Experimental Biotechnology
Prof. Vishal Trivedi
Department of Biosciences and Bioengineering
Indian Institute of Technology-Guwahati, Assam, India

Module-I
Preparation of Solution & Buffer

Lecture-06
Solution and Buffers

(Video Starts: 00:23) (Video Ends: 00:59) Hello everybody, this is Dr. Vishal Trivedi from department of biosciences and bioengineering, IIT Guwahati. Once you enter into a laboratory, the first thing what you are going to do is, you are going to prepare different types of solutions or reagents. Whether it is a reagent for performing the SDS-PAGE or the agarose gel electrophoresis or whether it is a reagent for doing some of the cell biology, immunology or the molecular biology experiments.

All these reagent preparation requires a definite training as well as the precautions what you have to take. So, in today's lecture, we are going to discuss about all these precautions, how to prepare the solutions and in addition, we have also going to discuss how to prepare the buffers. Because most of these solutions are made up of a buffers, so that it does not change the pH of that particular solution while you are doing the reactions.

So, we will start the lectures with the understanding how to prepare the different types of solutions, how you can what are the precautions you should take while you are preparing the solutions and what are the different ways in which you can be able to prepare the solutions.

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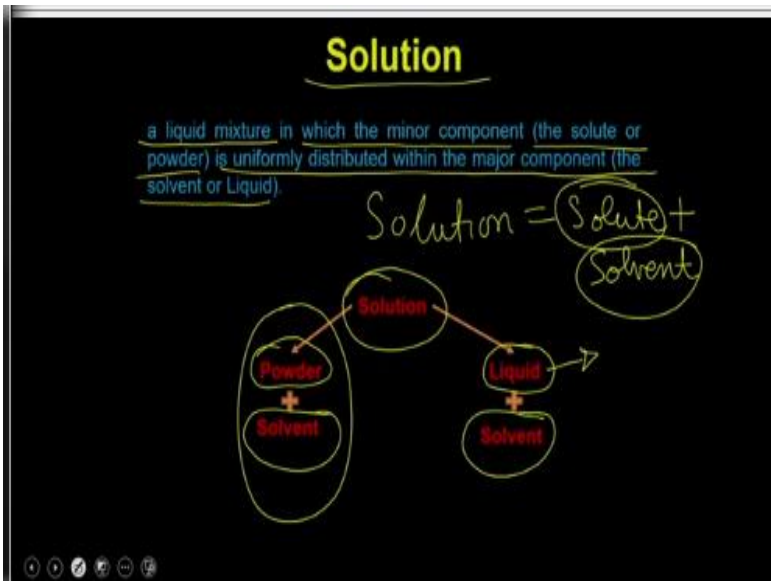

So, as the name suggests, the solution means a liquid mixture in which the minor component that is the solute or the powder is uniformly distributed within the major component that is the solvent or the liquid. Which means a solution is the summation of the solute which is actually the powder + the solvent which is actually the liquid part. So, solvent is going to be the major component, the solute is going to be the minor component.

But when you prepare the solutions in a life sciences lab or in a chemistry lab, you can actually prepare the solution by 2 ways. Either you take a solute which is actually going to be in the form of powder and then you dissolve that into a solvent and that actually is going to give you a solution. In the other way you can also have the liquid reagents like glycerol like for example.

And then you can add that to a solvent the, so there are 2 ways in which you can be able to prepare the solutions. Either you take the powder and mix it with the solvent and that actually is going to give you a solution or you can be able to just mix the 2 different liquids and that also is going to give you the solutions. So, either of this way the solution can be prepared in different ways.

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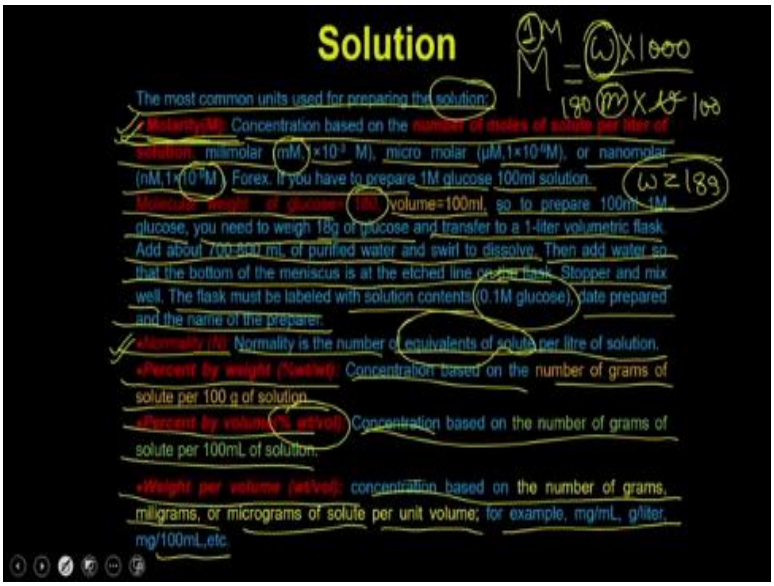

So, you can prepare the solution in the molar terms. So, when you prepare a solution, you can use the different types of unit to prepare the solution. For example, you can prepare the molar solutions like, so the molarity of a solution depends on the number of moles of the solute per litre of the solutions it can be millimolar. That is the millimolar means 10 to power -3 molar, it can be a micro molar, which means 10 to the power - 6 molar or a nanomolar solution which is actually going to be 10 to power - 9 solutions.

So, let us see how to prepare a molar solution. So, for example if I ask you to prepare the 1 molar glucose 100 ml solutions. So, the information what you require, if you want to prepare a molar solution is that you require a molecular weight of the molecule. So, in this case, the molecular weight of the glucose is 180 and the volume what you require, so volume is 100 ml. So, to prepare the hundred ml 1 molar glucose, what you need to do is, you need to weigh the 18 grams of glucose and transfer it to a 1 litre volumetric flask.

Then you add the 700 to 800 ml of purified water and you allow that to swirl to dissolve and then you can add water. So, that the bottom of the meniscus is at the line of the flask then you can use the stopper and mix well. The flask must be labeled with the solution consideration that is the 0.1 molar glucose which is 100 millimolar glucose. And date prepared and the name of the preparer.

This means the molarity what you can prepare is simply with this formula that is the w into 1000 divided by the molecular weight into the volume of that particular solution. So, if you put like 1 molar, for example, so in this case you put 1 here and weight you have to calculate and the molecular weight is 180 whereas the volume is 100. So, if I do that and if you do a math the w is going to be 18 grams in this case.

So, this is very easy to do because the molecular weight of a compound if it is dissolved in 1 litre, it is actually going to give you a 1 molar solutions. So, that you only have to remember, if you require to prepare the 0.1 molar solution then you just divide the molecular weight by one tenth. If you want to prepare the 1 molar solution but the volume is 100 ml then you just divide.

So, because when you prepare the solution in the lab you cannot do this kind of extensive calculations and you know. So, that is why it is very easy to understand that the molecular weight of a compound dissolved in 1 litre of solution or dissolved in solvent is actually going to give you 1 molar solution. If you dissolve the milligrams of solution and dissolve it into the 1 ml of solution or 1 ml of solvent that actually is going to give you the millimolar concentrations.

So, that is actually the way you have to calculate, so that when you are actually preparing these solutions, you should do very quickly. Because if I ask you 50 millimolar Tris pH 8.0 then you should not take time because you know the calculation for 18 millimolar or suppose 100 millimolar NaCl So, you know the NaCl molecular weight is 58.5, so you just divide that number by 10, so 5.8 grams is what required for 100 millimolar NaCl solution for 1 litre.

