Experimental Biochemistry
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Lecture – 06
MAKING PHOSPHATE BUFFER (100 MM)

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So, here we have taken sodium phosphate monobasic, here around 3 gram the weight. So, now, we will dissolve it in double distilled water.

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Here, we have taken double distilled water out here. Now, we will not add 250 ml double distilled water directly at the first go why due to volume adjustment or volume error might be there, since we are adjusting it around 100 millimolar, molarity calculation requires volume adjustment.

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So, we will be adding roughly around 200 ml. Here we can see the graduation is made around 100, 200, 300 and 400. However, note down that the graduation in case of a beaker is not actually accurate. So, the graduations were rough graduation never go by

the graduation mentioned in a beaker generally take the graduation mentioned, in a measuring cylinder which we will do later on after dissolving this thing. So, we will basically fill this to up to 200 ml, roughly up to 200 ml.

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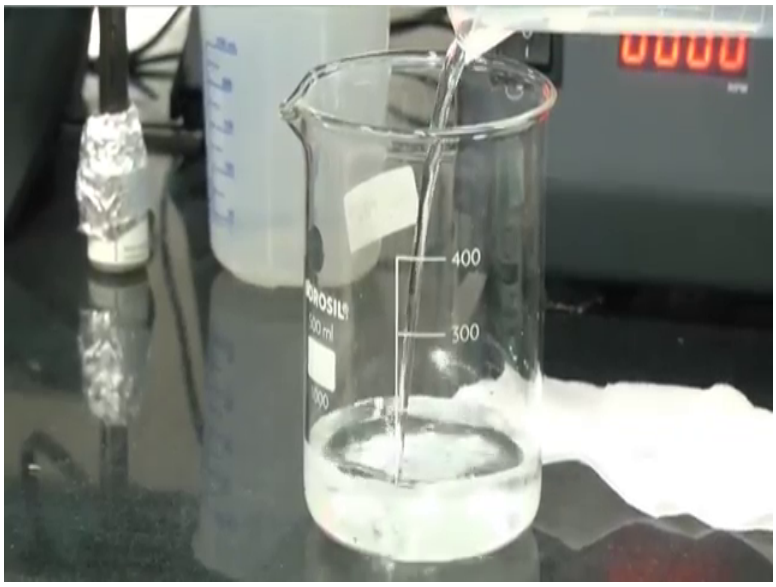

For that what we will do? We will add here, once we add we will just stir it gently.

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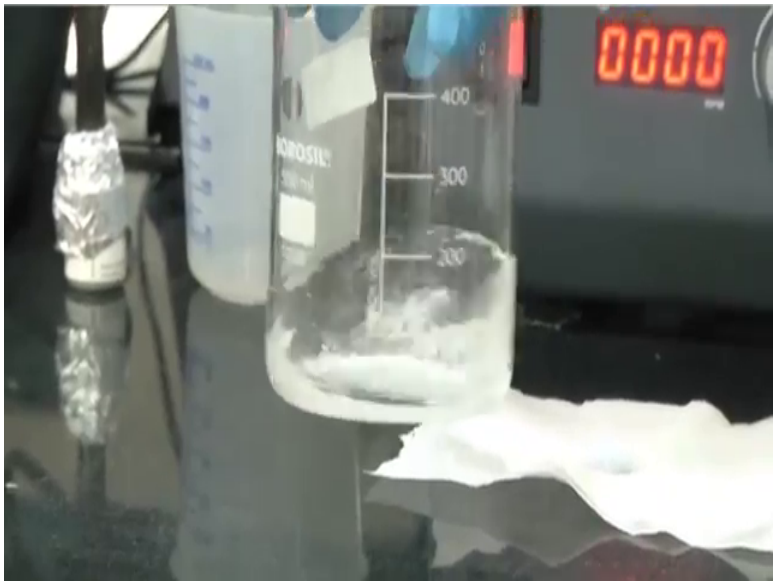

And again, go on adding. Here, we can see small chunks or small aggregates of this compound sometimes might be formed after adding double distilled water. So, how to

remove this? We cannot basically stir it and remove this one, for that we need to use this magnetic stirrer. And this magnetic stirrer calls for this magnetic bar.

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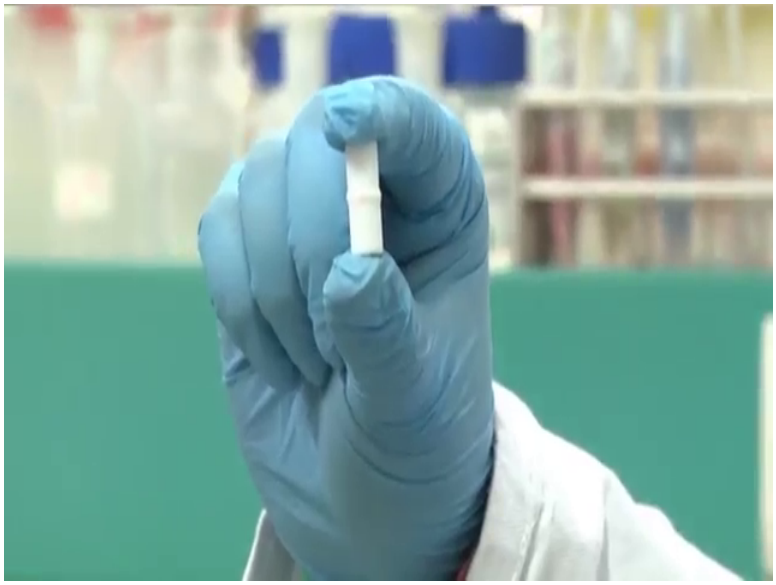

Now, these are the small magnetic bar. Some of you might be familiar with this one. Time to time we will be using this thing to dissolve the solid components in double distilled water or require solvents.

