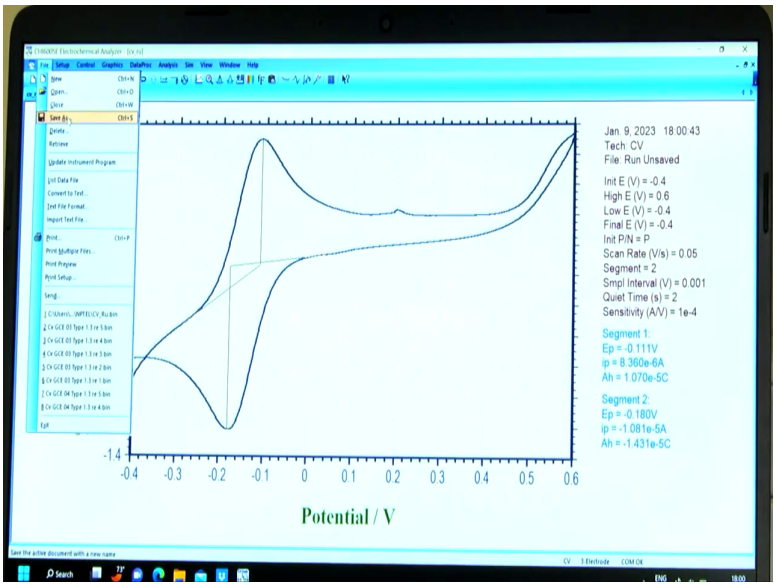

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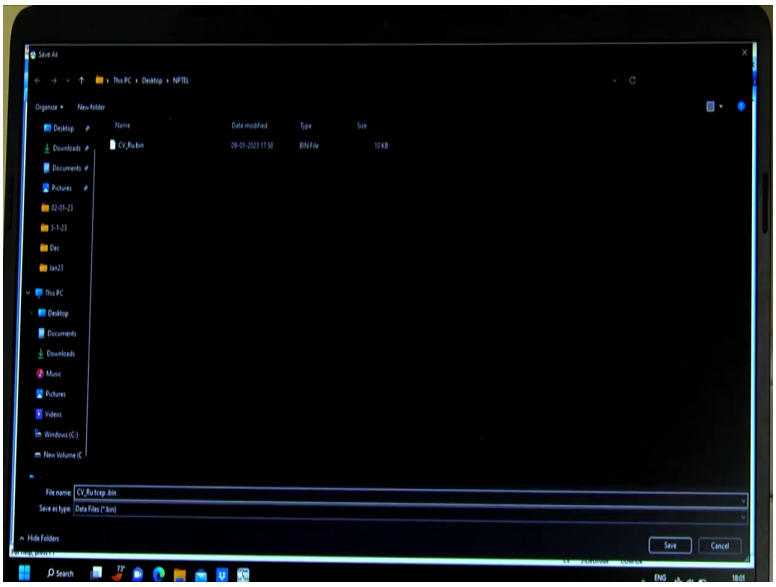

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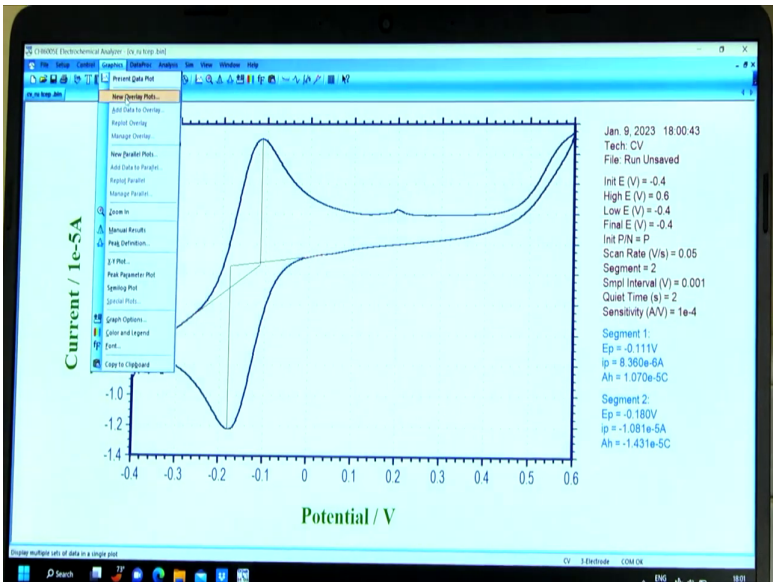

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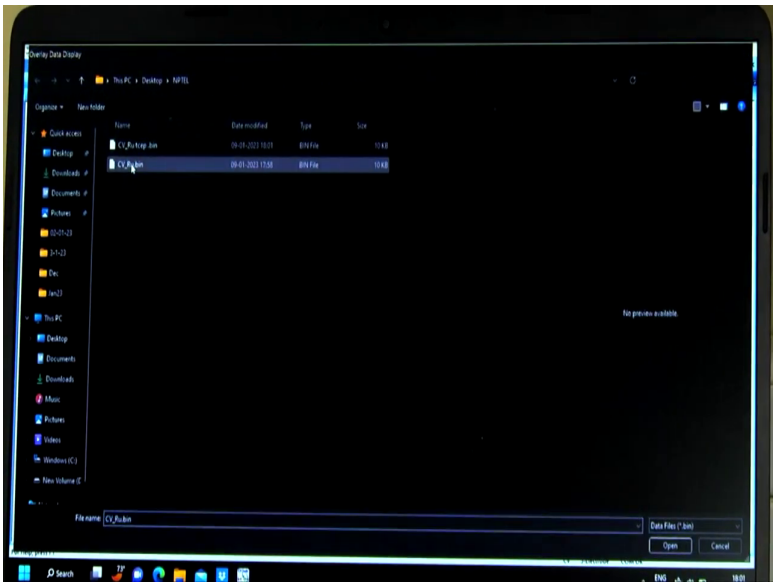

So, this CV done with the T-sep that is actually your background so, let us Save it. So, this one ruthenium and T-sep so, we are saving it and let us come I will overlay this one graphics overlay only ruthenium.