So, that is the way you have to do it in your lab, so initially when you are a new student in the lab, you might have to do calculation every time. But when you are slightly experience and then you know that what is the trick. The trick is you should remember if I have to prepare a 1 molar solution, I have to just take the molecular weight, I have to just go with the molecular weight which is actually being given on to the level of that particular bottle.

And then I have to just divided according to the volume. Similarly you can prepare the normality solution. So, normality is the number of equivalents of the solute per litre of the solutions. The

way that you prepare the molar solution the same way you have to prepare the equivalent solutions, a normality solution. The only difference is that you have to take the equivalent molecular weights of the equivalents.

Then you can also prepare the percentage by the weight which means the consultation based on the number of grams of the solute per 100 grams of solutions or per 100 grams of solvent. So, weight percentage is very, very difficult to do because you know in the case of solvent, how you are going to calculate the weight actually. So, if you want to calculate the weight, actually you have to take the formula of the water.

For example if I am using the water as for the weight, then the water molecular weight is actually the 18 grams. So, if 18 grams of water is actually going to give you so that is a way you have to calculate the molecular weight of your solvent. And the molecular weight the weight of your compound is anyway going to be in powder anyway. Then percentage by the volume which means the weight percentage by volume, so concentration based on the number of grams of solute per 100 ml of solutions.

Then you have the weight per volume which means the percentage based on the number of grams of or milligrams or the micrograms of solute per unit volume. For example the milligram per ml, gram per litre or milligram per 100 ml.

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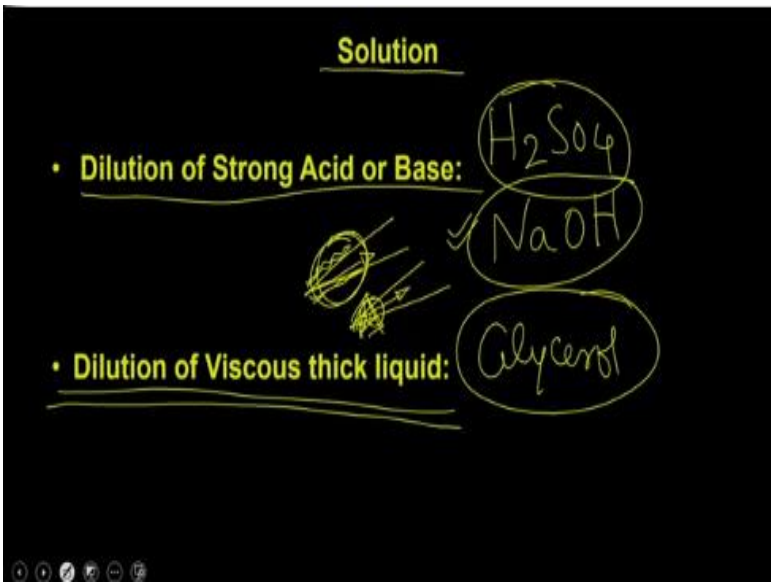

When you are preparing a solution you might have to do 2 processes one you can have to do a dilution of the strong acid or the base. So, when you are preparing a when you diluting a strong acid or a strong base. For example, if I am diluting like sulfuric acid which is actually a strong acid or if I am diluting the NaOH which is actually a strong base then I have to take a lot of precautions.

Because when you are diluting a strong acid you have to dilute the acid in such a way that you have to take the acid. And then you take that acid and drop wise you have to add that acid into the solvent system. Because you want to avoid the exothermic reaction, when you are actually diluting a strong acid it actually is a exothermic reactions. So, because of that the solution is going to be very, very hot, so if I take acid and if I start adding the water the exothermic reaction is going to be even bigger.

And that actually will sometime can actually damage the vessel where you have kept the acid. Number 2, it can sometime actually cause the injury because if the acid is very if the exothermic reaction occurs and that actually going to break the glass vessel or flask it actually can cause the, you know acid bond or the injury. The same is true for the base also. So, base when you dilute you have to be very, very careful because the base is also going to give you the exothermic reactions.

Similarly you might have to dilute like viscous thick solvent like for example the glycerol. So, if I have to take a glycerol and I want to dilute for example when you are going to prepare the sample buffer for the electrophoresis. The sample buffer actually contains the 40% glycerol which means you have to take the glycerol from the 100 ml from the 100% glycerol from the bottle and then you dilute it to 40%.

In that case taking out the viscous material like thick glycerol is actually quite a lot of precautions. Because of the only reason that these are thick solutions, so actually they are sucking through the pipette is going to be very, very slow. And they are also going to be deposit onto the outer surface of the tip. For example, if I am drawing the glycerol what happened is the glycerol role is going to attach to the outer surface of this tip.

As well as the movement of glycerol is going to be very, very small because the suction pressure is same whether t is the water or it is a glycerol. So, in that case what we normally do or what is recommended is that you cut the tip that top surface of the tip. And because of that the lower end of the tip is going to have the bigger diameter and then the sucking is going to be faster, you do not have to worry that you know I have removed the some part of the tip the accuracy of the volume what I am withdrawing is also going to be different.

That is not going to different because what the volume what you are going to withdraw is actually be proportional to the amount of vacuum what you have created in the pipettes, not to the tip shape or the size of the tip actually. So if I cut it actually I am going to suck the glycerol into much quicker. And on top of that because you are going to have large amount of glycerol on top of this tip, you also have to wipe this tip or across the, you know the bottle.

Because if that we would do, that you are going to remove all the excess glycerol what is present in. And then when you are dispensing this liquid into the next solvent, then also you have to be very careful. Because you have to keep pressing the pipette and you have to remain in that situation for very, very long time, so that the last drop of the glycerol is also going to be removed from your pipette tip.

Because as I said you know, the sucking of the thick liquid is also going to be a problem that the dispensing of that particular liquid is also going to be having the same trouble because it will take more amount of time for this liquid to come out from the pipette tip. And so that is why it is recommended yet you have to be very, very careful when you are handling the viscous thick liquids.

So, now once you have prepared the solutions, the solutions are actually been made in such a way that the solutions are actually going to have one component which actually going to resist for the change in pH. Because in most of the buffer most of the solutions you are actually going to add the buffer components. So, that when you are doing the reactions it should not change the pH of that particular solution because you want to keep the pH of that particular solution to be remained intact as the same.

For example if I am running the SDS-PAGE and if I am using the 1.5 molar Tris which 8.8, I want to ensure that the pH of this particular solution remain 8.8, it should not go like 6.8 or 9.5. Because if that happens then the resolution of that particular gel is going to be affected, the way the solutions are the way the molecules are going to be resolved onto the SDS-PAGE also may get affected. If there will be a change in pH.

So, that is why the change in pH is very, very crucial for performing the reactions as far as the you are talking about the biological reactions or the in the case of biochemistry.

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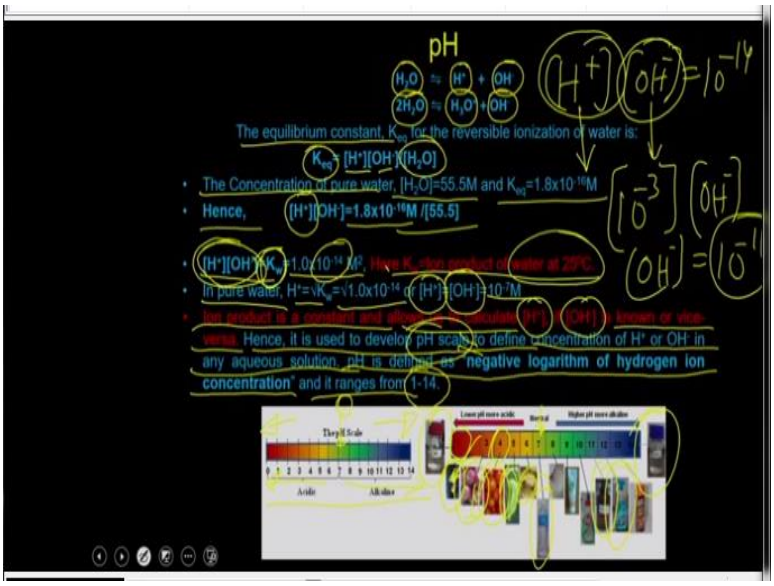

So, what is mean by the pH is that pH is actually a scale which actually measures the concentration of the hydrogen ion concentration within the solutions. So, you can understand that when a water is present it actually get ionized in the form of H + and OH -. So, if you take the 2 water molecules, it actually going to give you a hydronium ion as well as the OH -. Say if I ask write the equilibrium constant of this particular reactions, what I will do is, I will write the equilibrium constant of H +, OH - and the H2O.

