


Quantitative Methods in Chemistry
Prof. Dr. Aasheesh Srivastava, Dr. Bharathwaj Sathyamoorthy
Department of Chemistry
Indian Institute of Science Education and Research-Bhopal

Lecture-12

**A Comprehensive And Step-Wise Look At An Experimental Protocol Towards
Understanding Systematic Errors in An Experiment**

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Experiment 4: Determination of Critical Micellar Concentration of a Detergent Employing Fluorimetric and Conductometric Measurements



Introduction: Chemicals are categorized as hydrophobic (water hating) or hydrophilic (water loving) based on the chemical moieties present in them. A molecule containing both hydrophobic and hydrophilic groups within itself are amphiphilic (*amphis* – both, i.e. both loving) (Figure 1). Such amphiphilic molecules are common in nature, take for an instance the cell membrane that helps keep the contents of the cell intact. A class of amphiphilic molecules that are surface-active agents consisting of hydrophobic tail and a hydrophilic head group are called surfactants. Surfactants tend to lower the surface tension between two liquids, between a gas and a liquid, or between a liquid and a solid. When such amphiphilic molecules are dissolved in water, they tend to self-assemble that help in achieving an energy minimized state where the hydrophobic moieties group together to form the core while the hydrophilic head groups point towards the solvent surface. Such a self-assembly is referred to as a micelle (Figure 1). A variety of structures, such as bilayers and liposomes, are also feasible by varying the conditions of the surfactant and solvent.


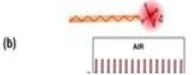
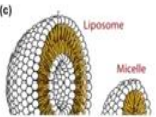

(a)  (b)  (c) 

Figure 1: (a) Chemical structures of negatively charged surfactant sodium dodecyl sulfate (SDS) and positively charged cetyl trimethyl ammonium bromide (CTAB) with the circle indicating the hydrophilic head and



Welcome to the last lecture in the third week in the course quantitative methods in chemistry, like in the last class we try to understand where systematic errors could arise from. We took an example of determining the critical micelle concentration of a surfactant. And we ended up using 2 different techniques to see whether a given measurement is repeatable and whether it is reproducible.

Of course, some numbers are given there where you were able to realize that one experiment done with conductometry did not agree with the other person. However an independent measurement with fluorimetry was able to achieve this. What we will end up doing today is to go to the nitty gritty details of this experiment and try to understand where systematic errors could arise from and what problems would end up arising when you do not set up your experiments carefully.

And for this purpose, I am going to be showing you the manual where step twice clear instructions would have been written down once again, towards making sure that the experiment can be repeated by anybody across the world, and trying to emphasize in points where things could be quite different. We also be taking a look at some video demonstrations, where different steps would be performed and we can try to also see where mistakes could arise from.

So, let me be also clear in the fact that we will not be describing the idea or the chemistry behind the techniques that are being used for instance, the flourimetry and conductometry the basic introduction that we had in last class should suffice for us to understand what the way we are trying to do okay.

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as bilayers and liposomes, are also feasible by varying the conditions of the surfactant solvent.

Figure 1: (a) Chemical structure of negatively charged surfactant dodecyl sulfate (SDS) and positively charged cetyl trimethyl ammonium bromide (CTAB), with the hydrophilic head (rectangle) and hydrophobic tail (zigzag) groups indicated. (b) Depiction of self-assembly processes: monolayer at the air-water interface, soluble monomer in water, and micelle formation. (c) Other architectural self-assemblies: liposome, micelle, and bilayer sheet.

Surfactants are categorized as cationic, anionic or neutral depending upon the nature of charge structure of the surfactant (Figure 1). Their unique property results in forming

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So let us quickly recap what we saw an example was in the case where you have micelles that are formed from surfactants, surfactants are chemicals that end up changing the surface properties of a liquid. In the case, we are looking at sodium dodecyl sulfate SDS, which has a long hydrophobic tail and a hydrophilic head, which is charged. So, what ends up happening is that all of the hydrophobic tail end up forming structure called micelles, where the hydrophilic outside is exposed to water.

And of course, there are different types of such aggregates that could be formed by the surfactants. One example is shown as liposome here, where it also has an hydrophilic interior

that is formed. The micelle is one where only the hydrophobic interior is formed where the hydrophilic exterior is facing water. And this is a very common scenario where lipid bilayer is formed in all of our cells in form of a plasma membrane, where the hydrophobic inside once again point with respect to each other.

