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Lecture – 05
Making Tris Buffer [pH=8.2]

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So, here we are showing three different types of weighing balances and depending on your requirement, you should choose which balance you need, because every balance has its own range. Suppose, this balance can maximum weight up to 820 grams and it goes up to 2 decimal point. So, if you can go precisely measure up to two decimal point of a particular sample.

In this balance, the minimum range is written as 100 mg and the maximum range is written as 310 gram and it can go up to 3 decimal point. So, like that, this one is a high precision weighing balance where it can go up to four decimal point. So, all this range will be written on that particular weighing balance. So, depending on your requirement you should check first, how much it can measure and how accurately or precisely it can weigh your particular sample.

Now, suppose you are making some kind of buffer or media, then you can choose this two type of balances because, their high precision is not needed. Suppose, you are measuring lb or tris, you can use this kind of weighing balance.

Where, suppose you are experimentally designing a particular acid, where you need to measure some inhibitors or small pectors or some kind of small molecules around a value of 1 mg or 10 mg, then you should always go for a high precision weighing balance like this one; where you can precisely measure that particular amount, those kind of measurement will not be happen here in this big weighting balances where it will not give you the precise measurement. So, depending on your requirement you should choose which p weighing balance you need to use.

Now, we will make 1 molar of 250 ml of tris. Now, what does mean the molar solution? So, 1 molar solution means, 1 molar particular solute is dissolved in a 1 liter of that particular solution. So, to make a molar solution, you need to know the molecular weight. Now, how will you know a particular samples molecular weight? Either you can in online or net it will be available or the best way to do is to check the bottle of that particular solute or the sample.

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In that bottle, it will be written, what is the molecular weight of that particular sample. Suppose, here I am showing a trizma base which is another name for tris and the molecular weight is 121.14 gram per mol; that means, 1 mol of tris weigh around 121.14

gram. So, if I need to; want to make 1 molar of 1 liter solution, I will weigh 121.14 gram of this tris solute and then dissolve it into 1 liter of solution.

So, it is a better habit to check the molecular weight in that particular box because different sample ha might have a different weight and always the online value may differ because, here I am showing EDTA. So, it is a common reagent for in biochemistry lab and here the EDTA is dehydrate. So, the molecular weight comes around 416.20 gram per mol, but we have another type of EDTA; so both are EDTA, but this is anhydrous. And the molecular weight differs and it becomes to 92.24 gram per mol.

So, suppose you are checking it the molecular weight for EDTA and the online it which it is showing some kind of value, it might differ what kind of sample you have in your lab. So, it is a best practice to always check, what kind of molecular weight it is written in that particular sample box and refer to that particular box and based on that you should calculate, how much you will need.

So, I have mentioned that molecular weight comes in gram per mol. So, if you want to make 1 molar solution of tris in 1 liter, you should measure 121.14 gram and then dissolve that particular tris in 1 liter of solution, but we will make 1 molar of 250 ml of tris solution.

So, for that there is a measurement. So, basically it goes half or one-fourth in this case. So, if you want to make 1 liter of 1 molar solution you may measure that much of gram 121.14 gram of tris and then dissolve it in 1 liter. If you want to make 1 molar of 250 ml of solution, you just follow a unitary method and calculate, how much tris you will need? So, here I have calculated how much tris I will need and this is almost one-fourth and that will be 30.15 gram.

Now, I will measure that particular volume or sample of this tris in a weighing balance and then I will try to dissolve it in water or basically the ionized water. So, now I will measure 30.35 grams of tris to make 1 molar of 250ml tris solution. For that, before we start measuring any kind of sample, first check the level of the particular weighing balance. So, all the weighing balance contains a small bubble which is a leveling bubble and you should check if the bubble at the proper at the middle center of that ring. So, that the weighing balance gives a accurate measurement.

And also make sure that, the weighing balance is properly cleaned. So, this kind of calibration and leveling is also required at a regular interval. So, after you are sure about the level of that particular weighing balance, then you open the sliding door of the weighing balance and you put the paper in which you will measure your salt.

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Now, the paper will have weight of itself and then you need to tear the weight of that particular paper. So, that the weighing balance make it a 0, then when you measure your sample it will show the sample weight, otherwise the additional weight can come from the paper. So, make sure you tear the paper or any kind of container which you are using for weighing.

Now, I will gradually add the salt. So, as I have mentioned that I will do into small batches and to measure the 30.35 grams of tris, first I have taken 15.35 grams of tris, I will take out this small volume and then put this sample into my clean beaker. Again, I will put this paper back into the weighing balance, again I will tear it to make it 0 and then again I will weigh another 15 grams of tris.