So, the concentration of the pure water, so if I put the values of all these things. So, the concentration of the pure water is 55.5 molar and the K equilibrium for this reaction is actually 1.8 into 10 to the power - 16. So, if I put these values what will happen is that if you calculate the H + into the OH- is going to be 1.8 into 10 to the power - 16 divided by 55.5. So, if you solve all these and put the value of this that you are going to get the K w.

So, see the multiplication of H + and OH - is actually going to give you a value which is called as K w. And the K w is actually called is the value of the K w is 10 to power - 14 M squared. So K w is the ion product of the water at 25 degrees Celsius which means the product of the H + and OH -. This means in the pure water, the H + is going to be the underscore of K w and that is going to be underscore of 10 to power - 14.

So, if you solve that the H + is going to be equivalent to the OH - and that actually is going to give you a value of 10 to power - 7 M. So, ion product of a water is a constant and that allow us to calculate the H + in case the OH - is known or vice versa. So, this means the H +, the product of H + and OH - is actually going to be 10 to power - 14. So, if I have H + I can calculate the OH -, if I have the OH - I can calculate the H +.

For example if I have a solution of 10 to power - 3 and it is HCL actually, so then what will happen let us see, right. So if I have to calculate the OH -, OH - is going to be 10 to power - 11 because you can just divide this and that actually going to give you the OH -. So, hence it is use to develop a pH scale to define the concentration of H + OH - in any aqueous solution. This means if I know the H + I can calculate the OH -.

And that is why this ion product is giving you a scale where on one side you are going to have the acidic range. On other side you are going to have the basic range and in the center you are going to have the neutral center. So, that is why see so it is developed a pH scale where actually you are going to have the - 7 on one side and + 7 on the other side. And the pH is defined as the negative log of the hydrogen ion concentration, so it is ranges from 1 to 14.

So, this is what you have to see that the pH scale, where you have a 0 scale which is actually the pH 07. And on this side you are going to have the acidic range, on this side you are going to have the basic range which means on this side you are going to have more and more acid and this side you are going to have more and more bases. For example, I have given you an example of different types of products what we use in a daily life.

For example you have the HCL solution which is actually going to have a pH of 1 then you have the lime which is actually or lemon which is actually going to have a pH 2. Then you have the apples which are actually going to have the pH 3, then you have tomato which is pH of 4. Then banana, then potato and then you have the water which is actually of the pH 7 which is actually the neutral pH.

Similarly on the basic side also you have lot of solutions like you have the detergent powders and you have the acids and you have the NaOH solution which is actually going to give you a pH of 14. So, these are, so pH is a very, very important scale to measure the acidity or the alkalinity of a particular solution. So, if the pH is less than 7 it is actually going to be acidic, if pH is more than 7 then pH going to be alkaline solutions.

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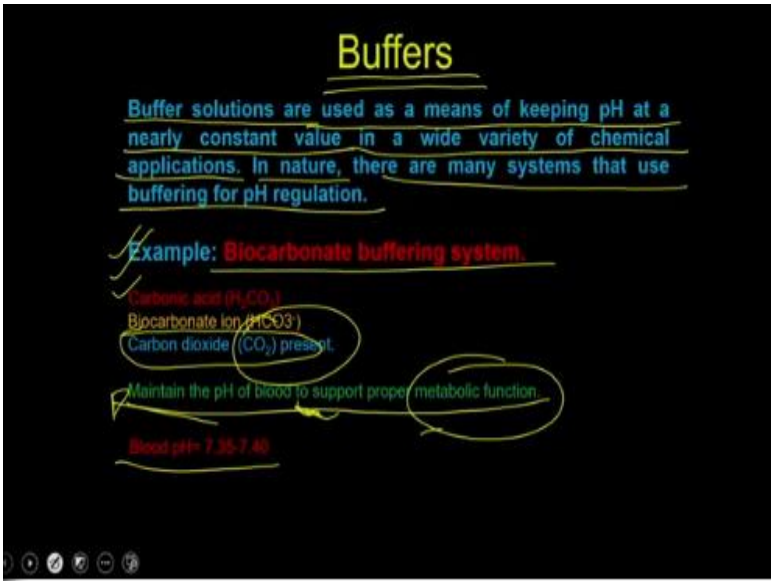

Now the question comes why you actually have required a buffer and what is mean by the buffer. So, buffer solutions are used as a mean of keeping pH at a nearly constant value in a wide range of chemical applications. In nature there are many systems that use the buffering for pH regulations. One of the classical example is the bio carbonate buffering system, so bicarbonate buffering system actually utilizes the 3 component, the carbonic acid H 2 CO 3 bicarbonate ions H 2 CO3 -.

And then the carbon dioxide which is actually been present into the air. So, by using these 3 molecules under the equilibrium the bicarbonate buffer is actually maintaining the pH of the blood to support the proper metabolic reactions. And you have many conditions or many situations where the slight change in the pH of the blood. For example pH of the blood is ranging from 7.35 to 7.4.

So, if you have slight change in the pH, for example if you have a pH of 7.2 you are going to have the acidosis problems like where the person is going to have the lot of problem in the breathing difficulties and all those kind of thing. Because what you remember is if it goes into the acidic range indirectly you are actually going to affect the carbon assimilations or carbon transport within the system, which means you are indirectly going to affect the oxygen transport as well.

So, if it is actually more and more as carbon dioxide is going to be associated with the body it is actually going to reduce the amount of oxygen within the body as well. So, that is actually is because the carbon dioxide is under the equilibrium within this particular buffering system. And that is how the carbon dioxide is been transported from one part of the body to another part of the body.

And eventually it reaches to the lung where the carbon dioxide is been removed from the body and the oxygen is been transported back. But if you actually going to change the pH of the blood and that actually if allows the accumulation of carbon dioxide within the blood, then it is actually going to change the overall respiratory activities and other kind of activities. Because the whole body depends on the respiration to perform the all the functions.

For example, even if it a liver which does not mean directly attached with the lungs and other places but it requires the oxygen to perform the functions. So, if there will be any change in blood of the pH that eventually going to accumulate the carbon dioxide into the liver or it actually not going to provide the enough oxygen for the liver to respire to produce the energy and to perform all the metabolic reactions. So, that is how the maintaining a crucial pH is very important for the normal physiology of a human being as well as for the other animals.