And the hydrophilic outside end up being one part outside the cell and the other being inside the cell and entities like membrane proteins which are present in between them help transporting the chemicals. So the aim of this experiment that we saw last week was to determine the critical micelle concentration of sodium dodecyl sulfate using 2 different techniques namely flourimetry and conductometry.


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Aim: To determine the critical micelle concentration of a detergent sodium dodecyl employing complementary techniques such as flourimetry and conductometry.

Materials Required:

• Volumetric flasks	(10 mL one unit)
• Beakers	(100 mL one unit, 50 mL two units, 25 mL two units)
• Micro-pipette	(2-20 μ L volume one unit, 100-1000 μ L volume one)
• Conductometer	(one unit)
• Dropper	(one unit)
• Glass rod	(one unit)
• Thermometer	(mercury or ethanol, one unit)
• Sodium dodecyl sulfate	(as provided)
• Potassium chloride solution	(0.1 M, 0.01 M)
• Fluorescent dye	(100 μ M, as provided)
• Nano-pure water	(from MilliQ system, as required)



So, now, having said that, let us take a look at what are all required as I just mentioned, we are talking about determination of critical micelle concentration. For a given detergent, in this case, sodium dodecyl sulfate, employing 2 different techniques such as flourimetry and conductometric. And as always, whenever you are setting up an experiment, it is a good idea to have every small thing that you might end up using for the experiment clearly written out.

In this case, you would need volumetric flasks, you would be using 1 unit of 10 ml volumetric flask, beakers, you would be having different volumes of breakers and different units of them and micropipette, micropipette is one instead of the regular pipe that we end up using with a

rubber ball, this one will be able to aliquot very small amounts of volume such as something like 2 to 20 microliters quite accurately and precisely and also something between 100 microliters to 1000 microliters accurately and precisely

As we just spoke about it we also need a conductometer in order to perform the conductometric experiments and other subtle things such as dropper glass rods and thermometer in order to do its purposes of storing and also measuring temperature in the case of thermometer. The chemical in this case that we are using the sodium dodecyl sulfate this of course we provided an experiment so that we can determine the necessary stuff.



We will quickly understand why you would need potassium chloride in this experiment as this would be used towards calibration of the conductometer, and the fluorescent dye since this is a surfactant that is not inherently fluorescent. In order to perform the experiment, you would have to add this fluorescent dye, and of course, water in order to make up the solutions as pure as possible in order to make sure any contaminants in water does not contribute towards error in your measurement.

Think about taking tap water. If it is too hard, that could be other thoughts that could change your conductometric measurements and would result in systematic error there.

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Fluorimetry Measurement –

- 2.1. Measure the room temperature using the thermometer. As this is an equilibrium experiment, temperature must be clearly mentioned.
- 2.2. Using the nano-pure water provided to prepare 10 mL of 0.5 M SDS solution in a volumetric flask provided to you. As SDS is a surfactant it will form bubbles that will make it difficult to make up the volume upto the mark accurately. Therefore, add around 5 mL of water to the surfactant shake vigorously till the surfactant dissolves. In case of incomplete dissolution, sonicate it for 5 minutes. After the SDS has completely dissolved, dispense off all bubbles using a hair dryer provided to you. After the bubbles have been dispensed off add nano pure water to make up the volume up to the mark (see lower meniscus). Molecular weight of SDS: 288.4 g/mol.
- 2.3. Turn on the fluorimeter 15 minutes before pursuing the experiment. This ensures that the source is well equilibrated to provide the expected response.
- 2.4. Dilute the provided fluorescence dye (at 100 μM) to 10 μM in the nano-pure water provided in 10 mL volumetric flask. Transfer, using a pipette, 2 mL of this solution to a clean cuvette after rinsing the cuvette with ~ 1 mL solution. Transfer this to a clean cuvette for fluorescence measurement.



To start with we can try to look at how the fluorimetric experiments could be set up. The first step almost in every equilibrium experiment is to measure the room temperature at which you are working, in the case that you are working in experiments where the temperature is set at a different value apart from the room temperature. For instance, if you are performing your experiments at 37 degrees Celsius, you got to be careful such that throughout the experiment, the temperature is maintained.

