Experimental Biochemistry
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Lecture – 02
Buffers

In lecture 2 of Experimental Biochemistry, we begin a discussion on buffers. We spoke about the pH yesterday, and how we can determine from the pH and a knowledge of the pK a value, the ratio of the salt by the acid.

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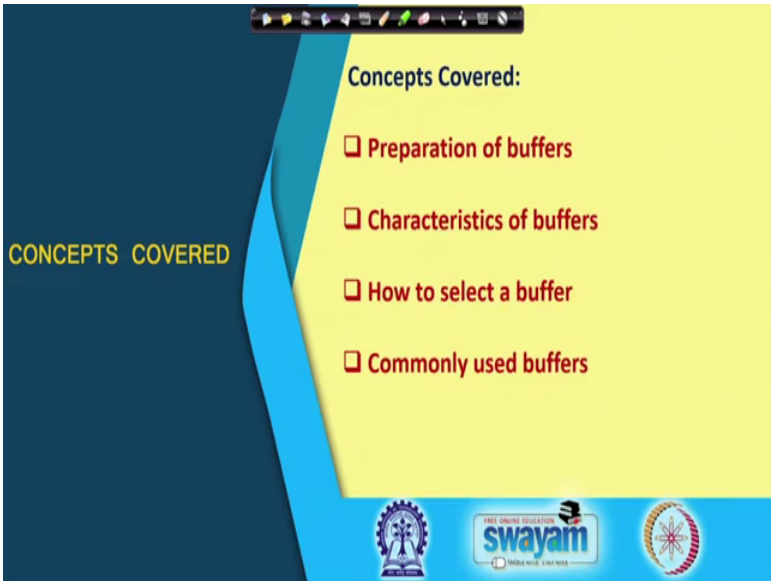

We will see today how we can actually prepare specific buffers, what are the characteristics of buffers, and what commonly used buffers we have for a biological or biochemical experiments.

First of all, what is a buffer at it is buffering capacity. A buffer as we saw in the previous class, maintains the pH of a solution by either taking up the protons that are released during the reactions or releasing protons, when they are consumed during the reactions.

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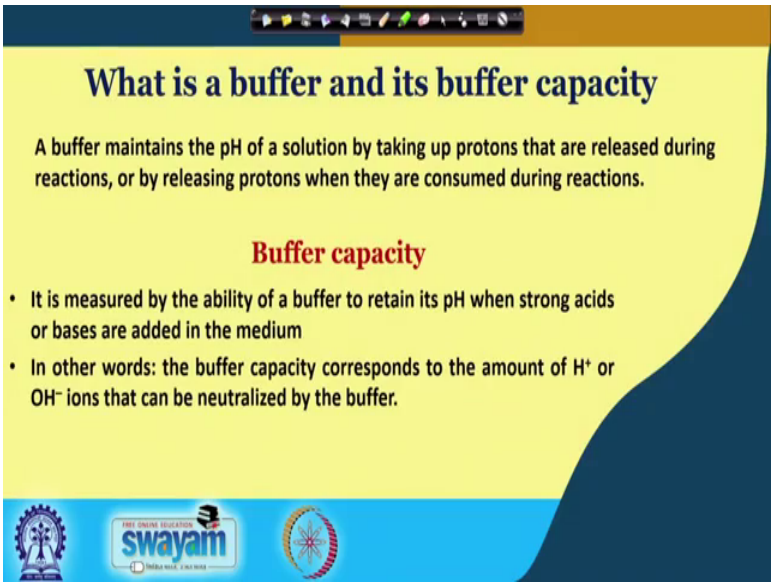

The buffer capacity therefore, is measured by the ability of the buffer to retain it is pH when acids or bases are added to the medium.

This means that the buffer capacity corresponds to the amount of H plus or OH minus ions that actually can be neutralized by the buffer.