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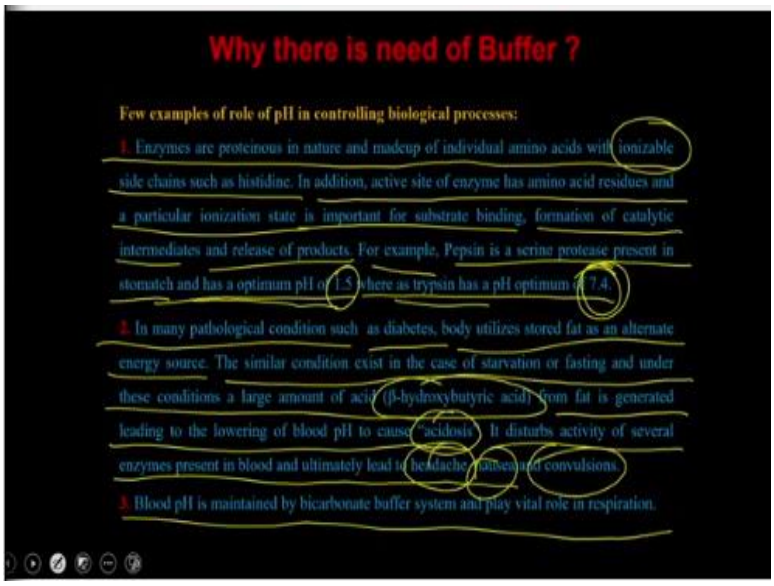

Apart from the physiology, let us see how the pH is also changing the other biological processes. For example, the enzymes or proteinous in nature and they are made up of individual amino acid with the ionizable side chain such as the histidine. In addition the active side of enzyme also has the amino acids. A particular enzyme is important for substrate binding formation of catalytic intermediates and the release of product.

For example the pepsin is a serine protease present in a stomach and has the optimum pH of 1.5. Whereas the trypsin has a pH optima of 7.4. So, that is why most of the enzymes are actually having the ionizable groups, they have the active side residues and all these active side residues are have to be present in a particular you know valency state and as well as the ionization states. So, because of that the particular pH of that particular value or the place where these enzymes are present it has to be in a perfect order.

So, that it should these enzymes are actually going to work very efficiently. One of the classical example is the pepsin which is actually present in the stomach and it requires a pH of 1.5 to digest the food. Whereas the trypsin which is another protease, require a pH of 7.4 to function. Similarly in many pathological conditions such as diabetes body utilizing stored food as an alternative energy process.

The similar condition exists in the case of starvation or fasting and under these conditions a large amount of acid like the beta hydroxy butyric acid from fat is generated leading to the lowering of blood pH to cause the acidosis. It disturbs the activity of several enzyme present in the blood and ultimately leads to the headache nausea and the convulsions. So, as I think we already discuss about the role of the blood pH. So, here are the few example like if a person is suffering from the diabetes and instead of using the glucose, if it starts using the fat and other kind of stored food material.

Then eventually it is actually going to produce lot of acidic bio byproducts like metabolic byproducts. And that actually is going to lower down the pH of the blood and that condition is called as the acidosis. And acidosis is directly going to affect the first organ that is the brain actually. So, if the acidosis is there, it actually going to affect the brain because the brain is going to deprived of oxygen.

And ultimately it is going to cause initially with the minor mild symptoms, it is going to cause the development of headache. But if the conditions continued and there will be no change in the pH of that particular blood. So, that there will be no supply of oxygen, then the headache is going to be turned into the nausea and convulsions. Similarly the pH blood pH is maintained by the bicarbonate buffer and it plays a vital role in respirations.

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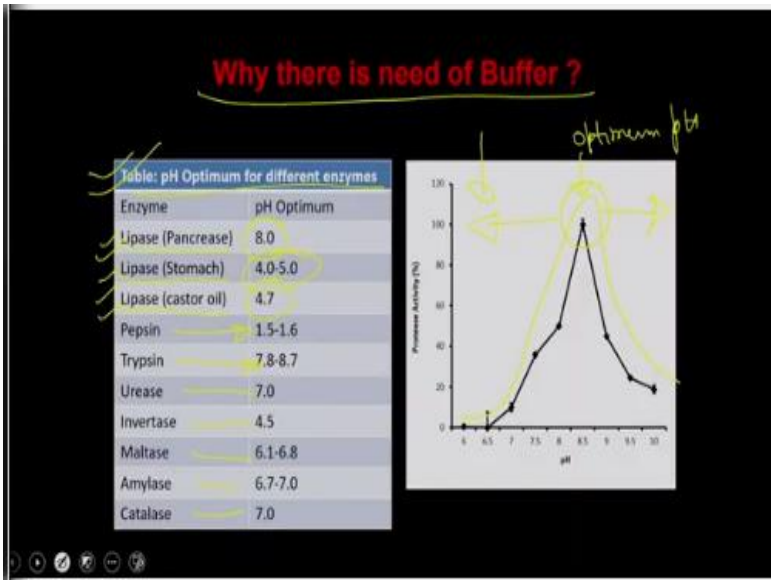

So, that means the buffer is very important for the enzymatic activity as well as the normal physiology for the body. Here are few examples where I have given you a table of showing that what is the pH optima of different enzyme. For example, even if you have a same enzyme you see the same enzyme is present in 3 different locations like the lipase which is present in pancreas having a pH optima of 8.

Whereas if the lipase is present in stomach has a pH optimal of 4 to 5, and if the lipase is present in the castor oil that if the plant the pH optima is 4.7. Then the pepsin which is the pH optima of 1.5, the trypsin pH optima of 7.8, urease 7, invertase, maltase, amylase and catalase. And in general what you see is if you plot the pH based activity of an enzyme what you will see is. It is actually having a biphasic behavior which means at this side you are actually having the optimum pH.

This is the place where the enzyme is going to work optimally on the both the side whether you were go on to the acidic side or whether you go on to the basic side you are actually going to affect the activity of these enzymes, why you are going to change the activity of enzyme. Because you are actually changing the ionization status of those amino acids which are either present into the crucial points where either they are crucial for stabilizing the structures or they are important for catalyzing the reactions.

So, either of these situations the change in pH is actually going to affect the ionization stage of the side chains. And eventually it is going to disrupt some of the, you know the electrostatic interactions or Vander wall interactions or the salt bridge interactions with the neighboring residues. For example, if you have a lysine and it is making a interaction with the glutamate and if you change the pH either of these pair are actually not going to be in a proper ionization state.

And that is how that particular interaction is going to be broken down. And once these interactions are going to be broken down, it eventually leads to either that particular portion of the enzyme is going to be moved or there will be a conformational changes in the enzyme. And that eventually is going to make the enzyme less efficient compared to that when it was present in the optimal pH conditions.

So, that is why the pH is very important and that is why the buffer is also very important that why to maintain a pH. Now the question comes how the buffer is actually maintaining the pH.

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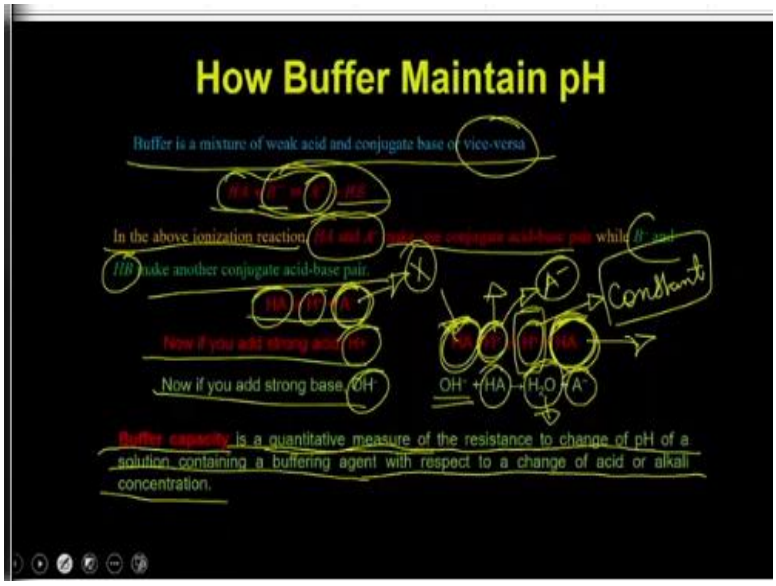

So, the buffer is actually a mixture of weak acid and a conjugate base or the vice versa. So, you can imagine that you have a condition like HA + B and that actually is ionizing to give you A- and HB. So, in this ionization reactions, the HA and A - are actually being part of the one conjugate acid base pair. Whereas the B - and the HB are actually making another conjugate acid base pair which means, the HA and B is making one pair and the B - and HB is making another pair.