Of course, the same problem would come up and room temperature where as a function of the time in a given day, the temperature could vary. It is always a wise thing to you have a thermometer and keep checking, what is the value or what is the temperature at which you are performing the experiment, let us say there is way too much fluctuation, it is better off that you don't perform such an experiment at that given point of time and ensure that in a given part of the day where the temperature is set constant, you can perform the experiment.

Most often labs end up having an equilibrium or atmosphere in terms of an air conditioner, which will ensure the temperature does not change too much while you are performing the experiment. Okay, now comes the first step. As I mentioned, you will have to add 10 ml of 0.5 molar SDS solution the volumetric flask provided to you. So this is the first step where you end up taking a stock solution.

So this is the first assumption you are making you are taking a stock solution of a known concentration and how would people prepared it. They would know the molecular weight of sodium dodecyl sulfate, they would have measured it in weighing balanced and added a certain amount of mass to given volume in order to make the 0.5 molar solution, as we saw in the first week of classes, how to prepare things of this sort.

So first step in this experiment is to prepare sodium dodecyl sulfate solution so that you can perform your measurements in fluorimetry and conductometry. The first step here would be to prepare 0.6 molar sodium dodecyl sulfate, how one end up doing it, you would end up measuring a certain amount of weight, depending upon the molecular weight of the given material. In this case, sodium dodecyl sulfate.

And then you would be making it up with a 10 ml of solution and a volumetric flask. First step that we are able to realize already here is that the mass that you end up weighing could be a reason for error where if you are not calibrating the weighing balanced, that could end up resulting in a systematic error. And on top of it, as I had introduced to you class, every volumetric flask is reference to a given temperature, let us say 20 degrees Celsius.

And an standard deviation that is associated with it. So one has to be careful. So the error here could come from the weighing of the SDS and the making of solution, the volumetric flask. One should remember since SDS is surfactant similar to your soap, if you are adding soap to water, it is going to result the formation of bubbles. And this could result in the fact that it is not easy for you to determine the meniscus of the solution in the volumetric flask.

Therefore, generally what people end up doing, instead of adding all the 10 ml to make up the solution in one shot, they end up adding just 5 ml of the solution. They shake vigorously. And in case the surfactant is incompletely dissolved, we end up sonicating it for a few minutes. So what this ends up doing is that instead of putting all the 10 ml and saying that it is not completely dissolved, people generally dissolved salts or anything that is solid in multiple phases.

So, we end up adding a certain amount of salt, add a certain amount of water not the entire amount, shake it or do whatever step in this case sonication is suggested, sonication is a technique, where you apply sound waves in order to provide energy and dissolve the material. So, you try to dissolve it within a few minutes and after all of the SDS has been dissolved in this 5 ml of water, then you ensure that all the bubbles that have been created are completely taken out of the solution by using a hair dryer.

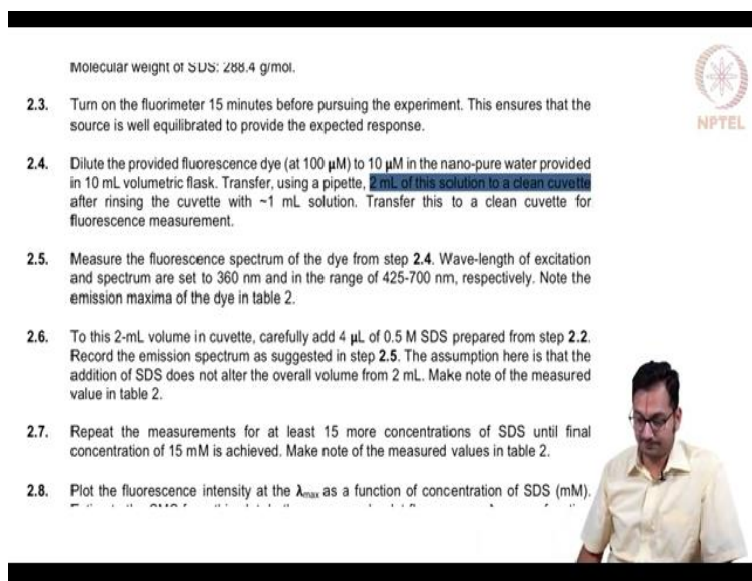
So basically, you warm it up such that you result in the bubbles bursting and no bubbles are present, so that you can carefully make up the solution to 10 ml. Once again here comes the problem where if this is not carefully followed, you might introduce systematic error into your measurement. After all of this has been done, you make up the solution to the entire market in order to get 10 ml of 0.5 molar solution.