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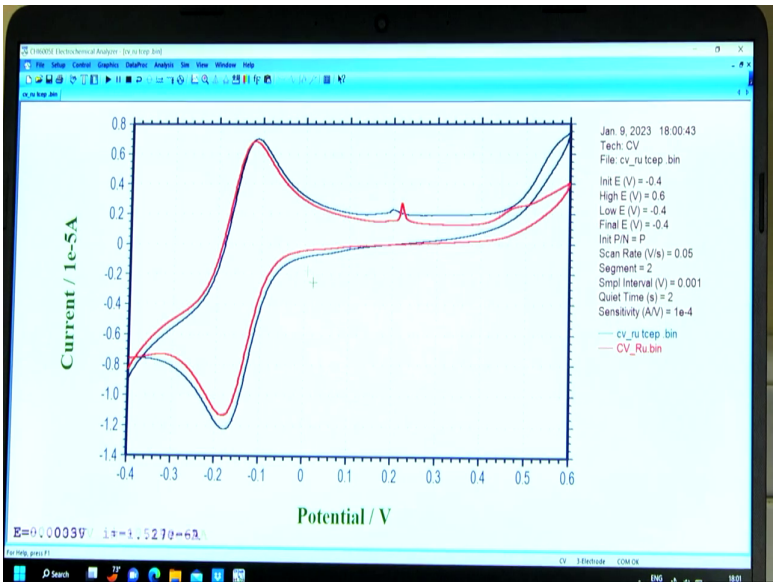

See, I will you can see let us change the color here ok, you can see here the two different cyclic voltammogram like one is just ruthenium and another this one I only ruthenium T-sep. So, there is not much change see almost they are similar. It means ruthenium hexamine and T-sep they are not reacting each other that is why you are not getting much difference.

Also, we can take like chrono calorimetry chronoamperometry as I told you to get the signal differences, but which potential will choose. So, as I taught you first, we will take the cyclic voltammetry then we will decide, which potential is good for chronoamperometry or chronocoulometric. See here oxidation complete after in this potential right minus 0.1.

So, we may take little higher where we will get the maximum oxidations or we may get the complete oxidations of the ruthenium right. So, we may choose 0.1 this potential, but the optimization potential I already thought. So, you have to go through different different

potential then you have to find out best potential for highest signal to background ratio. So, for example, now I will choose minus 0.1 for getting chronoamperometry.

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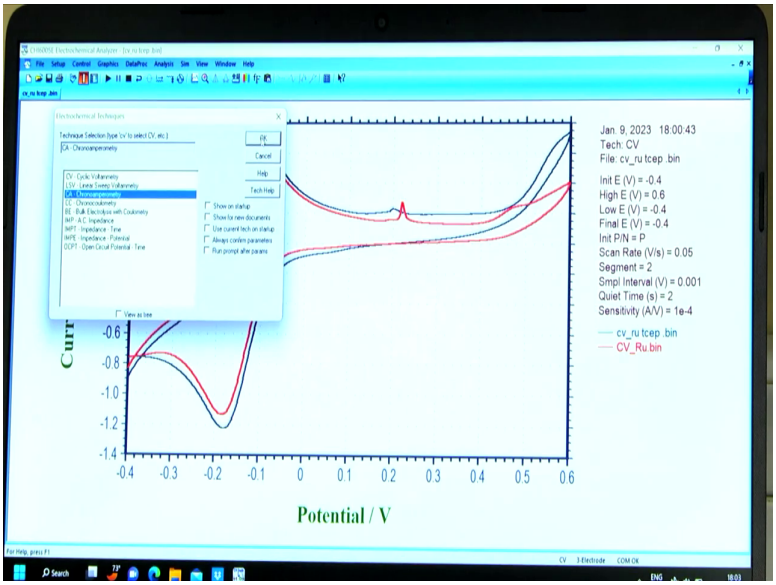

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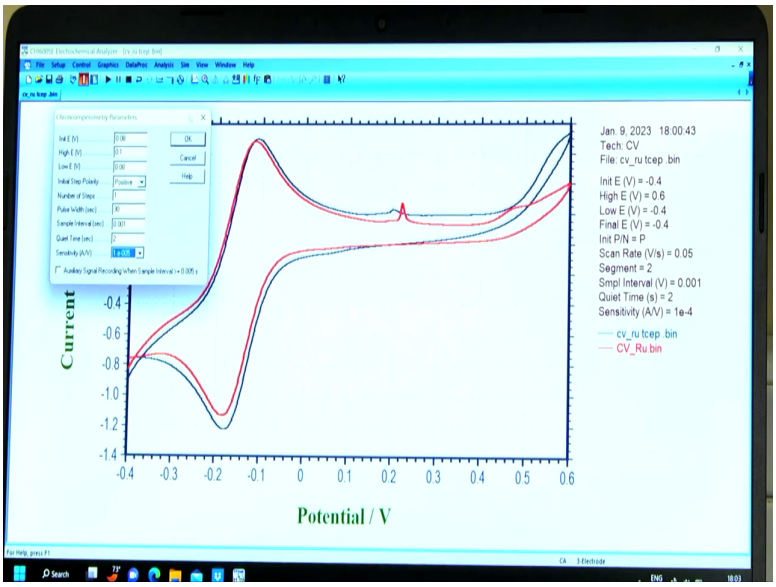

So, I will go to the chronoamperometry and 0.1 potential I will choose. So, your highest will be 0.1 and initial it can be 0.08 and we will oxidize this solution. So, let us make this positive. So, total step we will take 1 and we will take just for 30 second and the sensitivity means we are using, ok.