This means the HA can that is why the HA is weak acid which is been associated with a strong base and that actually is going to give you the buffer. So, HA is going to be ionized like HA H+ and A -, so that is the ionization of the HA which is a weak acid and with a strong base. So, if you add the strong acid like if you add the H + so what will happen is the HA + H +, so there should be increase in H +.

Because you are increasing the H + but what happen is the H + whatever you are adding is actually combining with A -. And that is how you are actually getting the more amount of HA instead of getting the more amount of + H +, which means the H + remained constant and that is

the resultant of the change in pH. Because when you calculate what is the pH of the solution you are actually going to consider only the H + or ionizable H+ present in this particular solutions.

So, even if you have added the acid which is a strong acid and that actually is going to combine with the strong base and that is how it is actually going to be get neutralized. Now imagine that if I have added these strong base OH -, then what will happen the OH- is going to be added to the HA which is actually a buffer. Then what will happen OH - should have increased the pH.

But what happen is the OH- is going to combine with H+ and it is actually going to form the water and the A- will remain the same which means the base component will remain the A - which is actually been responsible for that particular pH of that particular solution. Since you are adding the base you expect that the A - should go up because that is the base component of that particular solution.

But instead of that it actually been neutralized by the acid component and that is how it is actually going to be you know remain the same pH. That is how in a buffer you have a combination of the acid as well as the base. So, if you add the acid the base is going to react and neutralize the acid. If you add the base the acid is going to react and neutralize the base.

And that is how it is actually going to maintain the pH of that particular solution but how long that buffer is going to maintain the pH. So, that is in always been measured with a definition called as the buffering capacity or the buffer capacity. So, buffer capacity is a quantitative measure of resistance to the change of pH of a solution containing a buffering agent with respect to a change of acid or the alkaline consideration.

Because you can imagine the even we are adding the H + and that H + is acting neutralized by A - that is actually going to be equal or proportional to the amount of A + or A - what you have in the solution. So, once the A - are going to be exhausted which means keep adding the acid eventually what will happen is that the A - is going to be exhausted, which means there is no longer the A - is going to be available to take care of the H + what you are adding from the acid.

And in that situation, if you add another drop of H + that H + is not going to be neutralized and at that actually is going to lower down the pH of that particular solution. So, that is all the buffer is going to maintain the pH until you have some form of ionizable bases or some form of ionizable acid present in that particular solution. And that is actually decides the buffering capacity of that particular buffer solutions.

And that can be measured simply by quantitatively if you titrate a buffer solutions with the acid and the base. And that actually is going to give you that value, what is the buffering capacity of that particular solution. And is it advisable that you should work with the buffer within it is buffering capacity, which means you cannot work beyond that buffering capacity. Because if you work beyond the buffering capacity then as soon as you are actually going to have any change in the H + or OH- concentration.

Or if there will be any generation of H + or OH - within the solution it is actually going to change the pH of the solution because you are working beyond the buffering capacity of that particular buffer.

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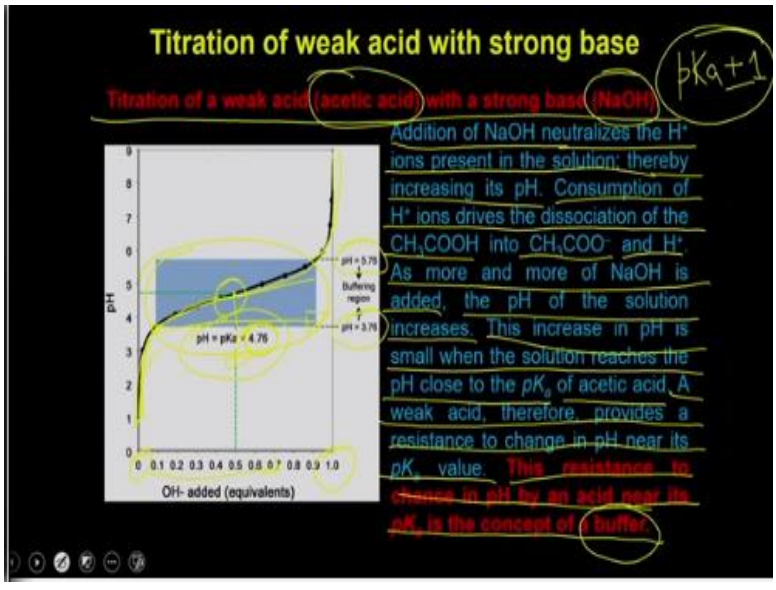

Now, let us see how you can be able to do a titration. So, if you titrate a weak acid, for example in this case, I have taken an example of acetic acid and with a strong base like the NaOH. So, addition of a NaOH neutralizes the H+ ion present in the solution, thereby increasing it is pH.

Consumption of H + ions drive the dissociation of the acetic acid into the CH 3 - NH + as more and more of NaOH is added the pH of the solution will increases.

Which means in the beginning you are not adding any OH this means pH of the solution is 1 actually. And as you are actually increasing the OH - you are actually taking up the pH of this you are increasing the pH and what you will see is that it is actually keep increasing as long as you are adding the OH equivalent ok. But at this point, ok you will see there are change of the pH is going to be very, very small.

So, this increase in pH is a small when the solution reaches the pH close to the pK of acetic acid. So, the pK of acetic acid is 4.6 and we will understand what is the pK. So, a weak acid therefore provides a resistance to change in pH near its pK values. This resistance to the change in pH by an acid near it is pK is the concept of the buffer, which means if you will reach to the pK of that particular weak acid you will see that it is taking longer time to change it is pH.

And that is how if you titrate that you will be able to know in what buffering range the change in pH or the slope of that particular. So, if you see the slope of this curve, the slope of the curve is very sharp here, but the slope of curve is very flattened in this particular region. Which means, in this particular region, the buffer is actually or the weak acid is actually resisting for the change in pH.

And that is how you can be able to calculate the buffering range of that particular weak acid, so the buffering range. For example in this case the pK is 4.6, the buffering range is starting from the 5.76 to 3.76 which means. As a thumb rule, if whatever the pK is there you are actually going to see the buffering range plus minus 1 which means, if your pK is 4.76 the buffering range will start from 3.76 to 5.76 which is actually in this case.

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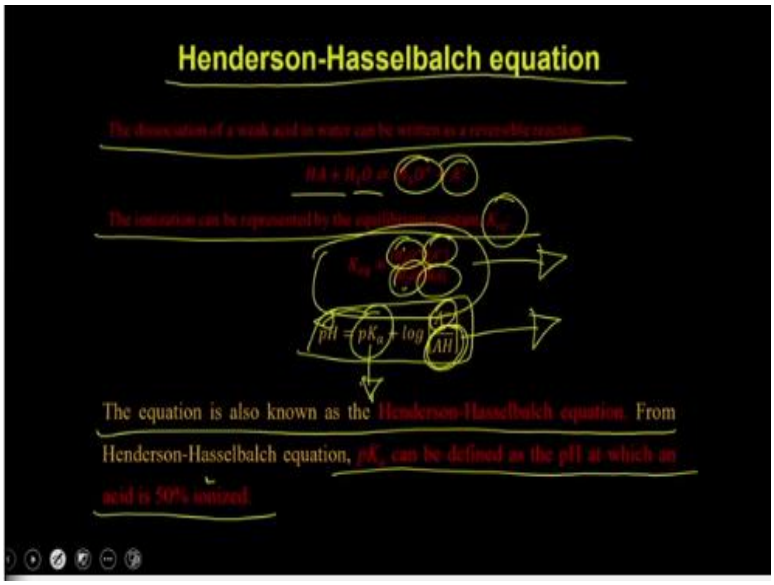

Now, we were understand what is pK. So, understanding the K will understand about the Henderson Hasselbalch equations. So, the dissociation of a weak acid in water can be written as the HA + water equal to hydronium ion + A -, the ionization can be represented by the equilibrium constant K equilibrium that is the hydronium ion A - water and HA ok. And if you solve all these equations and if you put the values of the H2 water concentration of water concentration of hydronium ion and all other concentrations, what will happen is.