Now, I have measured another 15 grams of tris. So, before you actually believe the value which is showing by the weighing balance, you should always close the doors because if there is any kind of air cannel it might change the readings. So, close the door and wait for sometime and make sure the value is stabilized and then you check the value. After

you are sure about the value, then again you take out the sample and again I am putting this thing in the same beaker where I have total 30.35 grams of tris.

After you are done with the weighing balance close the door, if there is any kind of spillage you should immediately clean it with a tissue paper and then you switch it off. Now, that I have measured 30.35 grams of tris, then I will close the main container of the tris properly, it should be air tight and do not use the spatula again to weigh something else; always use a clean spatula and you can wash it first with some kind of liquid detergent and clean it and then use it for another round.

The paper which we have used you, if it is a reusable both you can reuse it again, otherwise here I am using which is non reusable I will throw it. So, that people do not mistake it and next time when you measure something else, you do not contaminate that particular sample with something else. After you switch it off and then I have taken this 30.35 grams of tris.

Now, I will pour some deionized water into it, for that I have taken in a beaker some deionized water and I have also taken a clean properly washed measuring cylinder which is of 250 ml of volume. So, as I want to make 250 ml of tris solution, it is not advisable to add 250 ml of water. So, you need to adjust the volume because the salt itself has some volume on itself and again you need to adjust the page of that particular solution. So, we will be adding concentrated HCl to adjust the pH, it will have again some volume.

So, for that first we will add around 150 ml of deionized water. So, add some amount of water, so that the salt is properly dissolved before you start adjusting the pH, then after you adjust the pH then make up the volume till 250 ml. So, right now I will be storing this deionized water, later we will be again needing this to adjust the volume. And here, I have a clean dry magnetic stirrer, I will dip this stirrer into the solution. So, that it will help me to dissolve the salt.

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Now, I will put this container into a magnetic stirrer and where it will try it will make sure the salt is dissolved properly into the solution. As I have mentioned I will keep the solution in a stir bar; magnetic stirrer. So, I have kept the stir bur inside the solution and I will switch it on and it will rotate the stir bar and it will help me to dissolve the solute into the water. So, before we start measuring the pH, we have to make sure the whole solute or the salt is properly dissolved in the water.

Now, that the salt is dissolved completely in the solution. So, make sure you do not put too much of water, also you should not put too less, otherwise the salt might not get dissolved. So, if you feel that, the salt is not dissolving in whatever amount of water you have put you can again put some amount of water more and then try to dissolve the salt completely, before you go for pH check or check the pH.

So, now that my salt has been dissolved in the water, I will check the pH of the this solution and around that and tris pH is around 11 and I have to make the pH around 8.2. So, for that I will be adding concentrated HCl to change the pH from 11 to 8.2. So, for that as I have calibrated my pH meter, I will take out the probe from the storing solution and as I have mentioned earlier, always clean the probe with deionized water before you put the probe in another solution. Then dab it with a clean tissue paper to make the probe dry then open this cap before you start measuring the pH of a particular solution. Now, I will put the probe into the pH solution.

Now, we can seen I have dipped my probe inside my solution. Always maintain a certain distance between the probe and the glass wall of the beaker also the magnetic stir bar should not hit the probe, otherwise it might damage the probe and you will get erroneous value of the pH. So, now the pH of this solution is shown in the monitor, it is showing around 11.48. So, to bring down the pH of the solution from 11.47 to 8.2 which is my desired pH of the solution, I will be adding concentrated HCl to the solution.

Now, concentrated HCl gives fumes, so you can do this in a fume hood or otherwise if you are doing like me in a open space, maintain a certain distance from the concentrated HCl because it will harm you and always use a glass dropper or pipette and add this concentrated HCl in very small quantity to bring down the pH of the particular solution. Now, do not put the concentrated HCl at a one go or do not put too much of concentrated HCl or otherwise the pH will go down instantly. So, you should drop wise give the concentrated HCl and simultaneously check the pH of the solution.

Now, the pH is showing stabilizing, that means it has to be stabilized before we can actually take that particular pH. So, as I drop wise adding the concentrated HCl, the pH of my solution is going down. So, we want to make the solution around 8.2 because the buffering range of the pH, of that tris is around 8 to 9 and for physiological experiment or protein expression DNA, you need to have trace which is around pH 8.4 or 8.2. So, depending on your need you need to change the pH of your solution.