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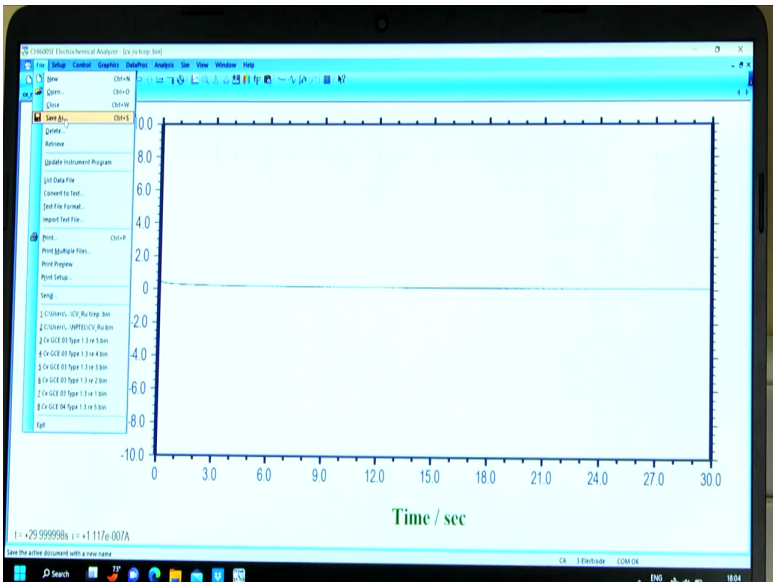

So, let us see now we are checking in the same solutions how the chronoamperometry it looks like then we will compare this background data with the signal data. Now you are getting the background data at 0.1 volt then we will compare at the same potential with your signal data that is ruthenium T sep and hydroquinone that I am going to take after this measurement, ok.

So, this is your background data ready. So, I will Save it for your comparison. So, this is chronoamperometry and this is your background, ok. Now, let us go back again the cyclic-voltammetry with actual signal data that is ruthenium hexamine T-sep and hydroquinone, ok.

Now again I will prepare the solutions that is the response that is the responsible for the signal. So, similar way I will take first the buffer. So, first I will take 7 ml phosphate of arsaline then 1 ml ruthenium hexamine 1 ml T-sep and 1 ml hydroquinone. So, final your

mixture each compound will be diluted for 10 times, right. So, let us take. So, I am taking first phosphate buffer saline PBS 7 ml.

Now, ruthenium hexamine so, as I told let us mix it properly and then put 1 ml then 1 ml T-sep I am just pipetting in pipetting out for making homogeneous or you can shake it and then here again I took this is the T sep 1 then I am taking the hydroquinone solutions.

See again you can see here the all the solution I wrap with aluminium foil if you have any solution light sensitive then let us try to keep them stable by making by wrapping with aluminium foil so, that you can store your solution for longer time, now I am taking the hydroquinone.

So, I will make this solution again homogeneous. So, let us make so, that each solutions will homogeneously will be diluted and you can get the reproducible data. So, now, your solution ready I will dip all the electrodes back to cell working electrode, counter electrode and reference electrode, ok. So, your cell now ready to take the measurement for your signal data, ok.

Now I will take first cyclic voltammogram you see that I am using the same potential window. So, this is your background now take the signal data. So, you can see the same parameter I am using. So, let us take the cyclic voltammetry first inside this solution, ok.

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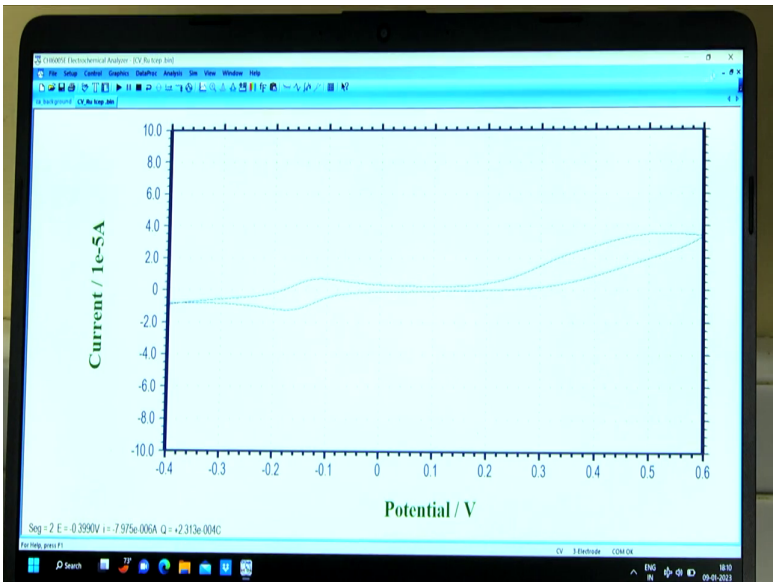

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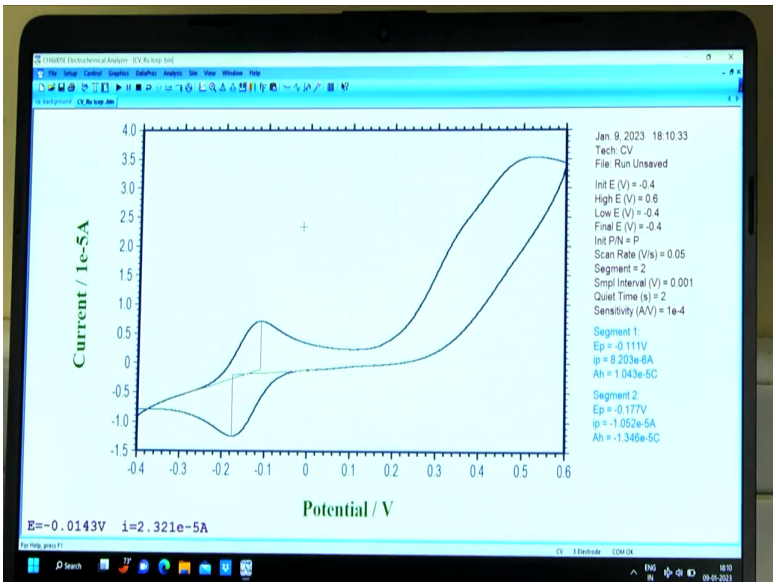