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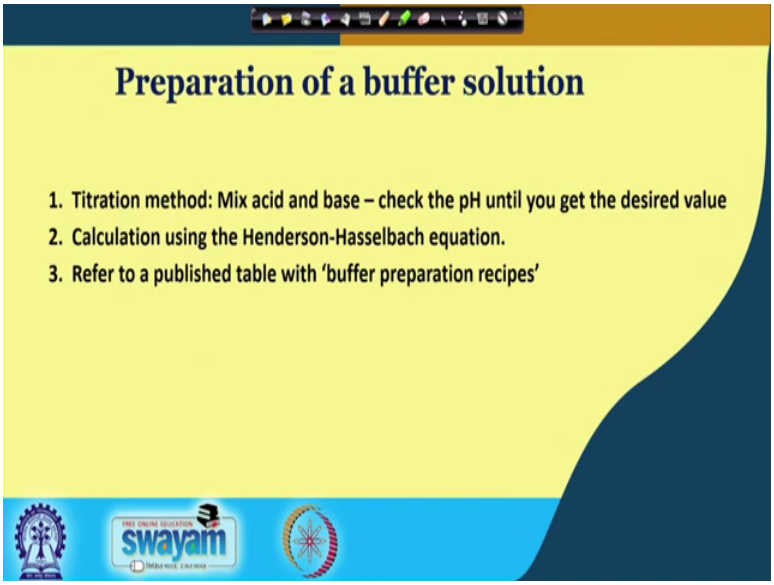

When we prepare a buffer solution, there are several methods by which this can be done. First there is a titration method, where the acid and base are mixed, and the pH is checked until the desired value is obtained. A second method involves the calculation of

the specific ratio of salt to acid that needs to be added based on the Henderson-Hasselbach equation.

So, a certain value would have to be determined and this specific proportion mixed in solution to prepare the desired buffer of the desired pH. Now a days there are published tables that have a buffered preparation recipes, where the amount of the salt and the acid and the total volume for which desired pH is required is already provided as a table available on the internet or in biochemical laboratory books.

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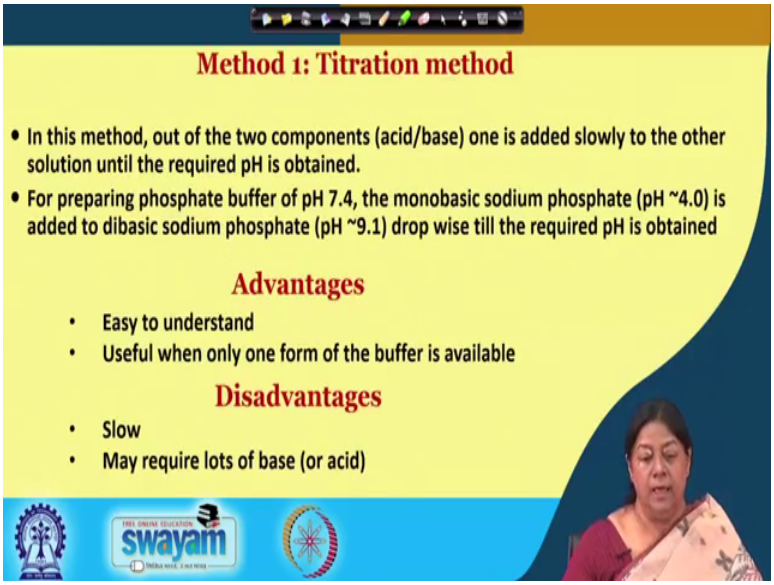

We will look at the several methods that are used for buffer preparation. And we will specifically see the preparation of phosphate buffer, we will then look at other biological buffers that are commonly used, the advantages and disadvantages of the buffers as well. In the titration method this method out of the 2 components the acid or the base, one is gradually added to the other solution until the required pH is maintained or other obtained. For preparing for example, the phosphate buffer of pH 7.4 the mono basic sodium phosphate is added to the die basic sodium phosphate, drop wise till the pH that is required is obtained.

The advantage of this is that is easy to understand and it is usually useful when there is only one form of the buffer that is available. The disadvantage is that it may require a lot of salt or the base or the acid, and it is relatively slow and the variations in pH may not be consider.