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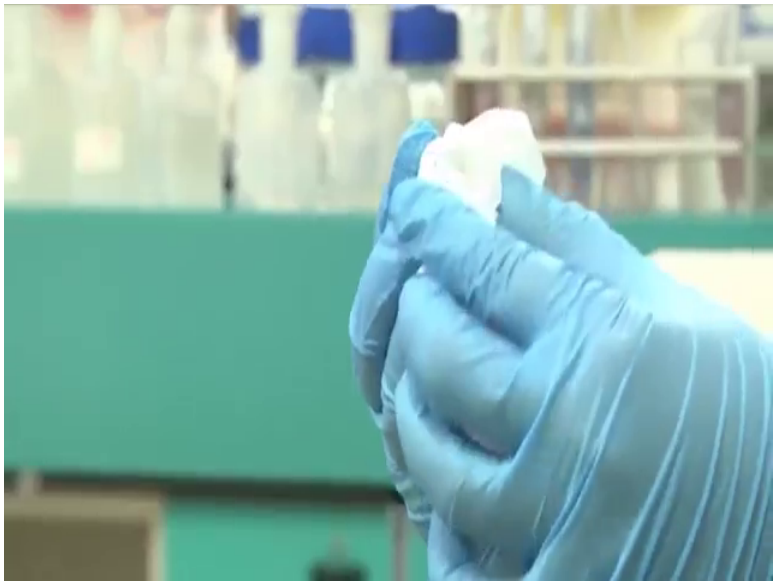

This magnetic bar has been previously washed however, I am again wiping this using a clean kim white tissue paper.

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And once I have wiped this one I am this letting it into this solution. Here I can here I have added this thing you have seen I have not used my hand while adding this one, I have just rubbed it using this tissue paper and directly from a tissue paper I have added it in this solution.

Now, I am pouring again double distilled water up to 200 roughly. So, here we have poured around 200 ml of double distilled water. Now, I will keep it here in this magnetic stirrer for dissolving of the solute components.

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For this, this is the magnetic stirrer, I am turning to switch on here. Here we can see the magnetic bar has started to rotate. We can adjust the speed out here using this regulator. We can increase it, we can definitely decrease it depending upon how required solution we can regulate the speed. Here if we keep it till here, we can actually see the solute components in this beaker we can go for volume adjustment.

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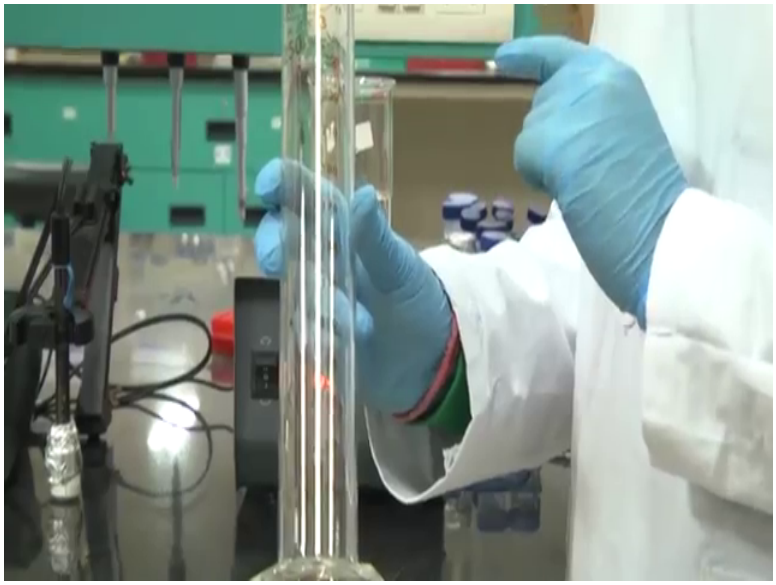

Now, once our solution is there in the magnetic stirrer and it is being dissolved, we have to take a measuring cylinder, if we have take a clean measuring cylinder out here we have to clean it using double distilled water. Now, the measuring cylinder we have taken is 250 ml because the solution we are preparing is 250 ml of this phosphate buffer in the component one, component of the phosphate buffer.

So, here we can see the graduation is there for measuring cylinder is exactly graduated and we can assume it to you basically exactly graduated much more accurate than conventional beaker. So, here we can see the graduation from 30, 50, 70 and till 190 and 250. So, we have to pour this solution out here in this beaker and we can adjust the volume then up to 250 ml. Now, we have to check whether this solid components is completely dissolved or not. Here we can see that the solid components completely dissolved and we can stop this magnetic stirrer. For stopping this magnetic stirrer you have to just decrease the speed initially and then you can switch it off like this way.

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Now, I will be pouring this solution put here. Now, please note that this magnetic bar out here, the magnetic bar out here do not drop in this measuring cylinder be careful while putting this solution in this measuring cylinder. So, I have completely transferred this solution from here from the beaker to this measuring cylinder.