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So, as I am adding drop wise the concentrated HCl, the pH in the pH monitor it is changing and as the stir bar is still there in my solution, it is constantly mixing the concentrated HCl into my solution, so that all the volume having a uniform pH. As you can see, the monitor is showing a stabilized pH value which is around 9.5 it is still not 8. 2. I will add again small amount of this concentrated HCl into my solution and then again wait for some time and we will give the pH meter some time to stabilize the reading.

So, until and unless the monitor is stabilized the reading is shown as a ready value we have to wait, do not drop, do not add too much of HCl or at a 1 go always add drop wise. Now, that again the monitor is showing some ready values and I still did not get my desired pH. I will again small amount of the concentrated HCl and wait till the HCl which I have put into the solution is properly mixed into the whole solution and a monitor is also stabilized; do not rush to make the page at a one go, always add small volume and then wait.

Be very careful while adding the concentrated HCl it pro gives fumes and always keep a water bottle or some kind of water beside you. So, if there is any kind of spillage or if you might get contact with the concentrated HCl, you just rinse it with clean water first. Now, then I have got the desired pH of my solution which is approximately 8.2. So, minor deviation from the desired pH around 0.01 to 0.04 is acceptable.

So, I will stop here. Now, after I got my pH around 8.2, I will take out the probe from the solution, remove the solution from the magnetic stirrer. And again, I have to wash the probe thoroughly with the deionized water, dab it with the tissue paper.

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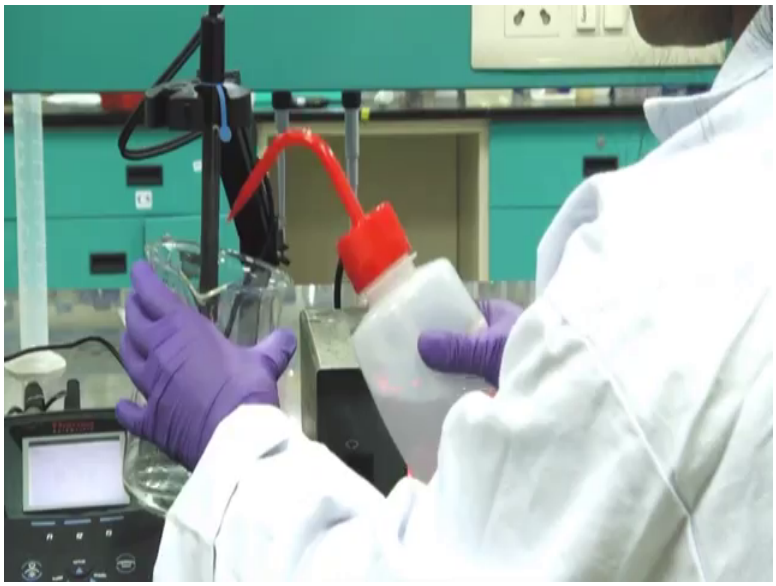

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And then for storage purpose, I will keep the probe inside the storing solution again and also I will make sure this cap is closed while storing and then switch off the pH meter.

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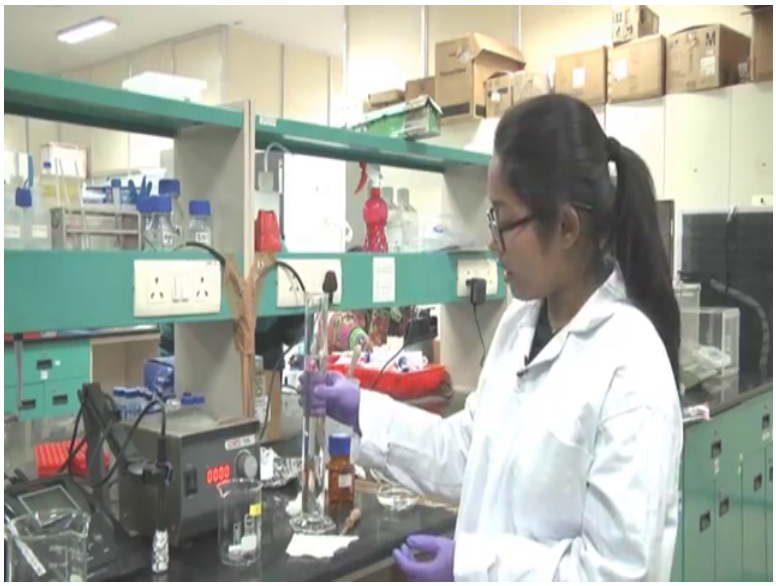

Now, in this container we have 1 molar of tris which pH is around 8.2, but still it is not 250 ml. So, we have to make up the volume to 250 ml, for that I am again taking the same measuring cylinder and I am going to pour all my solution in that multicular measuring cylinder without the stir bar. As you can see, while dissolving the salt I have put 150 ml of water, but now the volume has been increased to more than 170.