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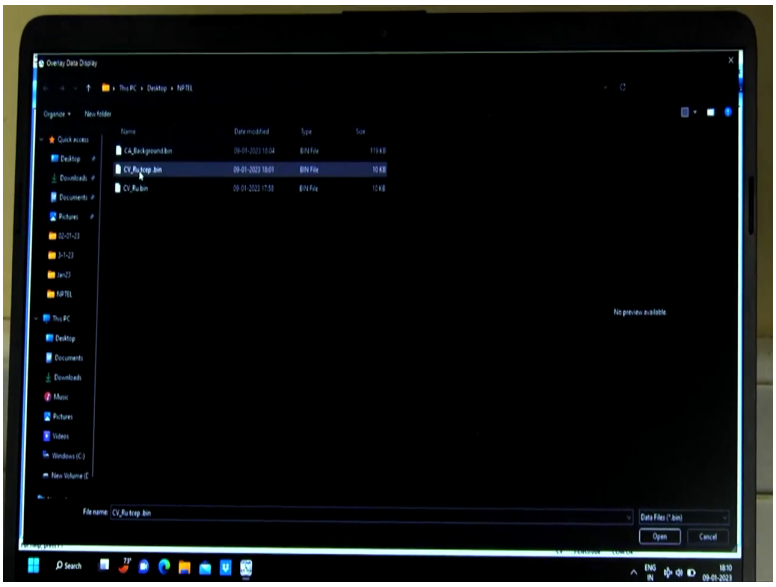

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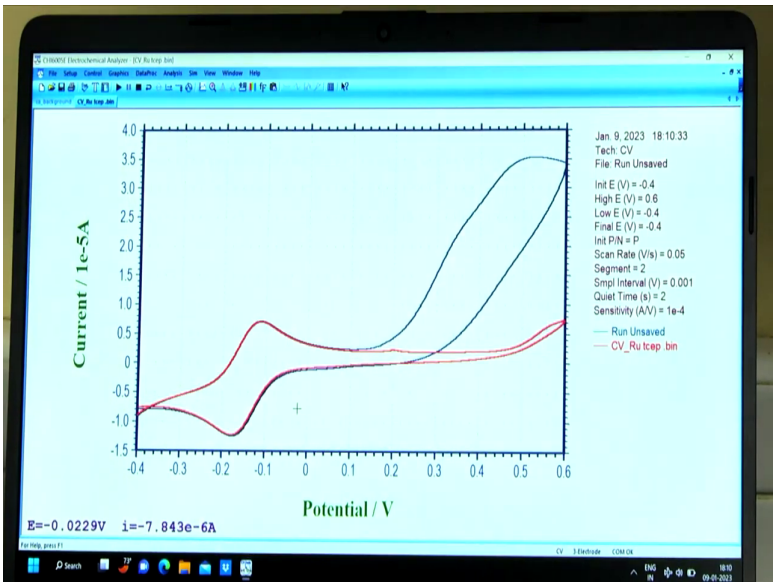

Your cyclic voltammetry ready, now I will compare and I will explain the distinguish peak. So, let us overlap. So, this is your signal let us compare with the background CV. So, ruthenium T sep you can see that here catalyst speeds come here this is due to the hydroquinone. And in the ruthenium side you can see the kind of the signal amplification not much this is because the three solution was not reacted properly.

So, why it is not reacted? Because ruthenium hexamine T-sep hydroquinone they should interact each other. So, we need some incubations. So, I just drop it and take it I took it immediately. So, let us incubate for some time and then I will take again the cyclic voltammetry then you can see the change. So, it means this three reactions was not completed. So, I will incubate it for 10 minutes then I will show you the difference of the cyclic voltammetry, ok.

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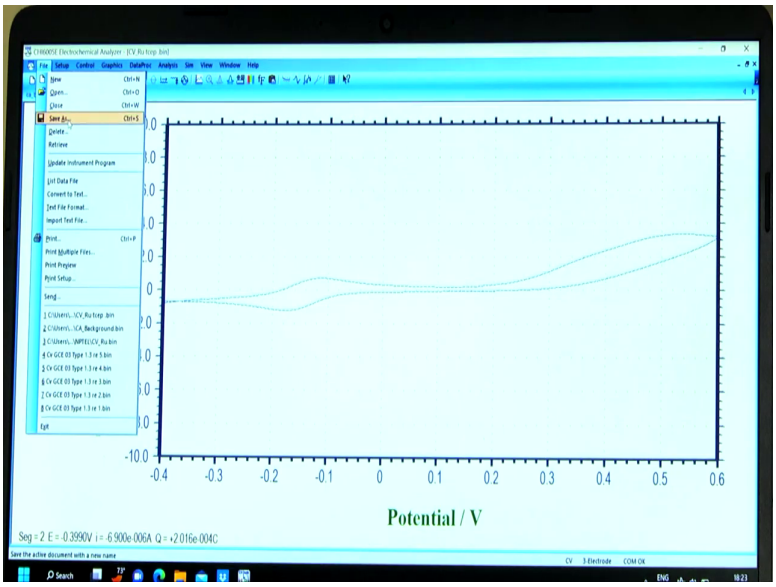

Your 10 minute incubation now already done let us take the cyclic voltammetry then I will compare after this incubated solutions and non incubated solutions background and signal value. And then I will take chronoamperometry then we will compare background and signal.