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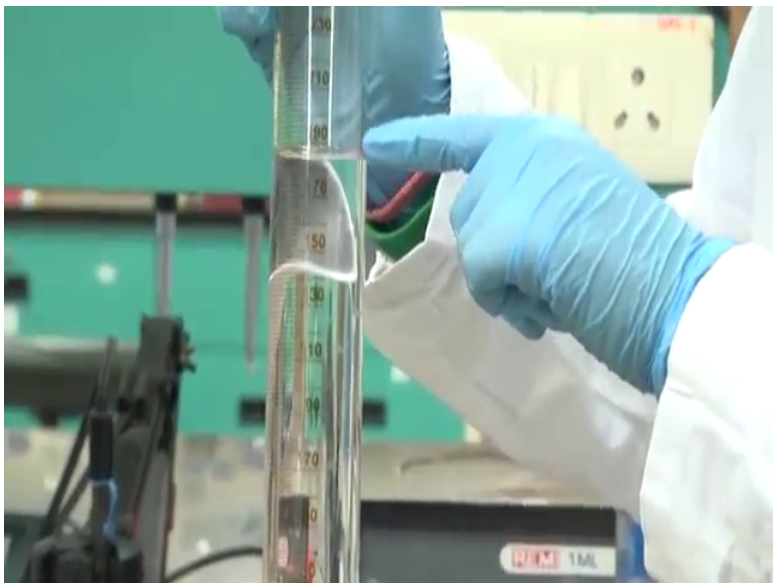

And one interesting to note down here is that, the volume the volume out here is basically less than 190, we can see the meniscus it is less than 190 or close to 190 ml. However, we have added it around 200 ml. So, the graduation in case of beaker is not

accurate. Here we can see in case of measuring cylinder its 190 whereas, in beaker it was around 200. So, better to use a measuring cylinder rather than a beaker

Now, we will take this one and adjust it till 250 ml.

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For that we will be taking a double distilled water.

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And we will be adding here.

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Now, once it reaches around 230 or 240, I pour this double distilled water gradually. While it reaches this 250 ml, keep it in a place that is a steady place and a flat place out here better do not take it in hand you know flat place and add double distilled water. Now, keep it bring it to your eye level for that we have to just take it or check it out here and its more or less, ok. So, here we have actually measured 250 ml of sodium hydrogen phosphate monobasic, and we will transfer this again in a beaker.

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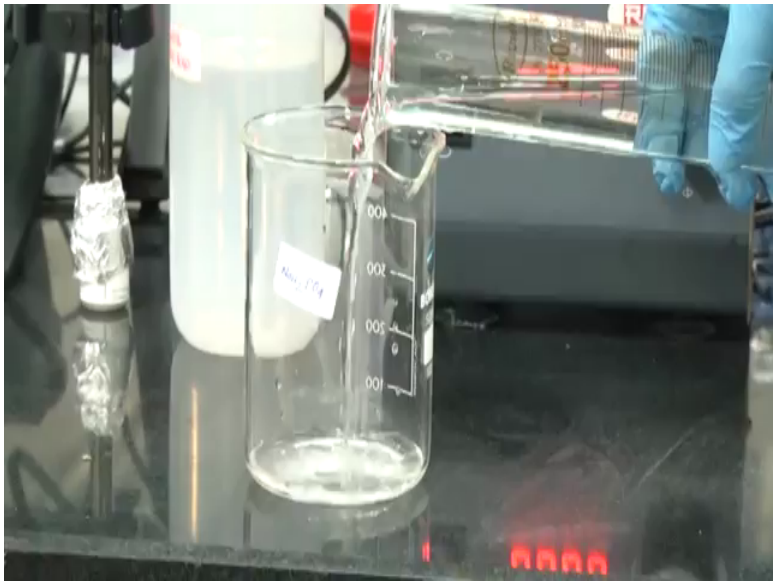

Now, we have taken we have taken this beaker and marked it as NaH 2 PO 4 that is monobasic one, and we are adding the solution to this beaker. And now, this one we have prepared 250 ml, 100 millimolar of sodium hydrogen phosphate monobasic.

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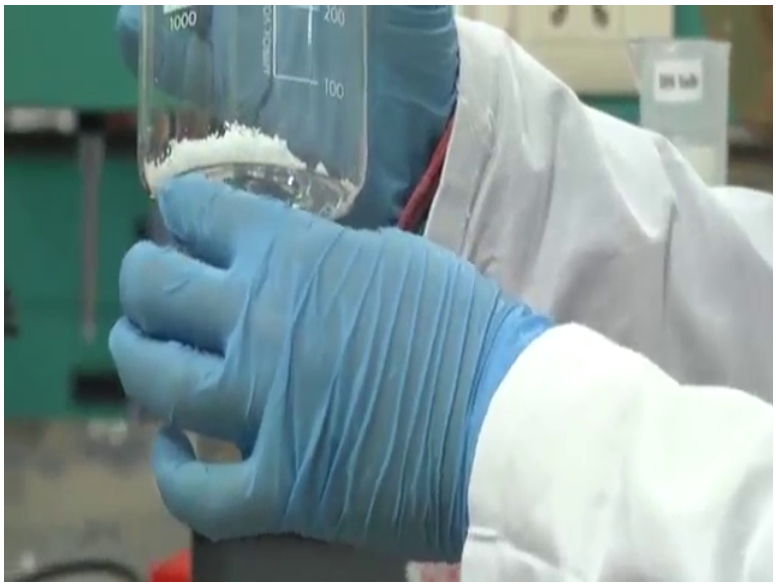

Now, we will dissolve the second one that is sodium phosphate dibasic. Out here we have taken this crystalline part sodium phosphate dibasic one, here we can see.