So what are the reasons you might get systematic error here, wrong measurement of the weight of sodium dodecyl sulfate, the volumetric making up as a solution to 10 ml where bubbles could be there or your SDS is not completely dissolved. Now, after having done that, one should be making a fluorometric measurement and it is quite common for many spectrophotometric experiments, where the instrument is turned on for at least a certain amount of time before we start the measurement.

In this case, it is recommended that you start the fluorimeter at least 15 minutes prior to your experiment. This is largely because many times the source that ends up exciting your samples has to be in its own equilibriums generally these are lamps which has to warm up to a certain temperature and the excitation bandwidth is uniform such that any measurement that you end up doing subsequently are also carefully represented in your data.

So this will ensure that the source is equilibrated okay, now that is done. So you are prepared the solution of SDS and you have equilibrated your fluorimeter.

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Molecular weight of SDS: 288.4 g/mol.

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- 2.3. Turn on the fluorimeter 15 minutes before pursuing the experiment. This ensures that the source is well equilibrated to provide the expected response.
- 2.4. Dilute the provided fluorescence dye (at 100 μM) to 10 μM in the nano-pure water provided in 10 mL volumetric flask. Transfer, using a pipette, 2 mL of this solution to a clean cuvette after rinsing the cuvette with ~1 mL solution. Transfer this to a clean cuvette for fluorescence measurement.
- 2.5. Measure the fluorescence spectrum of the dye from step 2.4. Wave-length of excitation and spectrum are set to 360 nm and in the range of 425-700 nm, respectively. Note the emission maxima of the dye in table 2.
- 2.6. To this 2-mL volume in cuvette, carefully add 4 μL of 0.5 M SDS prepared from step 2.2. Record the emission spectrum as suggested in step 2.5. The assumption here is that the addition of SDS does not alter the overall volume from 2 mL. Make note of the measured value in table 2.
- 2.7. Repeat the measurements for at least 15 more concentrations of SDS until final concentration of 15 mM is achieved. Make note of the measured values in table 2.
- 2.8. Plot the fluorescence intensity at the λ_{max} as a function of concentration of SDS (mM).

Then the first step in this would be to dilute the fluorescent dye that has been provided to you, as I had mentioned already SDS is not fluorescent, so, therefore you have to add a dye in order to follow the measurement, this is something very similar to adding an indicator in your titration

measurements. For instance, if you are doing acid based titration, you would end up adding phenolphthalein.

And you know for a fact that unless the pH goes above a certain extent phenolphthalein is colorless, so, you the basic assumption in this kind of experiments is that the indicator does not change the end point of the measurement. We are making a similar assumption in this experiment that the fluorescent dye that you end up using does not change the value at which the critical micelle formation happens for SDS.

So, this is one assumption that goes into it when you are setting up an experiment says assumptions are better laid out so that if there are any discrepancies you can go back and revisit what is going on. So, dilute the given fluorescent dye in a given volumetric flask, and then using a pipette, the micropipette carefully transfer 2 ml of the solution to clean cuvette. So why do not we take a look at how this is going to be performed oaky. **(Video Starts: 12:22)**

What you are able to realize here is that this is the volumetric flask we are talking about as you are able to realize there are some bubbles, we are assuming that the researcher has taken care of all those things. And what you see here is the fluorescent dye that we will end up using, this is the cuvette that will be using for the measurement. And of course, you always have a spare cuvette such that you can blank your solutions as required.

And this is the pipette with which we will be making our measurements as they are able to see the cuvette is transparent on all the sites as this is a fluorimetric measurement. If you are doing a spectral photometric measurement, you are using UV visible spectrophotometer, you would cuvettes that could actually be only transparent one parallel set of planes, not the perpendicular ones.

So the pipette would help you measure very small volumes as we had mentioned already, you have 2 different pipettes, one that will help you measure 2 ml one that that will help you measure 2 to 20 microliters. Now, you are able to realize that the researcher is carefully taking out the

desired amount of the ANS dye the fluorescent dye using the micropipette and carefully adding to 2 ml of it into the cuvette as you see here.