Then you can learn how we are comparing the background signal and your actual signal value. And if your scheme work then we will see the difference if your scheme does not work we will not see any difference between the signal and background. So, as I mentioned this is because of your hydroquinone and this is because of your ruthenium. So, let us Save this one this data. So, this is your CV for signal, ok.

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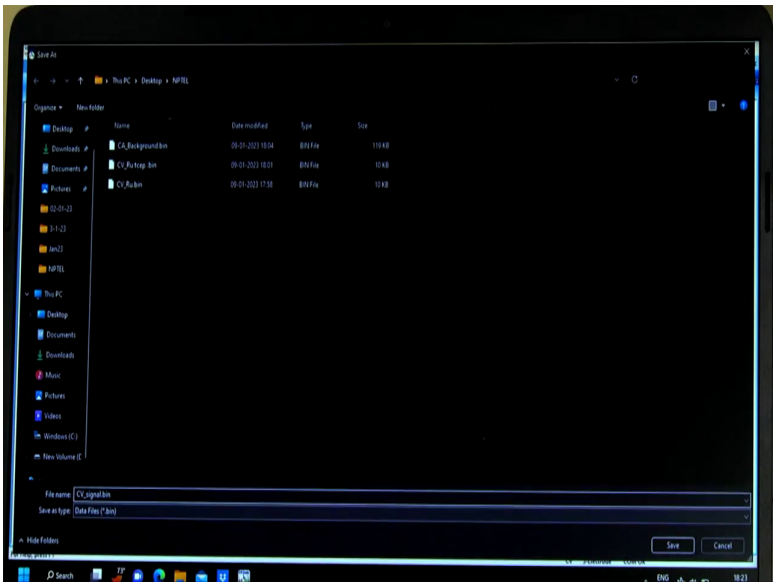

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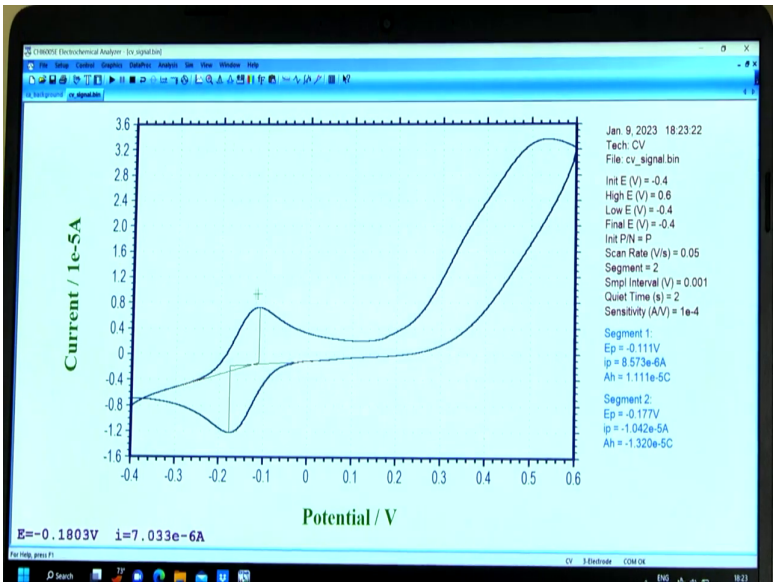

So, you see this is the two distinguished peak hydroquinone and your ruthenium. So, they are actually reacting each other that we can observe if we take the chronoamperometry. So, let us compare here just only your see.

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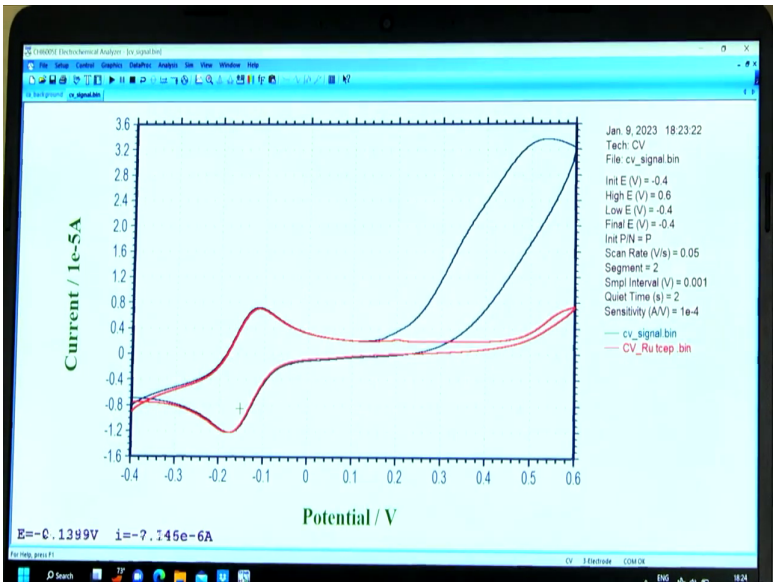

So, still see it means ok, you can see here is not that much difference your difference came actually this side. And we want to we want to check their reactions actually more than your ruthenium potentials and actually we have to find out which potential is based for this reaction. So, you can see if you take if you take the potential near the negative side there is not much amplifications.

So, you have to take the optimal potential near this window that is why optimizations of a ECC redox cycling is also very important. So, let us take that is why first chronoamperometry. So, I took the background in 0.1 then I will take the signal also at the 0.1. So, let us open your background its.