So, for this purpose you should not put the whole water at a one go, you should put sufficient water so, that the salt is dissolved and then, after you adjust the pH make up the volume with deionized water till 250ml. So, make sure you use deionized water, do not just add any water you front, because then the pH also will change and also it will put some other contaminants into your buffer which might be harmful for your sample. So, now I have 250 ml of 1 molar pH 8.2 so tris buffer, after this you can keep this buffer in a container and which will be readily used by other purpose.

Hello, hello everyone, this is Pritham Roy from Department of Chemistry, IIT Kharagpur. I am working under the supervision of Professor, Swagata Dasgupta in protein chemistry lab. I am one of your TA. So, hope we will come across various new and interesting experiments where we can learn various types of biochemical methods; see you soon, thank you.

We have seen, how to prepare tris buffer; it is tris excel buffer. For that we have taken powdered tris solution in double distilled water and to which we have added HCl drop

wise and regulated the pH. Now, what was the exact problem out there? Now, in that case there were small confusion about the ionic strength. We have added HCl to it, but we cannot regulate the ionic strength of HCl out there. Why? Because we did not measure the amount of HCl we have added into tris, while doing the pH adjustment.

Moreover, the HCl we purchased commercially, the exact concentration of HCl is not accurate all the time. As a result, ionic strength of the individual components in that case it cannot be accurately determined. Hence, the tris excel buffer preparation has some short comes. In this case we will prepare phosphate buffer in a defined method. For preparing phosphate buffer we will take two different components of known strength. So, out here, we will basically fix the strength, the concentration of the individual components; for that we will take sodium monobasic and sodium dibasic hydrogen phosphate.

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Now, let us see how to prepare the buffer? Now, the two components that we require for preparing this phosphate buffer are number 1, sodium phosphate dibasic. This is sodium phosphate dibasic, one component and the second component is sodium phosphate monobasic. Now, one thing we have to carefully note down, while using this chemicals are its molecular weight.

Now the molecular weight are generally mentioned either at the bottom of this bottle or on the side ways. Now, here the molecular weight is me written, the molecular weight of

sodium phosphate monobasic having a molecular formula of NaH 2 PO 4, the molecular weight is 119.98.

Now, why do we require this molecular weight? Now, why calculating the concentration, that is the required concentration for preparing buffer individual components have to be prepared of desired concentration; as we know that for making a desired concentration we need its molecular formula. So, this molecular formula as well as the molecular weight is required out here. Now this is one is for sodium phosphate monobasic.

Now, let us see the second component. The second component which we will be using is sodium phosphate dibasic heptahydrate. Now, in some bottles we can see this heptahydrate is not mentioned; however, we have to checkout whether this heptahydrate is mentioned or not, depending upon this its molecular formula will vary as well as the molecular weight.

So, if heptahydrate is not mentioned if you seen any bottle, where sodium phosphate dibasic is written and the formula mentioned is NA 2 HPO 4, then the molecular weight will be adjusted accordingly. However, out here since, heptahydrate that is 7 H 2 O is there. So, the molecular weight will increase depending upon this formula. So, the formula out here is NA 2 HPO 4 dot 7 H 2 O, it is written here NA 2 HPO 4 dot 7 H 2 O and the molecular weight written out here is 268.07. So, this is the molecular weight of sodium hydrogen phosphate dibasic.

So, we have taken these two components the monobasic 1 and the dibasic 1 and now we have to weigh the amount of this components required. Now, before proceeding for weight, we have to calculate the amount of this individual component required. For that we need to actually sort out the concentration of this component for the concentration of buffer we require the so, concentration of phosphate buffer. The concentration of phosphate buffer we will be preparing is 0.1 molar or 100 millimolar.

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So, let us see the amount or the weight of this individual component required. Before proceeding for weighing this define components, we need this spatula here and the second thing we require is this butter paper. Now, before proceeding to spatula, I want to show you something about this butter paper for weighing, what we need is this butter paper and the butter paper should be folded accordingly. Here we can see, we have folded it in a specific way.

So, how to fold the butter paper? Here, what we can do, we can basically fold it in this way we can all the four sides we can fold it out here and then diagonally we have to fold it, it takes something of this shape. So, basically it actually depends upon your convenience. Generally, if you do it in this way the chances of this reagents or whatever solid substance we are taking of coming out or falling from this butter paper reduces, when you take this sort of boat confirmation or boat type of structure of this butter paper.