The eventually you are going to get a equation called as the pH equal to pK + log concentration of A - divided by AH and this equation is called as the Henderson Hasselbalch equations. And according to equations the pK can be defined as the pH at which the acid is 50% ionized which means if A - is equivalent to A + AH, the pK is going to be equivalent to the pH. So, the pH at which the acid is going to be 50% hydrolyzed that is the pH what is called as the pK.

And PK is a very important information because it is tell you that at this particular point, the buffer is going to resist for change in pH. And the pK is also going to allow you to calculate the buffering capacity.

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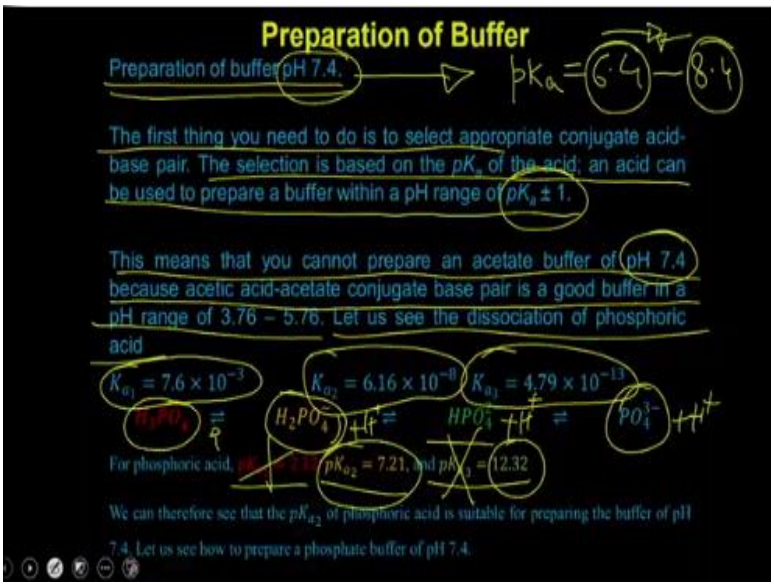

Now, let us understand how to prepare the buffer. So, I have taken an example of how to prepare the buffer at pH 7.4. The first thing what you have to need is because you want to prepare a buffer at pH 7.4 which means you are looking for a pK of 6.4 to 8.4 which means you are looking for some solutions which should have a pK of 6.4 or 8.4. Because you can easily go 1 unit down or you can go 1 unit up.

So, the selection is based on the pK of the acid and acid can be used to prepare a buffer with a pK range of pK plus minus 1 which means I am looking for acid which has a pK range of 6.4 to 8.4. This means that you cannot prepare an acetate buffer of pH 7.4 because the pK is 4.6 actually. Because the acetic acid-acetate conjugate base is a good buffer in a pH range of 3.76 to 5.76, let us see the dissociation fastened of phosphoric acid.

So, in the case of phosphoric acid which is actually the color H 3 PO 4 is been dissociate in 3 different forms. So, H 3 PO 4 is been dissociate first in the form of H 2 PO 4, so 1 hydrogen is been dissociated, so you have 1 H + here right. And then you have H 2 PO 4 which has been ionized to form the HPO 4 -, + H + 1 right. And then the HPO 4 is been hydrolyzed HPO 3 - + H +.

This means the phosphoric acid which is H 3PO 4 is being ionized in 3 different forms. And that is how you are actually going to have the equilibrium constant of these reactions. For 3

equilibrium constant and the pK of this H 3PO 4 is also going to be 3. So, pK 1 which is for this reaction is going to be 2.12, so that is not useful because you are you know this is very far away from 7.4.

The pK 2 is 7.21 which is for this reaction and pK 3 is 12.25, 12.35, so that is also not correct. So, this pK of 7.21 can be used which means you can be able to use the phosphoric acid to prepare the buffer of 7.4.

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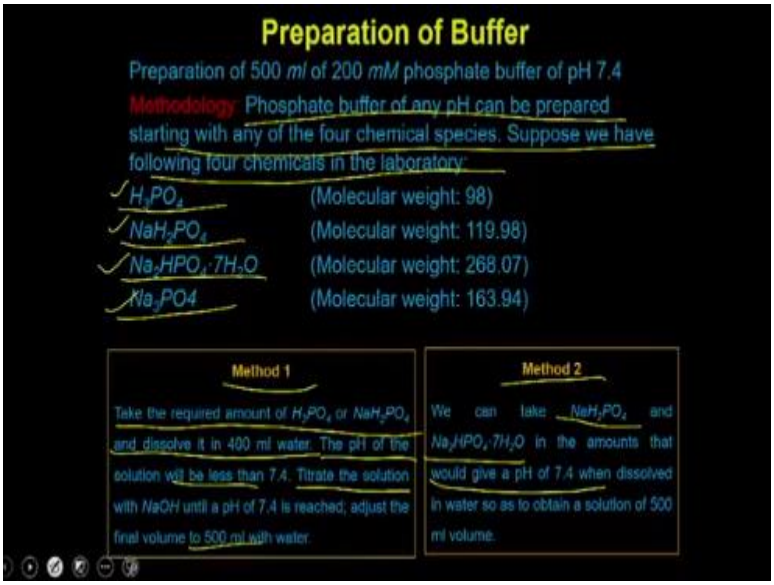

Now to prepare the buffer what you have to do is the phosphate buffer of any pH can be prepared starting with any of the 4 chemicals. Suppose we have the following 4 chemical in the laboratory like a phosphoric acid, NaH 2 PO 4, Na 2 HPO 4 and Na 3 PO 4which means all these are actually the derivatives of the phosphoric acid. So, this is the phosphoric acid, this is the sodium phosphate, this is a sodium di hydrogen phosphate and this is a sodium phosphate.

So, and the molecular weight is given. So, you have the 2 method in which you can be able to prepare the buffer what you can do is you can take the required amount of phosphoric acid or NaH 2 PO 4, you dissolve it in a 400 ml water. The pH of this solution will be less than 7.4 then you can titrate this with the help of the NaOH. And eventually you bring the pH to 7.4 and that is how you can be able to prepare the buffer.

The second method is that you take a mixture of NaH 2 PO 4, Na 2 HPO 4 and you bring the pH to 7.4 with the help of the solving the percentage of the both the components using the Henderson Hasselbalch equations.