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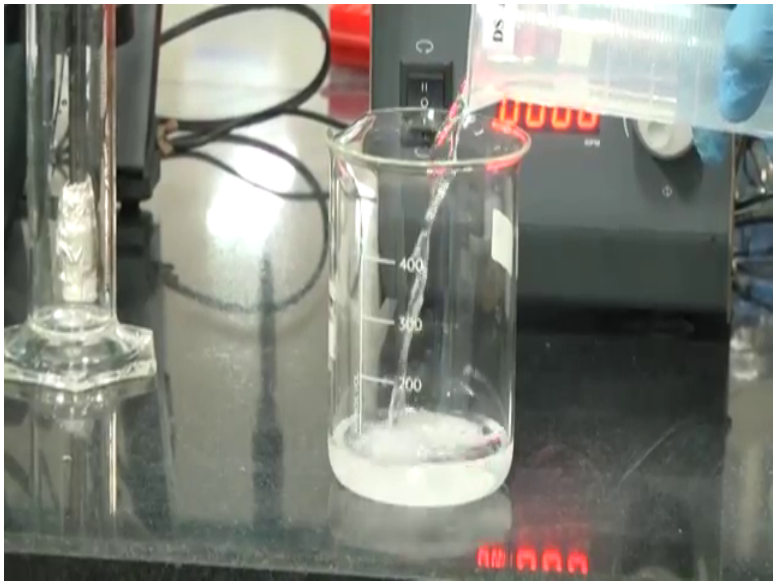

Now, I will be adding this is the graduation here we can see the graduation part is there around 200 ml of double distilled water.

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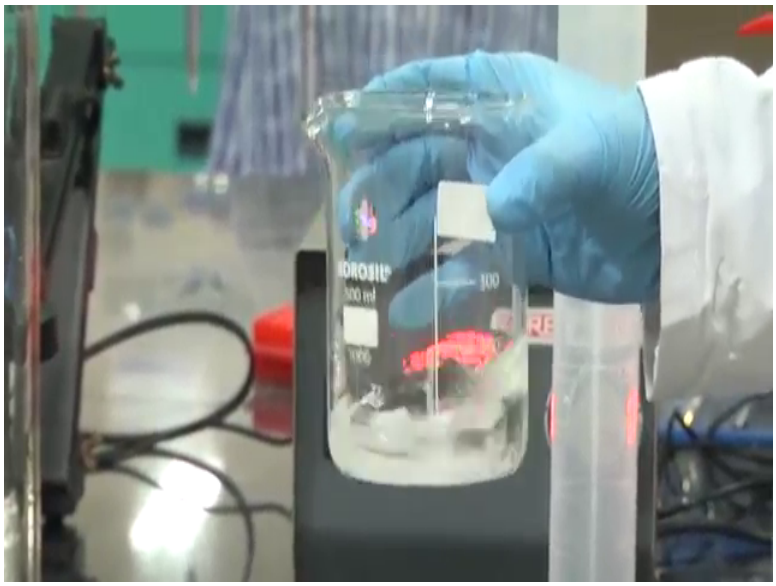

I will initially I will stir it gently; however, I can see basically the solid components is there undissolved within it. I have added roughly around 200 ml double distilled water here. Here we can see the solid component this have not been dissolved out here, so we will take it in a magnetic stirrer for dissolving.

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Here we have added the magnetic bar, so we will take it in a magnetic stirrer. I am placing this one in a magnetic stirrer.

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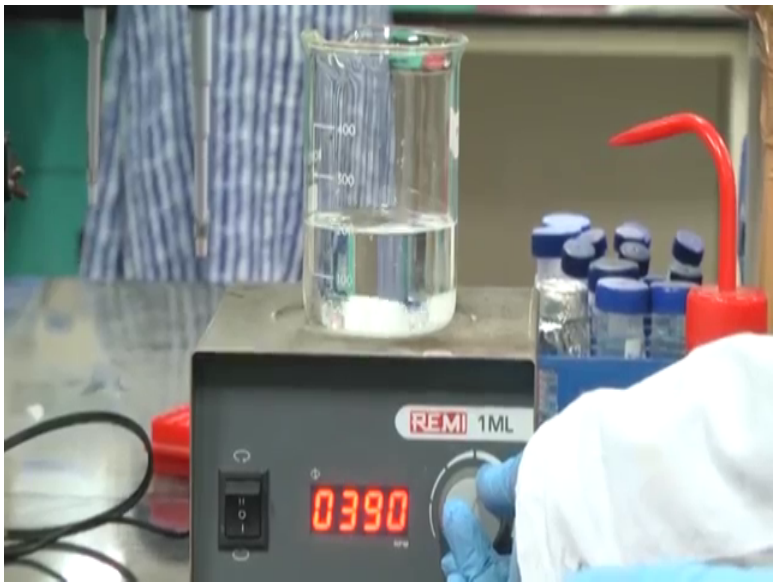

So, we have to leave this one until and unless the entire solution becomes clear and the solid components is completely dissolved.

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Now, we will check the pH of sodium hydrogen phosphate monobasic one that we have actually prepared and dissolved in double distilled water.

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For that, this is the pH meter as we have seen in earlier during Tris buffer preparation. Now, this pH meter we will just wash this bulb. We will be washing this bulb with double distilled water.

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Now, I will be washing this bulb with double distilled water and again wiping it with a clean tissue paper we have to try this procedure 2 to 3 times carefully. We are initially washing it using a jet of this double distilled water.