Now, the second thing we require is the spatula ok. This spatula is a very common thing in a lab; however, it is very important for us to note down very crucial thing, that is we have to use a clean spatula each and every time. Before using the spatula we have to see, if any impurities are there in the spatula or not. Visually, we can see actually, whether any impurities are there or not. If any impurities are there, we can actually clean it using a tissue paper either dipped in moist tissue paper, dipped in water dad a double distilled water; however, the spatula I am using out here is completely clean and it has been washed with double distilled water and; however, before proceeding for this weighing

we have we are actually cleaning it using the scheme wipe, this part we are cleaning it. Now, let us proceed for this weighing part.

So, we have taken this butter paper and kept it in this weighing balance. Now, we will pressed here, anyhow we can see it becomes 0 0 everything; now we will add, the sodium phosphate monobasic. We have calculated it for 250 ml 100 millimolar sodium phosphate monobasic, we will require 3 gram of this chemical.

Now, we will be gradually adding this one. Here we can see, we have added just one spoon and it has become is 1 around 1.2 gram ok, so it is 2.5 gram. Now, once it reaches this 2.5 gram, we know that our desired weight is 3 gram, we have to add in small amount. You can actually better adapt a smaller spatula with a smaller mouth, we can get actually small spatula out here, but; however, for large calculation, large weight like 3 gram or fixed gram you can generally use this type of bigger spatula.

Now, here we are taking a very small amount of this sample out here and we are adding out here, now we have to add again a bit, again we are taking a pinch or a small amount of the sample. So, better if we use smaller spatula it will be much more convenient and easy to use it is almost 22.99 you can stop here, but I will take a pinch excess. Again, a pinch of this sodium phosphate monobasic I have taken and I have added out here.

Now, you can question actually, the weight I require is actually 3 gram and it has crossed from 3 to 3.032. So, is there any error or not basically for small change in this weight error would not be cause that much, but it is recommended to take oh some amount of excess weight, why? Because once you pour this substance into beaker or any conical flask or whatever thing you take, some amount of this chemical will stick in the butter paper. It generally do not stick in the butter paper, but if you use some other thing or any paper apart from butter paper it generally sticks there. So, the entire thing we have taken 100 percent does not come out. So, better to use an excess of this chemical once you take any weight.

So, now we will transfer this one to a beaker. Now, we will take this carefully. Now, once you take it here try to fold it in such a way, do not keep it flat ok, so it will fall. So, try to keep it in such a way and do not keep any beaker away from this weighing balance keep it close to this weighing balance. So, I am pouring here, I marked it basically this beaker, I am pouring here.

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Now, we will measure the other part that is, now we have measured monobasic we will measure the dibasic 1. Now, we will measure sodium phosphate dibasic heptahydric, for that we have taken this a clean butter paper, not the butter paper we have used previously, but a clean butter paper, we have teared it the weight is actually 0.00 now, the spatula we will clean it, I have cleaned it, actual previously after using the monobasic one. Now, I will again clean it. Actually, if the salt is if any salt is attached out here you can visually see it; however, I think it is free no impurities are there out here, I am cleaning it once.

Now, the weight of this sodium phosphate dibasic required for preparing 100 millimolar 250 ml solution is around 6.7. So, we will be taking 6.7 gram of this component, here we are adding in parts using this spatula. Now, we have reached 6.3, we have to add in small proportions; however, before proceeding I want to discuss something out here, if that suppose the butter paper we are using for weighing purpose is overloaded with the chemical you are adding; you required actually. So, what you can do is that you can actually note down the weight transfer this part into a clean beaker and again weight and again weight the remaining portion.

Now, if you actually take this overloaded butter paper, what we can do is that? It can actually, come out of this, it can fall from the butter paper. So, this you can order to prevent that you can basically take in two different steps, two or three steps; however, in

this case we will be carrying out using this single in a single step only, since I think it will not overloaded out here.

So, now I am adding in small proportion as we can see out here it is in small proportion I am adding, since it is 6.3 and the weight we require is the 6.7 adding in small proportion. Here we can see it has reached 6.726; however, an extra 0.026 or 25 gram we can actually neglect it or its better to take a bit excess.

Again, we will be taking our this butter paper carefully, use both of your hands if you are not confident enough to hold it with one hand, now take this beaker and add this one. So, here we can see we have taken 6.7 gram of sodium phosphate dibasic, this one is sodium phosphate dibasic heptahydrate. And once again I am reminding you before calculating the weight note down, whether heptahydrate is mentioned or not.

Now, what we have to do? We have taken this NA 2 HPO 4 in one beaker and NaH 2 PO 4 in another beaker. Now, we will add 250 ml of water and we will dissolve this using a magnetic stirrer.