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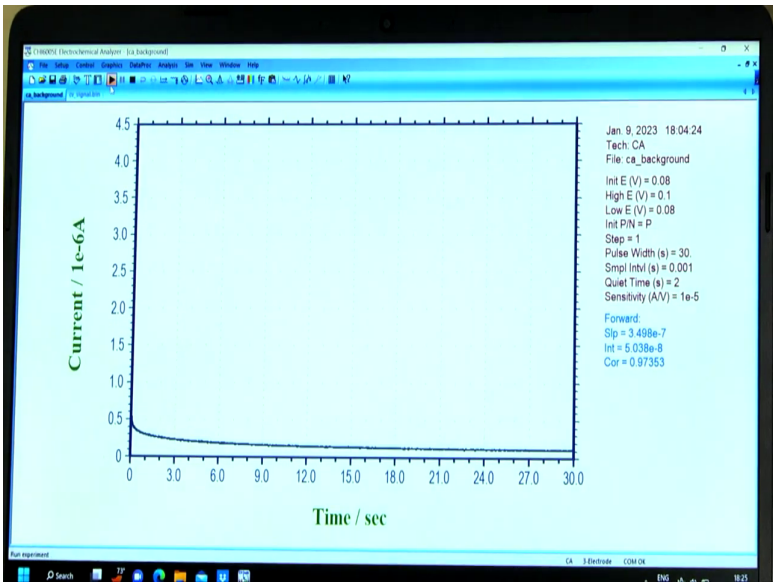

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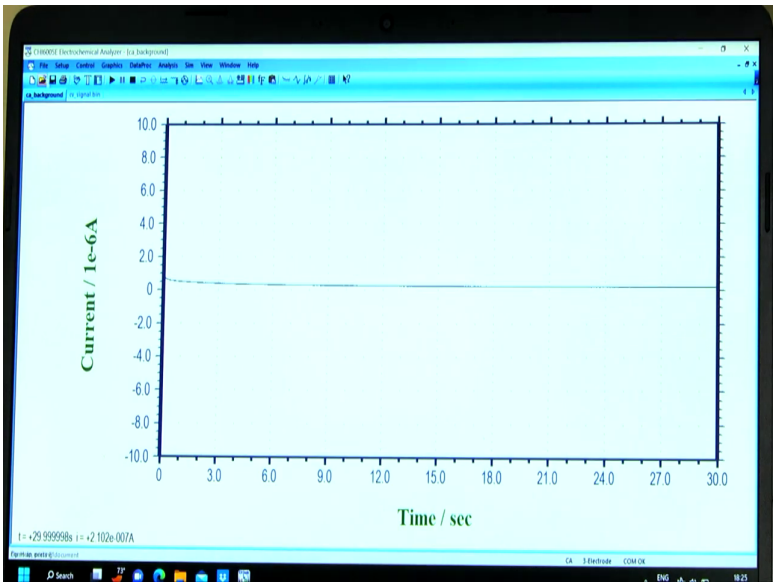

So, this is your CA of your background and we applied 0.1 volt and same potential I will apply signal let us see, ok. Your chronoamperometry done let us Save this data. So, this CA for your signal say.

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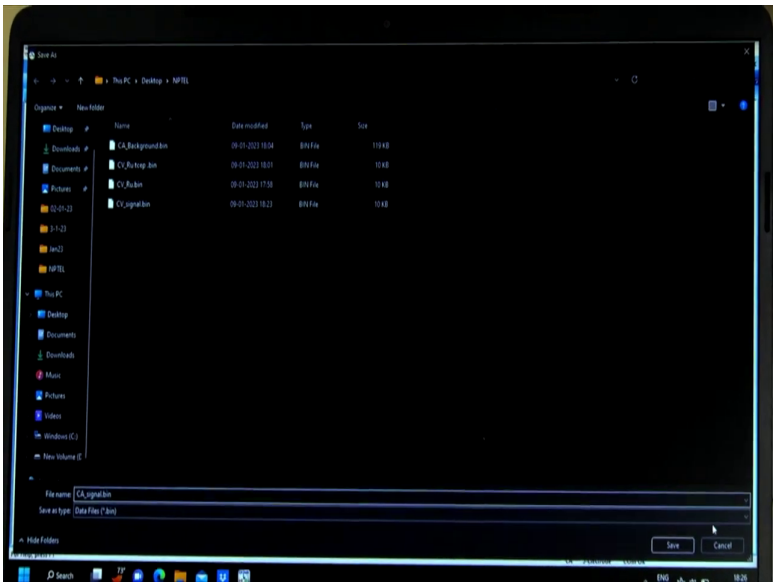

So, I will teach you today how we can convert this chronoamperometry to chronocoulometry we can convert these things also at the same time, but let us compare first the chronoamperometry with background and signal then again I will compare the background and signal with chrono coulometry also. So, let us overlay the data with the background data CA, ok.

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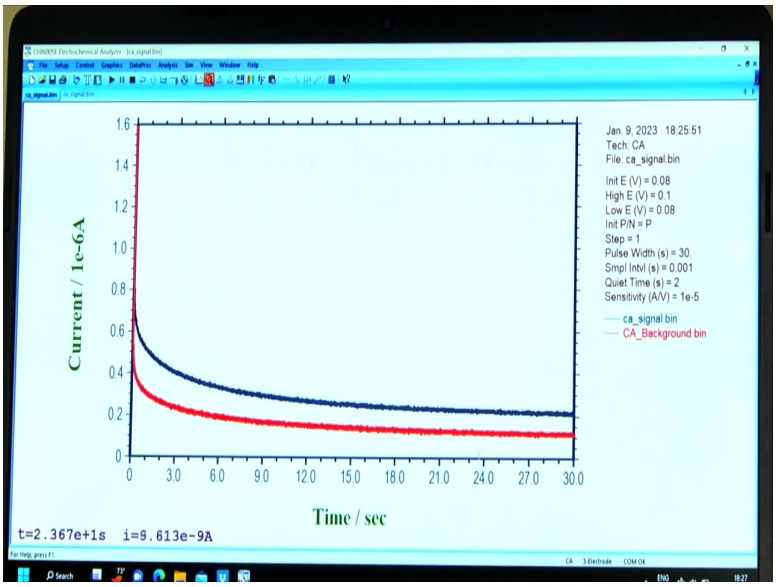

So, you can see there is a difference right. So, this is the signal data and this is the background data. So, if we see if you want to make if you zoom this ok, area. So, you can see actual the difference in the signal and background ok so, but one things now why I taught you the chronocoulometric also chronoamperometry case you are getting this much difference.