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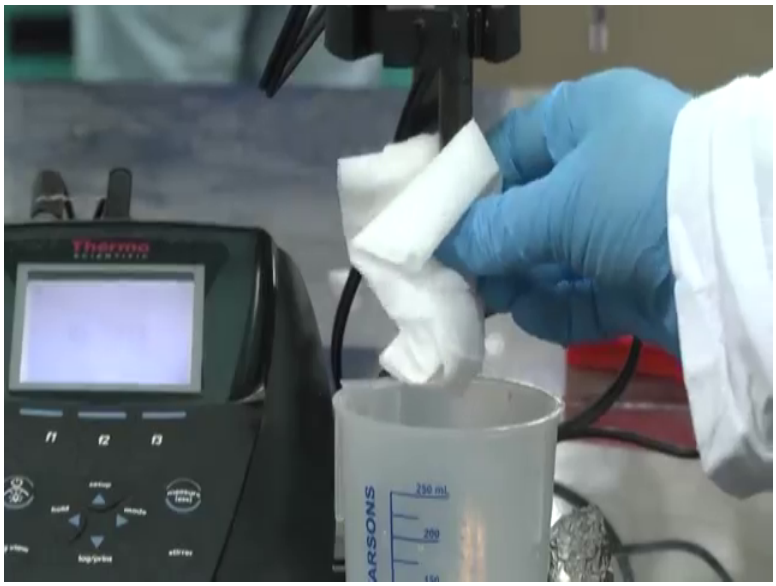

Then after that will wiping it using a clean tissue paper. Again, we will adding double distilled water, and cleaning it, we have to repeat it 2 to 3 times. Now, we will transfer it in the solution. Better do not keep it here in dry for a long time.

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Now, we are transferring it in the solution. Now, I have transferred this pH meter the bulb of the pH meter in this solution, and here we can see the reading of the pH of this solution it comes to around 4.35. So, basically the pH of this 100 millimolar, 250 ml sodium phosphate monobasic it comes to around 4.35 that is in the acidic region. So, we

have dipped this pH bulb in the solution, and here we can see in the pH is coming to 4.34. So, the pH for sodium phosphate monobasic lies in the acidic region around 4.34 the value mentioned is generally 4 to 4.5 and its coming 4.34. So, we have prepared a 100 millimolar sodium phosphate monobasic solution, 250 ml whichever pH of around 4.34.

Now, we will see the pH for the dibasic solution. So, here we can see our solution containing the sodium phosphate dibasic one this is almost clear and the solid components is dissolved. So, we can basically take this solution for volume adjustment that is take it up to 250 ml.

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For that we have to lower this regulator the speed of the magnetic stirrer again and then switch it off. Take this solution in a clean graduated measuring cylinder the same way that we have done in the previously for monobasic acid.

Now, we will take this solution in a graduated measuring cylinder. The measuring cylinder we have taken is similar to that we have used for monobasic acid. The measuring cylinder we have taken have been similarly used in case of monobasic acid for volume adjustment. Here we have taken a clean measuring cylinder and now we will pour this solution here.

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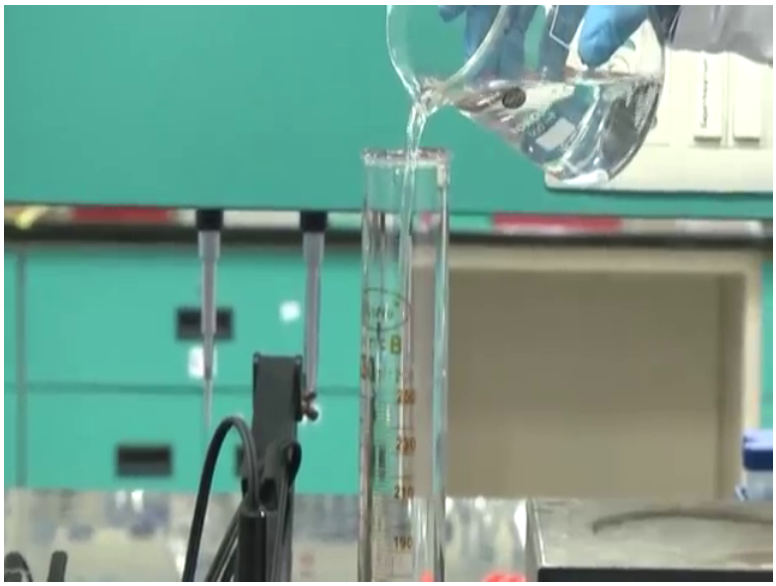

Now, before pouring this entire solution we can see the magnetic stirrer or the magnetic bar out here is there in the solution.

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So, one thing I would like to introduce you to all that is a tong in order to remove this magnetic bar.

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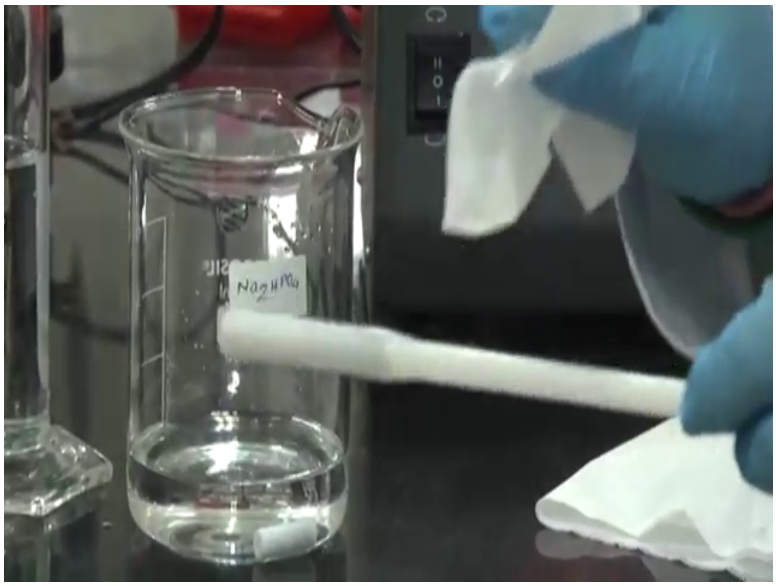

In order to remove this magnetic bar from the remaining solution we will be using this tong. This tong is basically the magnetic again a magnetic stick, we have to clean this one this has been previously cleaned and I am just rubbing it using this kim wipe tissue paper.