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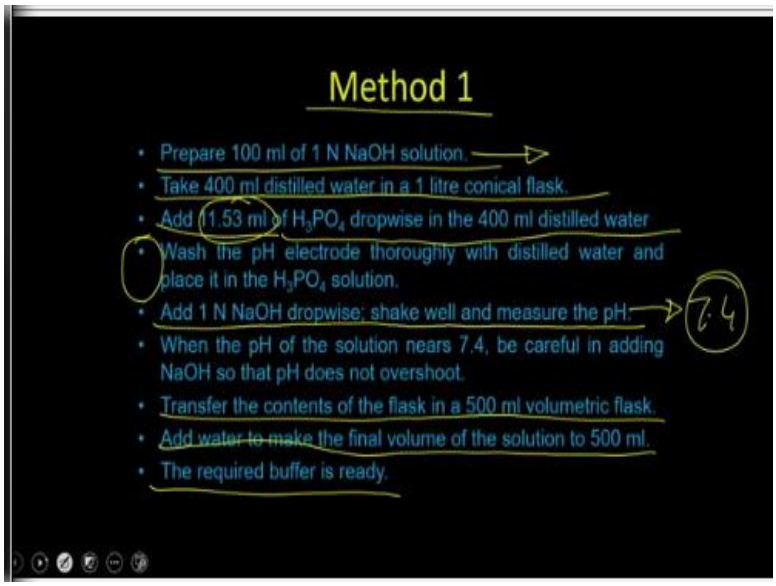

So, let us see how to prepare with method 1 and method 2 also. For the method 1 what you have to do is first you have to prepare the NaOH because that is what you are going to use to raise the pH of that particular solution. Then you take the 400 ml of distilled water in a conical flask, you add the 11.53 ml of the H 3 PO 4 dropwise in a 400 ml distilled water, you calibrate the pH meter or pH probe that we have already discussed in our previous lecture.

Then you add the one normal NaOH dropwise, shake well and measure the pH and you keep measuring the pH until it reaches to 7.4. And once it reaches to the 7.4 then you can transfer the content of the flask in a 500**,** you add water to make the final volume of 500 ml and the required buffer is ready.

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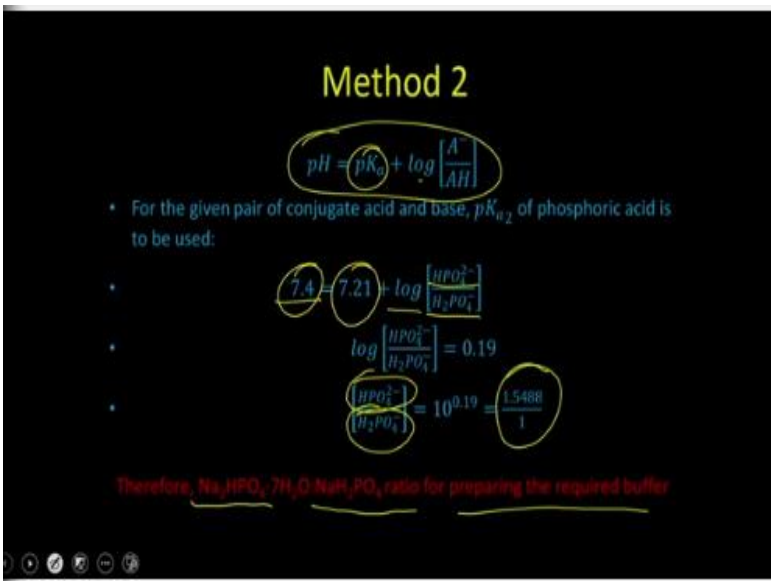

In the method 2 you have to use the Henderson Hasselbalch equations. So, the pH is equal to pK + log A- by AH. If you put all these values, so pH is 7.4, the pK is 7.21, the log A - versus AH. So, you calculate then it actually is going to give you the proportion of the HPO 4 2- versus the H 2 PO 4 - and that is 1.54 which means you have to take the Na 2 HPO 4 and NaH 2 PO 4 in a ratio of 1:1.488 .

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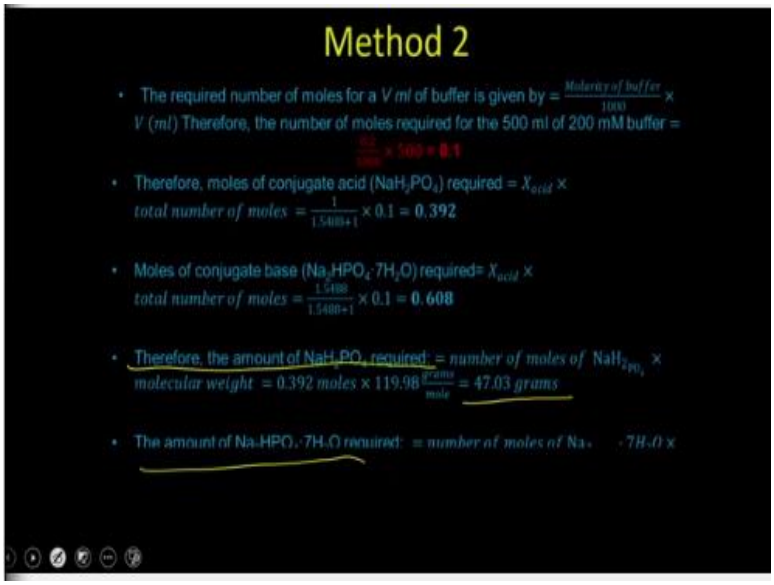

So, if you calculate all these, what will happen is the amount of NaH 2 PO 4 required would be the 47.03 grams and the amount of Na 2 HPO 4 would required would be the . If you can just put the value into this equations it actually going to give you the amount for the Na 2 HPO 4 as well.

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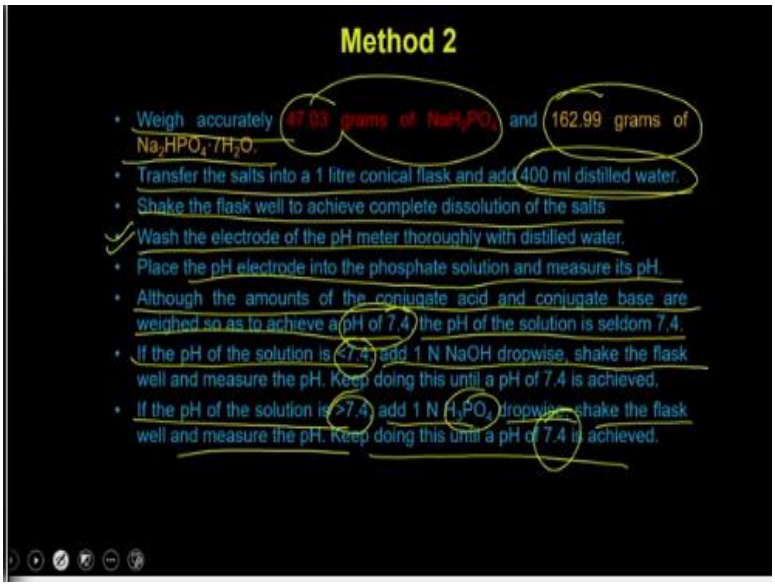

Then what you have to do is, you weigh the 47.03 grams of NaH 2 PO 4 and if you calculate you will get a value of 162.99 grams of Na 2 HPO 4. And that anyway you can calculate the transport the salt into 1 litre conical flask. And add the 400 ml of distilled water and then you shake well to achieve the complete dissolution of the salt. Then you, you know prepare the electrode for measuring the pH.

You place the pH electrode into the phosphate solution and measure it is pH, although the amount of the conjugate acid and bases are weighed. So, as to achieve a pH of 7.4 because as per the Henderson Hasselbalch equations the pH should be 7.4. If you are adding these 2 component in a proportion what is been calculated from the equation but it is not been always been achieved. Because there could be some variation and that is how the pH is roughly you will find the 7.4.

So, if the pH of the solution is more than 7.4 or less than 7.4 then you add the 1 normal NaOH and you can adjust the pH to 7.4. If the pH of the solution is more than 7.4 then you add the phosphoric acid dropwise and shake the flask and measure the pH, keep doing this until the 7.4 is achieved. So, if you have the less than 7.4, you add the NaOH and you adjust the pH to 7.4 if you have more than 7.4. Because in case some component are more and less then you can actually add the phosphoric acid to bring the pH down to 7.4.