But you can convert it to the chronocoulometry and you will get the much better difference than the chronoamperometry because why we are getting the better difference because we are actually integrating the current right, in the chronocoulometry that is why you will get the much better difference let us show you here. So, your signal and background the difference is there means your scheme actually working.

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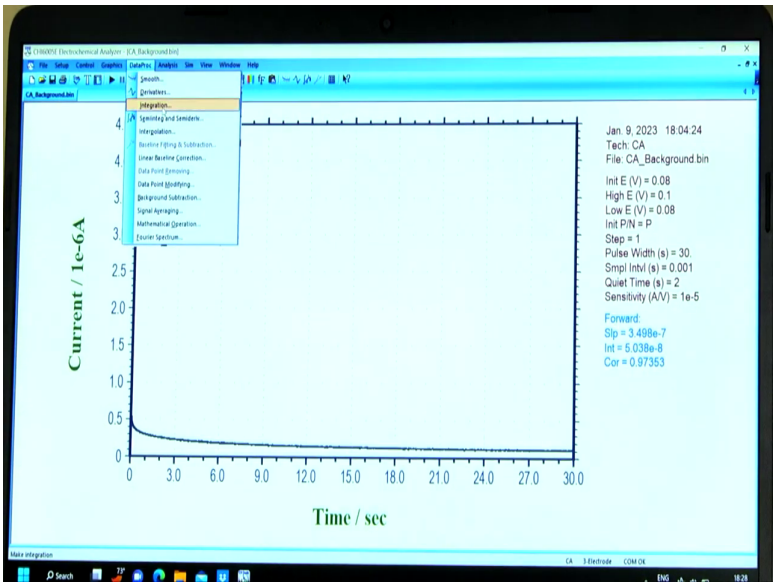

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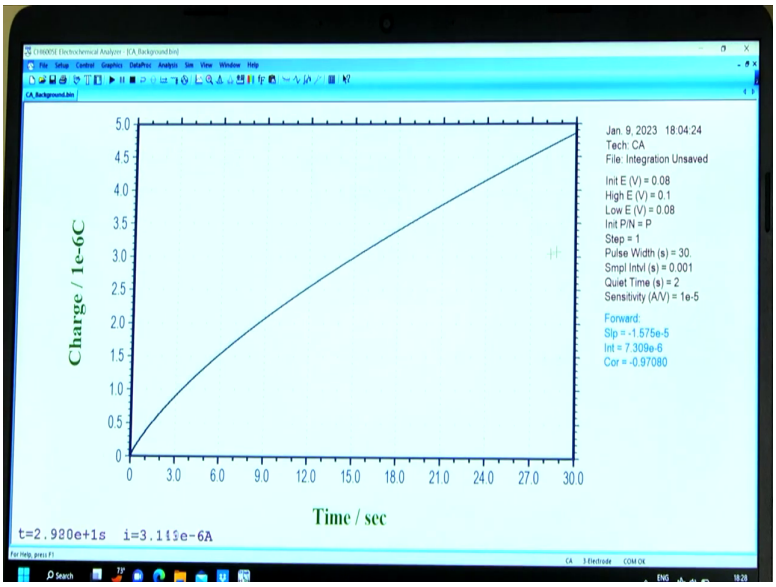

So, let us open your background signal CA of this then you can integrate it integration say here is the option is the integration you can integrate it and you will get it the chronocoulometry. So, that is why you do not need to take the separate the chronocoulometry and we can integrate the current of the chronocoulo amperogram and you will get the chronocoulogram.

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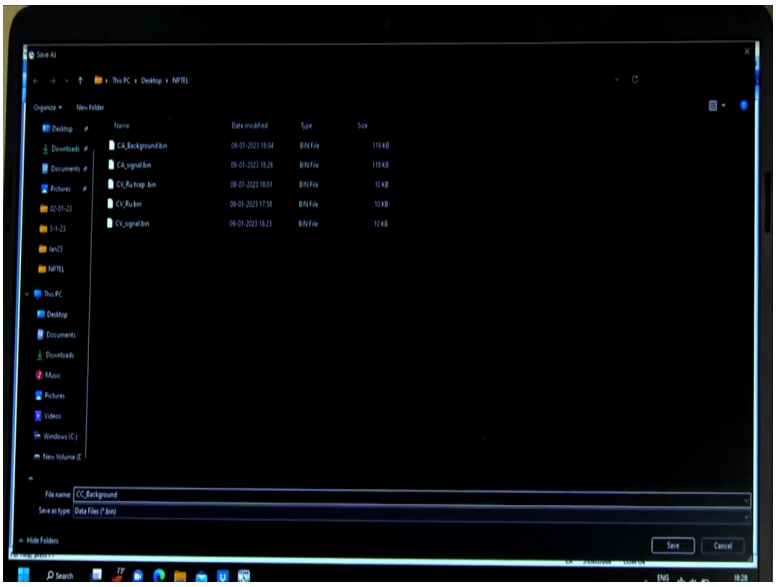

So, this is basically your background. So, let us Save this one CC Background, ok.