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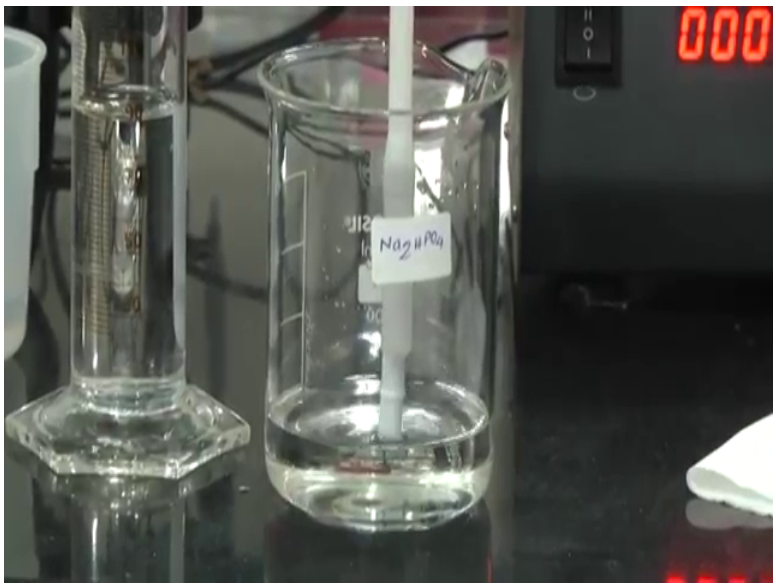

And then one interesting thing you can see here once you keep it here, take it here the magnetic bar will attach with this magnetic tong and we can remove this thing and then we can proceed with volume adjustment we can pour the rest of the solution freely.

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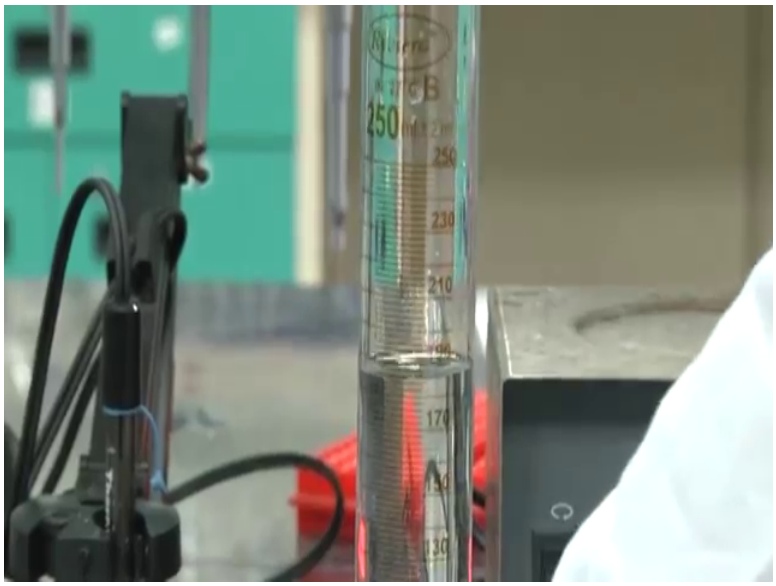

Then, we can see it is around 180, it is less than 190, so we can adjust it again using double distilled water.

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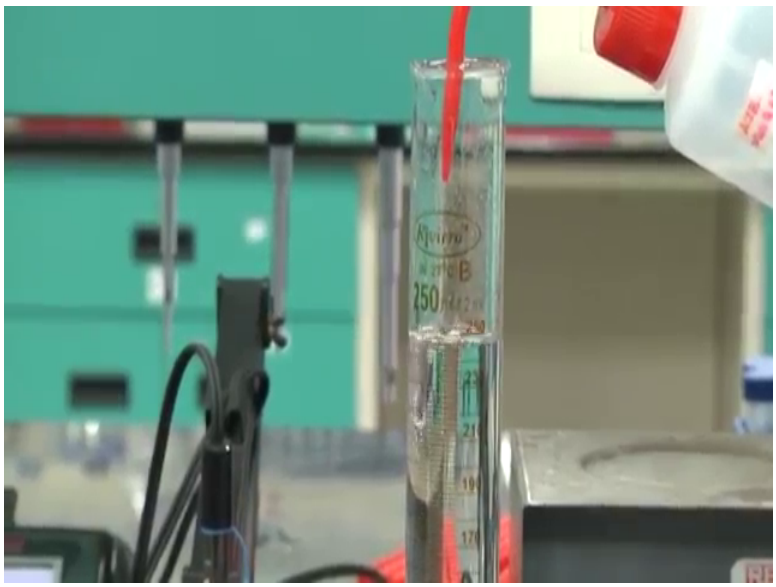

Again, once it reaches around 240 ml slowly add double distilled water.

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Now, we will take it in a eye level and it is around 250 ml. And now we will again pour this solution in a clean dry beaker which we will be containing our sodium hydrogen and phosphate dibasic solution.