Since it is a 1 ml pipette, we are to do the same pipetting out 2 times, so as to ensure that you add 2 ml and what could this end up happening. Let us say you have one microliter error in each of the 1 ml pipetting out. This is going to end up as a 1.44 microliter due to the error provocation that we have learned so much in the last few classes. So, now, that is being done, one has to ensure that the size of the cuvette are clean enough before setting up the fluorimetry experiment.

And here this is fluorimetry that we will be measuring our data from one carefully opens the chambers such that the sample can be carefully input within this fluorimetre will also be the same as a top down view of this so that you understand how the cuvette is carefully placed. As you are able to realize the cuvette has been placed within the sample chamber. As always with modern equipment, you will end up recording the data using a computer here we are just trying to show the software which we end up using in order to make a measurement.

So, you start the manual initialization process such that all the parameters can be carefully set. So, once the initialization comes up, then you are all ready to start your measurement. So, in this case, we are just going to be measuring spectra and we are going to be measuring emission spectra. So, you carefully choose what you want to choose, one must be careful in doing this because if you choose excitation spectrum, maybe the experiment is not good enough to capture the changes that you would like to capture.

And when you are doing excitation spectra, you have to be careful about the wavelength that we are using and the range at which you will be recording the data. You will soon realize that these changes are made based on the manual that we will be seeing in a moment. So, the first step is to change the excitation wavelength to the desired number which is 360 nanometers in this experiment and change the emission window that will be measuring between 425 and 700 nanometers.

Then you also ensure that the detectors are carefully chosen. In this case, we are using 2 detectors such that it will help us get the values carefully and not going into the details of it. But this measurement should also be carefully done such that from these 2 detectors, you take the ratio of the signal that ends up coming from these 2 different detectors. Once that is done, we end up setting it up that way that is not important for this.

But all it suffices for us to understand currently that whenever you are setting up any experiments, each parameter that you set up has to be carefully thought out has to be put in making sure there are no errors that propagate due to them. So once you have done that, you end up trying to measure a spectrum. So when you are able to measure a spectrum, you are able to realize as a function of the emission wavelength, the intensity keeps increasing, it reaches a certain maximum and it slowly start to drop off as a function of the wave length okay.

If you might remember the last week classes, what we ended up understanding there is that until the micelle is formed, you are not going to have the fluorescent dye that is trapped within this micelle which will result in low fluorescence intensity. Basically the intensity maximum that you ended up seeing just a while back is going to be almost constant, but as the micelle is formed this fluorescent dye gets trapped within this and the fluorescent intensity will increase.

So, this experiment is nothing but having the ANS dye in water, measuring fluorescence and adding small portions of the sodium dodecyl sulfate the stock solution that you are prepared into this cuvette and see how the fluorescent intensity changes. So, that is all the experiment is going to be about. And many times you will have to save your data as and when you measure your data. And this is just trying to show you as an example in a given lab experiment how you save your data okay.

Now that has been done, the next step that you would end up doing is to add a small amount of the sodium dodecyl sulfate into the cuvette. So, why do not we take a look at the protocol before coming back to this.

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So, if you are able to realize the point 2.5 is what we have done such that you set the excitation wave length to 360 nanometers and get the emission spectrum between 425 and 700 nanometers and you would want to make a note of the emission maximum which is saved in the data file that you ended up saving. Now to this 2 ml cuvette carefully add 4 microliters of the SDS solution that you prepared in the initial step.

So, basically in step 2.2, you ended up preparing 0.5 molar stock solution of SDS. So, carefully add only 4 microliters of it. And this is important because once again, you have to do everything quantitatively adding 4.1 or 3.9 or a 3 or 5 microliters would end up resulting in a systematic error. So, one has to be careful that once that is done you theory should let the solution equilibrate for a few minutes before you do your measurements.

As you are able to realize, although we are doing a thermodynamic experiment, the kinetics of formation of the micelles may not be always fast in this case, it is indeed fast, but if in case it is slow, you have to let it equilibrate for finite amount of time, generally something between 10 to 15 minutes for a fast aggregating system is good enough, such that when we make a measurement, it is indeed in its equilibrium state.