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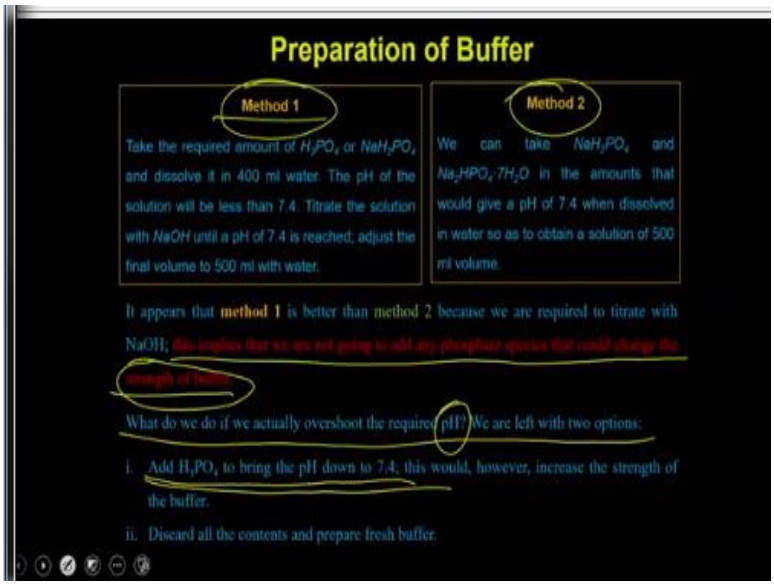

So, now what we have done, we have measured we have discuss about the preparing the buffer with method 1 or method 2. And I am sure you might have realized that the method 1 is much easier compared to the method 2. Because method 1 require just the phosphoric acid, you add the phosphoric acid, you adjust the pH with NaOH. Or in the method 2 you might have to do lot of calculations then you have to bring you know determine the ratios of the 2 components and then you have to adjust the pH at the end.

So, you know but the problem is that if you do method 1 or the method 2 irrespective of any of these method. Eventually as you are actually going to change the strength of the buffer which cause if you remember in the beginning itself we said that we have to prepare a buffer which is 200 millimolar phosphate buffer pH 7.4. Because if you do all these addition of phosphoric acid and addition of NaOH and all that.

It is actually going to change the buffer strength of that particular solution. So, if you have to very strictly calculate or you want to make the 200 millimolar phosphate buffer pH 7.4 then you cannot use either of these method. Because the both of these methods are actually going to give you the different concentration of the phosphate at the end because you have to use either NaOH or the Phosphoric acid to adjust the pH.

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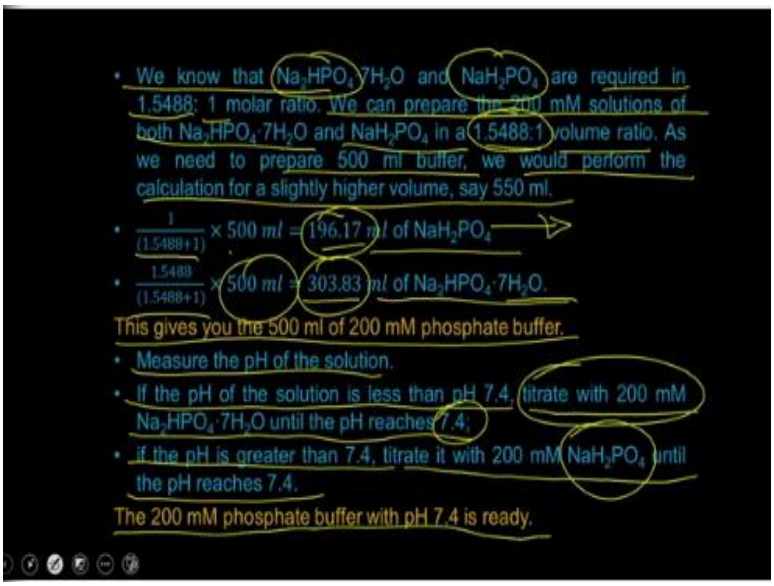

Then you can actually follow the third method and the third method what you have to do is, we know that the Na 2 HPO 4 and the NaH 2 PO 4 are required in the ratio of 1.54 versus 1. So, what we can do is, we can prepare the 200 millimolar solution of both the Na 2 HPO 4 and NaH 2 PO 4 in a. And then you mix them in the ratio of 1.54 versus 1 , so as we need to prepare the 500 ml buffer, we would perform the calculations for slightly higher volume for example 550 ml.

So, 1 by 1.54 + 1 into 500, so 196.17 ml of NaH 2 PO 4 and 1.54 divided by this and if you calculate it is actually going to give you 303.83 ml of Na 2 HPO 4. So, what you do is, you take the 196 ml of this 303 ml of this and then you mix them and that actually is going to give you the 500 ml of 200 millimolar phosphate buffer. Now you measure the pH it is possible that the pH of this solution may not be 7.4.

But in that case what you have to do is, if the pH of solution is less than 7.4, then what you can do is, you can titrate this with 200 millimolar Na 2 HPO 4 until the pH reaches to 7.4. If the pH is more than 7.4 then in that case what you do is you titrate with 200 millimolar NaH 2 PO 4 until the pH reaches to 7.4. So, irrespective of this buffer or irrespective of final pH whether it is above 7.4 or lower to the 7.4 then final buffer strength is going to be remain as 200 millimolar.

Because the solution what you are adding for titration is with a 200 millimolar strength. Earlier we were adding the concentrated NaOH or to the concentrated phosphoric acid and that is

actually is going to change the molarity of that particular final buffer. Whereas in this case you are actually adding the 200 millimolar strength of the solutions to adjust the pH. And that is how actually you are going to get the final buffer which is actually going to be 200 millimolar phosphate buffer.

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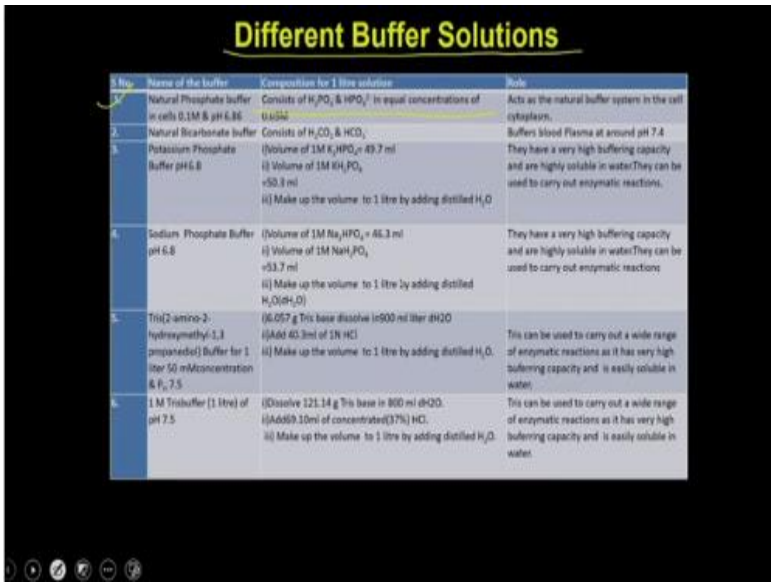

So, this is the different buffer solution what I have given you, I have given you the recipe as well. So, this is just for your information that you have multiple types of buffers and the recipes or multiple components what you have to dissolve and prepare the buffer. And then what is their applications in performing different types of reactions or different types of essays. And so with this I would like to conclude my lecture here.

And in this lecture we have discuss about how to prepare the solutions, how to prepare the buffers, what is the significance of the buffer in the biochemical reaction as well as in your experiments as well. So, with this I would like to conclude my lecture here, thank you.