We will again wash this bulb of the pH meter since we have previously used this for measuring the pH for monobasic which was acidic, and rub it once again using this tissue paper. Repeat it 2 to 3 times. And then we have to transfer this thing into the baker containing the solution of sodium phosphate dibasic.

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Here we have immersed this pH meter bulb in the solution. Here we can see the pH comes around 9 around 9.1 to 9.09, here we can see so; the pH for this dibasic acid is basically in the basic region or in the alkaline region what we can say it comes around 9.09. So, once we have done with measuring the pH of both the solution, we will again wash the bulb, we will take it carefully from the solution and again wash this one using double distilled water.

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Now, we will again wash this using double distilled water and wipe it using a tissue paper and then immerse it in the solution out here. We have prepared two different solutions one is sodium hydrogen phosphate monobasic and another is a dibasic. The two different solutions have two different pH values, one pH values that is for sodium hydrogen dibasic is around 9 and the other for monobasic its around 4.

Now, what we will do? We will proceed with preparation of phosphate buffer say for pH 7.4. Now, the concentration for both of this components are fixed that is 0.1 molar or 100 millimolar. Unlike the Tris part where the individual component concentration cannot be exactly or precisely determined out here we can basically consider the concentration of each and every components that is for monobasic and dibasic. Now, how to proceed for preparation of phosphate buffer?

Now, phosphate buffer pH may range from 8 to 6. Now, 6 like 6.5 or 8.2 or something like this and the most common part is 7.4 which we are demonstrating currently. Now,

how to proceed? For preparation of phosphate buffer having pH 6 or 6.5 we have to take one component in a beaker and add a second component gradually in a titration method which component to take is the primary question.

Now, the component we have to take in a beaker should be or rather the component which have the maximum volume should be that solution whose pH is near to a desired pH. Now, let us take an example for this one.

Suppose the solution or the pH we are bothered with is pH 7.4 or not 7.4 currently the pH we are bothered with is basically 6.5. So, we have two components, number one component is one is the monobasic part which is having a pH of around 4 and a dibasic part which is having a pH of around 9. So, which one to take? Basically, we will take that pH which is closer to 6 or 6.5 in this case we are having the pH that is 4 that is for the monobasic part we will take in a beaker, and then we will add a dibasic part gradually and so that the pH 6.5 can be achieved.

When we are going for pH 8 or 8.2 something like that for phosphate buffer, in the similar way we will take that solution whose pH is closer to 8. Whose pH is closer to 8? The solution that is dibasic solution having the pH of around 9. So, dibasic solution have pH around 9, and hence we will take the dibasic in a beaker and gradually add the monobasic part to it in order to take the pH from 9 to 8. The basic component for preparation of phosphate buffer is that, we have to take that solution whose pH is closer to a desired pH in a beaker and add the remaining and the next solution gradually using a be pipette to it.

So, the solution we are preparing is 7.4. So, which one to take in a beaker? Yeah, actually you guessed it right the solution we take in a beaker is the dibasic one having pH 9 around 9 and we will add the monobasic one having pH 4 in order to bring the pH the net pH to around 7.4.

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Here you can see we have taken the dibasic part in a beaker and we have immersed the pH electrode out in this beaker, and the pH of this solution is coming around 9.07. It is closer to 9, this is a dibasic one. Here we have taken the monobasic one and this pH is around 4 which you have measured just a few minutes ago.

Here what we obtain is that we have taken a magnetic bar and kept in a magnetic stirrer. Now, one thing we have to be careful is that kindly check that the pH electrode does not come in contact with the magnetic bar which is actually rotating within the solution. What will happen is that if the magnetic bar heats this pH electrode the pH electrode is very delicate and it will affect the pH electrode it might break or it might crack. So, it is better to maintain some distance between the pH electrode and the magnetic bar.

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I have taken 1 ml micropipette. Here you can see the 1 ml micropipette and this is the 1 ml tip, I will be taking this solution that is the monobasic one using this pipette and gradually adding in this dibasic solution.

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Here I am immersing this pipette, taking the solution and I will be adding drop wise.

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Here we are gradually adding this one and the pH is being getting reduced. And once you add it kindly give it some time for the mixture to become homogeneous and again start adding the solution.

So, here we can see the pH of the solution gradually decreases. While adding the solution we should be careful not to pour the solution directly into the pH electrode and try to pour the solution into the dibasic one kept in the beaker carefully. And again, I am giving it sometime in order to mix and become homogeneous. Do not increase the speed too much of the magnetic stirrer, keep it gentle. From 9 it has already come to 8.36. So, the pH is gradually decreasing it has come to almost 8.3. At one point we can see the change in pH becomes very slow and that is basically the buffer region. In a in the initial part that pH decreases in a spontaneous manner in a quite smoothly, but later in the part where buffer region is reached that pH becomes the pH change becomes quite slow.

Here we are preparing around 200 ml of the desired pH solution pH 7.4, that is why we have taken the volume which is lower than 200 that is the basic one dibasic one and we are adding monobasic one drop wise. Earlier what is to happen is that for one ml of addition of this monobasic acid we can see the change in the pH was very high, but here the change in pH is around 0.1 to 0.2 units or the change is around 0.01 to 0.02 units. So, the change is very slow here we can see.

So, it has reached 7.74. Our aim is to reach the pH 7.4. Whatever happens please note in a hurry do not pour bulk of the solution into a dibasic one in the buffer region. Sometimes it might happen that one might get impatient and add a large volume of solution in the dibasic one, but prefer not to do that because that will not give you the desired pH you have to add always drop wise. And what might happen is that if you add bulk amount of the monobasic one the pH of the desired pH might get crossed, like if I add a large volume like 5 ml of this solution in spite instead of adding this drop wise. Then what might happen instead of 7.4 it might go to 7.0 or even up to 6 in the range of 6.8. So, it is not desired. In case such thing happen, we have to adjust it using the dibasic acid that is using the sodium hydrogen phosphate having a higher pH value.