So, once that is done, once again, you repeat the fluorimetric measurement, you assume that the overall volume is 2 ml if you realize you are adding 4 microliters to 2000 microliters. So, it is an assumption that goes into place. So, any volume that comes up, you should be carefully getting the molarity of the final concentration of SDS by using the formula that you have seen before that is $c_1 m_1, m_1 v_1 = m_2 v_2$.

So, this experiment is nothing but repeating that for several different concentrations of SDS and getting the emission maximum, and then plotting the emission maximum as a function of concentration of SDS.

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concentration of 15 mM is achieved. Make note of the measured values in table 2.

- 2.8. Plot the fluorescence intensity at the λ_{max} as a function of concentration of SDS (mM). Estimate the CMC from this plot. In the same graph, plot fluorescence λ_{max} as a function of concentration of SDS to estimate CMC from this. How well these two measurements agree within themselves?

Table 1: Conductance measurement for determining CMC of SDS

S.No.	Concentration of SDS (mM)	Conductance (mS)
1		
2		
:		
:		
:		



This is exactly what we saw in the last class, then what ends up happening is that we start finally getting data that looks of this sort. So, you are going to measure concentration, you are going to get fluorescence intensity as a function of concentration. So what ends up happening, you get a constant intensity, and then it increases where at the point of break, is where you are able to realize that there is a critical micelle concentration which we saw in the previous class.

Now this is done, what you are able to realize one could have errors that could come up in very many different steps. Let us reiterate that for the fluorescence experiment. It could come to the being of SDS, it come from volumetric making up of the solution, it should come from the fact that the amount of ANS that you have added could interfere with the micelle concentration at which they form the surfactant concentration at which you for micelle.


It could come from the fact that when you are adding a certain amount of SDS to the solution, you are not equilibrated it enough such that the fluorescent intensity does not reach the equilibrium state that you might want to get. And it could also be the case that you might not have set up your excitation wavelength and emission spectra, the wavelength between which you want to record your spectra. So all this has to be carefully done.

One other variable that we set there as a slit which one has to be careful how the points are digitized, so that the maximum is carefully determined. So what you are able to realize that all

this could result in a systematic error. And let us assume that you have done an experiment such that everything is calibrated, including a pipettes and your weighing balanced, you are trying to minimize the systematic errors that could come up where only finally the random errors that are associated with the measurement itself would result in small standard deviations that would end up coming.

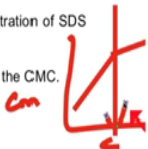
So now that we have seen how the fluorimetry experiment works, we also would like to see how the conductor metric experiment would be performed.

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Conductometry Measurements –

- 1.1. Measure the room temperature using the thermometer. As this is an equilibrium experiment, temperature must be clearly mentioned.
- 1.2. Calibrate the conductometer using 0.1 M and 0.01 M KCl solutions.
- 1.3. Obtain the conductance of nano-pure water using 15 mL in a beaker. This would provide the reference/background for the measurements that would be subtracted from the measurements that follow. Make a note of the conductance measured in table 1.
- 1.4. To this 15-mL solution, add 40 μL of 0.5 M SDS and **measure the conductance**. Make a note of the conductivity measured in table 1.
- 1.5. Repeat step 1.4 for at least 20 more such additions until the final concentration of SDS reaches 24 mM. Make a note in table 1.
- 1.6. Plot specific conductance as a function of SDS concentration to determine the CMC.



So for this similar protocols would exist. The first step in this would be to measure the temperature, you are to ensure that the experiment that is done in the fluorometric condition and the conductometric conditions are in fact very similar to one another. If this is not the case, you are going to have different measurements. Remember, all these aggregates that end up forming could be subtly different as a function of temperature.

There once again imagining an equilibrium constant between the micelle and the monomer and this could easily shift based on the entropy of formation of these species. So the first step that we are going to end up doing in this case is calibrating the conductometer using some standard solutions, if you remember, when we were talking about the materials required, we said we will

be using a small amount of standardized solutions of KCl in order to calibrate the instrument. So let us take a look at how does this work. **(Video Starts: 22:58)**

This to show a little bit on how the data is we ended up saying for the fluorimetric experiment looks when you save the data, you start ending up getting something, a wavelength and the intensity. So if you want to be able to plot this use a different spreadsheet software, or even any other program that you might end up using, such that you can reliably get the measurement that you are talking about.