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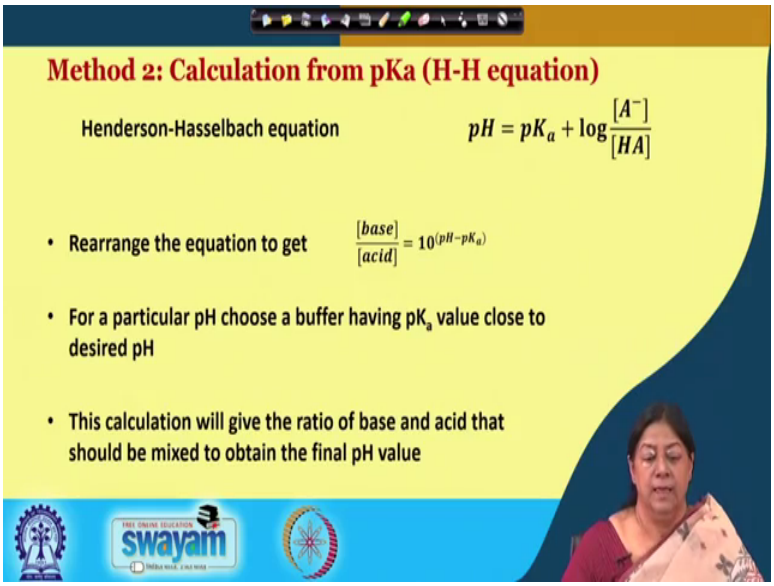

In method 2 where we are calculating the ratio of the salt and the acid using the Henderson-Hasselbach equation. We learnt in the previous class the Henderson-Hasselbach equation we get the pH with the knowledge of the pK a plus the log of the salt by the acid.

The salt in this case is we can call it the conjugate base. So, if we have the acid H A disassociating into H plus plus A minus, we know that we can get the form of the Henderson-Hasselbach equation as shown here, where we have the pH is equal to the pK a plus the log conjugate base by the acid. If we rearrange this equation, we can write this as the ratio of the base to the acid as 10 to the power of pH minus pK a. This means that for a particular pH, we choose a buffer having a pK a value close to the desired pK H, and from a calculation we can determine the ratio of the base to the acid. That should be mixed in that specific ratio to give you the final pH value.

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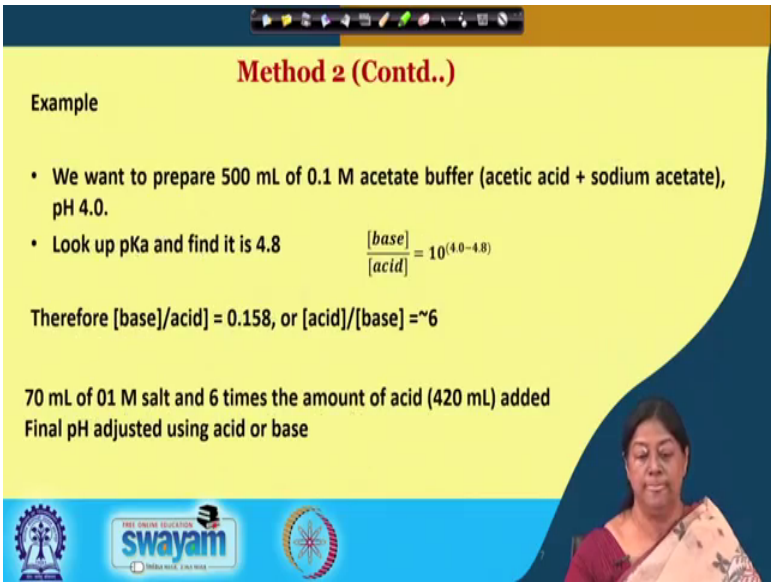

For example, if we want to prepare 500 ml of a 0.1 molar acetate buffer, there to prepare the acetate buffer we saw in the previous class we need acetic acid and we need sodium acetate the salt a weak acid and the salt of the weak acid.