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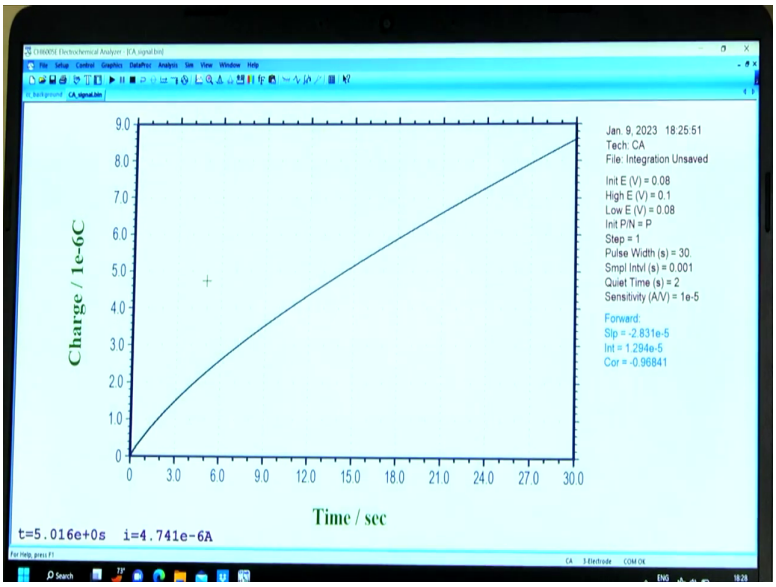

And now we will open the signal value of your chronoamperometry. So, this is your chronoamperometry of the signal. So, we can that is I as I mentioned thus, we can integrate this one with the software and we will get the chronocoulogram. So, let us save this data signal let us Save it, ok.

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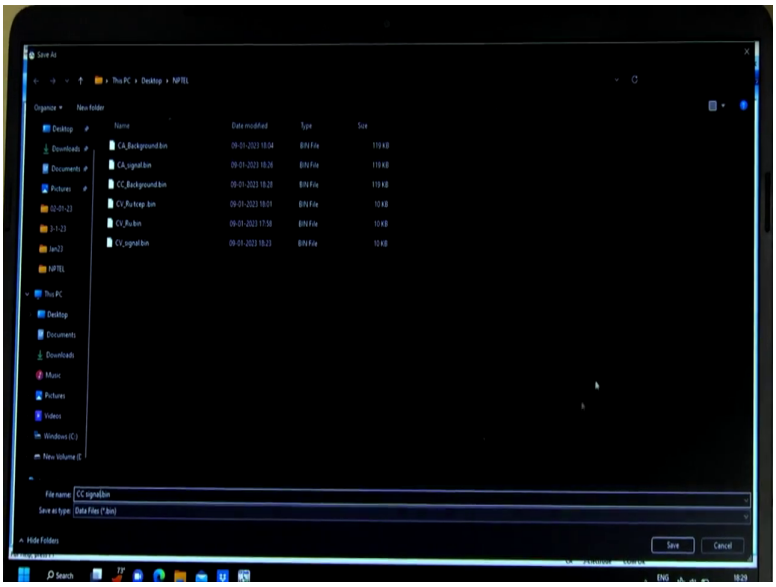

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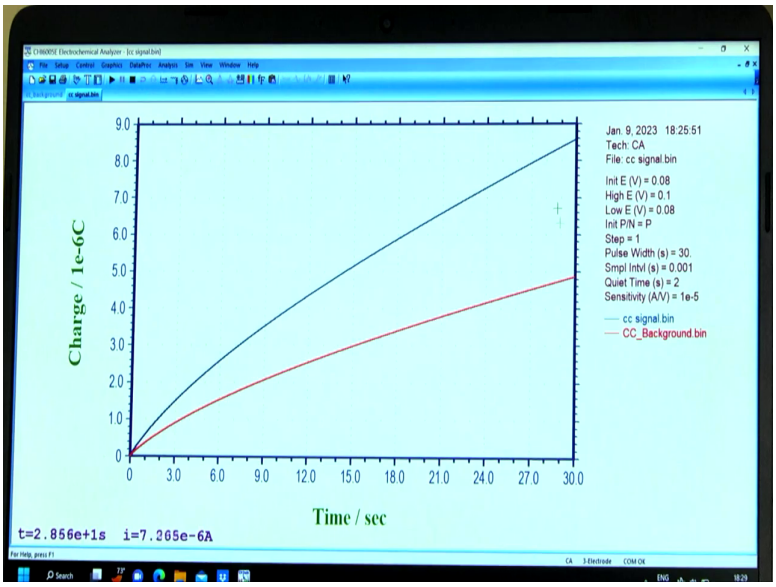

So, let us compare now background CC all the background ok. So, see here is the difference is there like background and signal, but one thing I wanted to tell you this difference is not enough still. So, there is there may be some issue can be there your reagent can not be a good if it is if you want to get the better signal you have to optimize the concentrations of your reagent.

Different concentrations of the reagent may give you the better these amplifications as I taught you in the class. And another optimizations you may need that is your very important optimizations that is the potential you can see I choose here 0.1 then I got that much difference, but if you compare your reactions with your signal and background and your signal CV your CV of ruthenium CV of signal.

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See is difference is coming, but your actual difference is actually is much better in this region. So, you can choose much higher potentials also for getting the much better signal to background differences. Accordingly, you can optimize the best potential for your ECC redox cycling's.

So, that is why see here is not that much difference, but actually hydroquinone and ruthenium can take part maybe in this region that you have to optimize and you may get the better signal to background ratio, ok. So, that is all for the ECC redox cycling and I think you learned now all the theoretical demonstrations.