And if you are able to realize since fluorimetric experiments are so sensitive, you are able to see so many decimal points that ends up coming. Just because you get so many decimal points does not mean it is as precise one has to repeat the measurement a few times in order to determine what is the position of the measurement, now to the conductometric experiments. So, this is a conductor meter.

This is what will help you get the reading for the solution that you ended up preparing that basically on what is the conductance. And if you see here, this is the probe which helps being in contact with the solution with from which you want to measure the conductance. It is connected to a cable which has this conductor meter interact with the probe, which hands ends up measuring the conductance of the solution that you would like to measure.

As we just mentioned, please ensure that you measure the temperature at which you are making a measurement. And it is a good idea to always put the thermometer within a water bath such that equilibrium measurements can always be taken care. What you are able to see the scientists doing here is that you are not able to read a conductance value here, this is how many meters end up working, they have their own ways of measuring things are different precision.

If you are having very low conductance, one might have to change carefully the settings that go here, the first thing that one has to end up doing is to tell the conductormeter that they are going to be measuring it at a certain temperature and if you are able to realize the scientist is actually

keeping it the temperature column and reads the temperature that is 25 degrees Celsius. And whatever temperature has been measured using the thermometer is carefully adjusted here.

Since we mentioned at 27 degrees Celsius, the research first changes the temperature at which you are making such a measurement. Once that is done, what he is changing it is studies changing to conduct a metric measurements and he realizes that he reads a value that does not make sense. So what he ends up doing is to change the least count at which he can end up measuring. So what he is able to see is that when the meter is kept at 20, micro Seamen measurement, you are actually not able to read anything.

So what he will end up doing is to change it higher to see whether some value comes of it, so it is just changing it from 20 to 200 micro Seamen and then immediately what you are able to realize is that you are able to read a value or which 71 micro Seamen. So, this makes total sense because if you are having a reading of 71 micro Seamen, if you are putting at a 20 micro Seamen as the maximum measurement, the conductormeter is not able to read.

This is a common problem that will come up with different equipment where they have their own range of measurement, and any measurements that you might end up doing higher or lower might not be detectable. So, this is something that each one of us must have in mind, before making a measurement, what is the correct value at which you should be measuring this, we will see more of this instance as we go forward. Of course, one can always argue is a keeping at a 200 micro Seamen, what will happen if I keep it at 1000 micro Seamen, basically 1 milli Siemens, we are going to see that in a moment.

So once you are able to change it to 1 milli Seamen, you are able to realize that the value from 71 goes to 0.07. You are losing the last decimal that comes which is 1 here meaning the front 2 significant figures, you are reducing it to one so the able to realize that if you are not setting this value, right, you might start slowly introducing systematic shifts that comes up due to digitization of the equipment that you end up using.

So therefore the scientist is switching it back to 200 micro Seamen in order to make his measurements. So the first thing that we would end up doing is to calibrate this conductometer, so is adding the 0.01 molar KCl solution from the volumetric flask that he has made it up in to the solution. And before you end up using the probe, the first thing that you must end up doing is to always wash the probe irrespective which one you are doing.

Wash it carefully such that no damage occurs to the probe, wash it enough with water and dry it with a paper that does not get stuck or results and scratches. So you have to be extremely gentle. When you remove the water you just touch it and it actually absorbs all the water. Then you calibrate the probe and the conductometer with the standard solution of 0.01 molar KCl. So what has to be done is that you generally have a sheet that tells what should be the conductivity of the standard solution at that given temperature.

And at the back of this conductometer, you will be able to adjust this value, you will soon see that the researcher will carefully change this in order to match with the tables that he has obtained. So, at that concentration of KCl, the value is set to that of the literature value, which in this case is 1.47 micro Seamen rather 1.47 million Seamen. So the next measurement that he ends up doing is for higher concentration of KCl.

So you are going from 0.01 molar to 0.1 molar. As you realize conductance increases with the concentration of the ion. When you increase the concentration, you are able to realize the value went outside the detection limit at 1 milli Seamen setting. So, therefore, what one has to do is to change the change the equipment such that it can carefully measure values until 20 milli Seamen. And that is essentially what is being done.