So, we have reached around 7.5. Now, we have to add very carefully add drop wise our desired pH is 7.4. So, here the pH changes very slow out here we are adding drop wise patiently and allow the magnetic stir to make it homogeneous. It is fluctuating between 7.47, 7.46. So, initially what is to happen is that for 1 ml of this monobasic acid solution the change in pH was around 0.3 to 0.5, but here the change is pH is 0.01. If it is completely in the buffer zone and it is reaching our desired pH it is 7.43.

Now, we will be adding 7.42. I am giving some time in order to mix the solution 7.41. Hopefully this would be the last 1 ml addition add drop wise just drop wise here we have reached 7.4, and just let it be there for some time. So, in certain times fluctuation might be there we can get 7.41 or 7.39. So, let it be there for a few seconds around thirty seconds or so.

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So here we can see that the solution is stabilized the pH meter is stabilized and it is showing 7.4 value. Thus, we have prepared our phosphate buffer of pH 7.4 by using two different components, number one is sodium phosphate dibasic one which we have taken in a beaker and to it we have added the second component sodium phosphate monobasic part.

By this say, we have taken it to 7.4 and the advantage of this method as we have disused earlier also, that we can actually monitor the concentration of the two components unlike the Tris part where we cannot exactly monitor the ionic strength of H Cl or the other component. Hence, we have seen how to prepare our phosphate buffer solution we have started by weighing the two different components, and then we have dissolved each of those components in double distilled water and adjusted the volume and then we have prepared our desired solution of pH 7.4.

So, while measuring the pH make sure the electrode is properly dipped in the solution, and also take care of the electrode in a properly manner because the electrode is sensitive and fragile.

So, today we have seen how to handle a pH electrode. Initially we have seen the how to take care of the electrode bulb and other basic precautions for handling pH electrode. And next we have seen how to prepare phosphate buffer. Initially we have prepared phosphate buffer of pH 7.4 using two components, one is the dibasic one and one is the

monobasic one using defined ratios of the two solutions. And secondly, we have prepared Tris H CL buffer of pH 8.2 these two buffers are required for various biological experiments. And apart from this there is several buffers are defined pH values which are required for specific experiments. Hope we have shown you how to handle the pH electrode and prepare buffer in a proper way.

Thank you.

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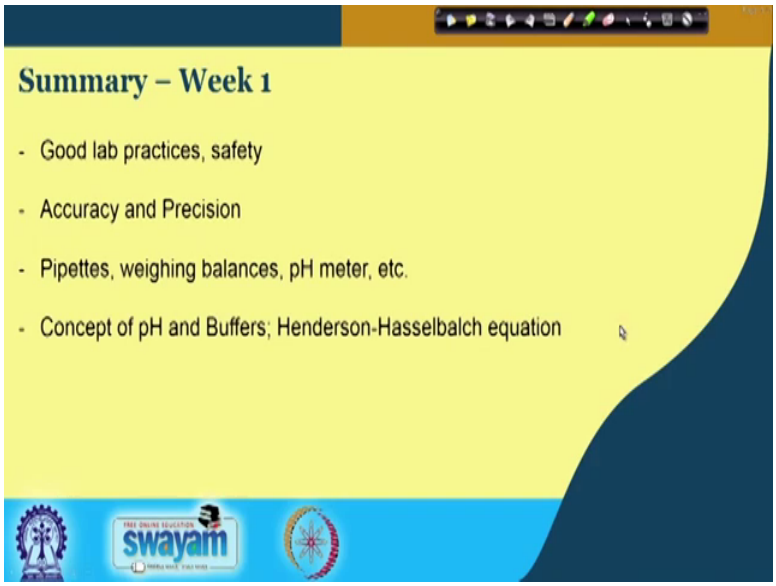

Hello. So, in this first week of lectures we have talked about several things and in this so I am going to summarize the important points of what we have learnt in this first week of our course. So, we have primarily, talked about good lab practices and for example, you should wear a lab coat, you should wear shoes in your lab, you should wear goggles when you are working with acids or basis you should wear gloves things like that.

So, the other important point is that you should always maintain a safe work environment. So, you should maintain, you should follow the proper safety guidelines which are important not only for your safety, but also for the safety of your co-workers. So, other students who are working in the lab. We discussed about accuracy and precision. So, this concepts are introduced and these are something that are used regularly in the lab, whether you are making your reagents or whether you are doing an actual experiment and getting your data. So, it is very important to know what is accuracy and what is precision.

We introduce some of the commonly used equipments in the lab. So, these are all small equipments that we use in a biochemistry lab on a daily basis. So, to accurately measure volumes we use pipettes or micropipettes, we use weighing balances, pH meter etcetera. So, you have seen all that being used in the lab.

And finally, we have talked about the concepts of pH and buffers. So, buffers we have seen that there are so many different buffers we talked about, goods buffers, and we have specifically talked about and also prepared in the lab two buffers, Tris buffer and phosphate buffer. These are the two buffers that are most widely used in a biochemistry lab. And we have also talked about the basic concepts of a buffer preparation which is Henderson-Hasselbalch equation. We have talked about conjugate acid and conjugate paste. So, it is important to know these principles because based on this you are going to design your experiments and make your buffers. So, these are some of the concepts we have covered, and we will see you again in the next week.

Thank you.