When you increase the concentration of KCl conductivity increases, and you are able to carefully measure it by changing the setting in the conductometer. If you have not done this, you are going to have trouble. So therefore, could also result in systematic errors. And you realize that the researcher make sure the value agrees with the literature value, which we just saw a moment back, which is 13.4 million Seamen.

So now that you are calibrated it at 0.01 molar KCl and 0.1 molar KCl, you are all set to make your measurements. Once again you are to ensure that you wash the electrode and gently and carefully and dry it so that any amount of water that might be sticking onto the electrode does not result in a measurement error. Once again, systematic. In this case, let us say more water less water gets stuck. You might have an issue where the solution of SDS that you end up adding will be more or less.

Actually in this case will be less because you have some water that is going to end up diluting the solution with which you are working. When you have one the pure water, there is no conductance that comes. Of course, there will be a small amount of conductance, which the scientist is able to change it to the 200 micro Seamen value and he is able to read about 63 micro Seamen that comes up okay, now that has done.

Of course, he is just trying to show you if the setting is kept at 20 micro Seamen and you do not read anything proper. So whenever you do not read anything proper check whether your instrument is carefully kept at the setting that it has to be kept in okay. So now that is done. So, this is the correct value one has to be careful about that alright. Now to this solution, you add a known amount of SDS that you are prepared.

If you remember that was the first step where we ended up preparing 10 ml of SDS in a volumetric flask. Now the scientist is carefully adding that solution into the beaker. And after having done that one must carefully store it. Although the researcher is just using swirling as the mechanism to mix it properly, it is better done with a glass rod such that this whole solution is homogeneous. And let it equilibrate again.

So, it is a good idea to let it equilibrate for a few minutes. So that diffusion also is complete, and the whole solution is homogeneous. Now that is done, you are able to realize the value of conductivity has now increased from 63 micro Seamen to 79 micro Seamen. Of course, he will probably try to show that if you are increasing this to 1 milli Seamen measurement, you are losing the last significant figure that comes up and it ends up rounding of the figure to 0.08 which is not a good thing. So do not do that. So one has to be careful doing this otherwise, this

also results in a systematic error in measurement is also not a lower value, because that ends up creating trouble. **(Video Ends: 32:05)**

What we have seen so far is that whichever equipment that you end up using, it is always a good idea to calibrate it before you start end up using it. And you are able to realize that the rest of the protocol says the same thing, you add 15 ml of a solution and carefully add a known amount of SDS using a micropipette to this solution of water and measure its conductance as we have seen right now and carefully make a note of what you end up getting.

Once having done that, you are going to have a measurement that looks like this, where you are going to have a certain curve until here and then the slope ends up changing after a point. This is of course concentration versus conductivity. So what ends up happening at this value of concentration is well, it is when you start forming the micelle. So what have we learned so far, that could be systematic errors that could come in terms of the room temperature measurement, it could come because you did not make up the standard solution of KCl properly.

Once again coming up, due to the fact that the weighing balances are not properly calibrated, or you had made mistakes where when you are transferring the KCl, from the way both into your standard flask, you may have lost some of it. So one has to be very careful there. On the other hand, let us say you are trying to add 15 ml of solution to the beaker, you add 16 ml or something slightly different from that, or when you are adding certain amount of SDS, you add varying amounts one time you are 39 microliters, when you are 41, sometimes 40.

This ends up also resulting in the curves that do not look perfect. If you remember the first researcher that we ended up seeing 6.3 millimolar was the SDS concentration for CMC, we realize that that is wrong and it comes up close to something like 7.3 or 7.4 millimolar. And that could be one of the reasons why the mistakes came up. And of course, you have to do enough measurements such that the curve looks proper such that you can carefully estimate the CMC of that given surfactant.

So, to conclude this lecture, I would like to make you understand that we have seen 2 independent measurements of the same experiment, and one is able to realize there are subtle points that one might end up missing, which could result an overall error that is not good enough for your experiments such as you can compare values. So one must always take care and also write the protocol in its excruciating, comprehensive detail.

So that such mistakes do not happen. I hope this helps you understand how experiments must be set up, and how to minimize any systematic errors that could come up. In the assignment that we will take a look at for this week would be setting up an experiment and I asked you to take a look at what all errors could prop up when you are making such measurements. Thank you very much.