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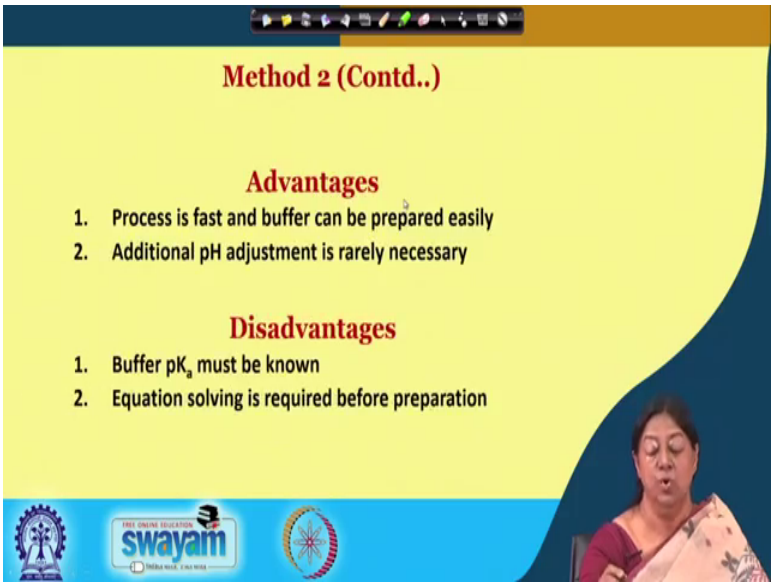

So, acetic acid and sodium acetate of pH 4. So, this is what has to be prepared 500 ml of a 0.1 molar acetate buffer of pH 4 if we look at the pK a of acetic acid we find that it is around 4.8. So, based on the previous expression that we found the ratio of the base to

the acid is 10 to the rate pH minus pK a which gives us a base to acid ratio of 0.158 which means acid to base ratio is about 6.

So, we take 70 ml of a 0.1 molar solt solution and 6 times the amount of acid added and the final pH can be adjusted by using acid or base. The advantage of this is that the process is fast and the buffer can be prepared quite easily and accurately. And at the additional pH adjustment is rarely necessary, because we have already calculated the ratio of the base to the acid that is actually required to get the specific pH required. But in this case the pK a of the buffer is has to be known, and equation solving is required before the preparation.

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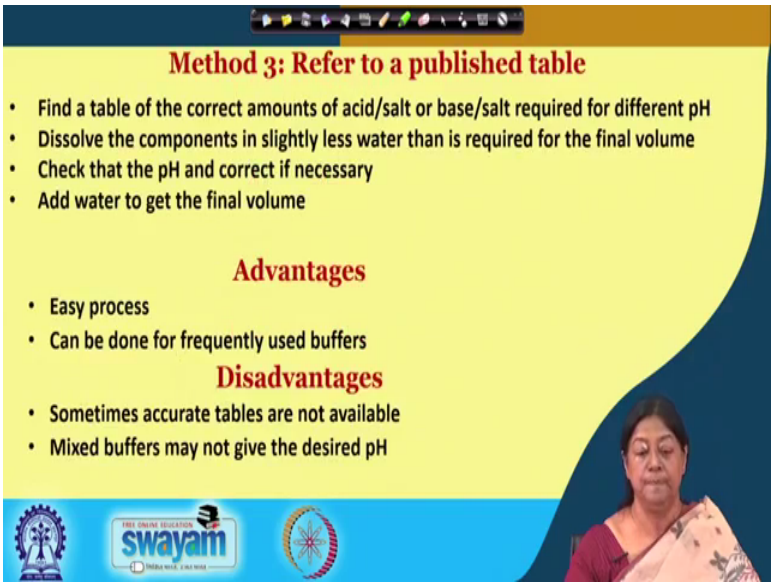

The other method as I mentioned before is referring to a published table. So, we find a table of the correct amounts of acid salt or base salt required for the different pH, in the tables usually you have the molarity of the specific buffer that you require, the pH that you require, and the specific recipe is given to you to tell you how much has to be weighed of each component, and how much water has to be added to give you the final desired buffer of the specific pH required. The advantages is that is it in a very easy process, and it can be actually done with frequently used buffers.

But the disadvantage is sometimes accurate tables are not available, and mixed buffers sometimes do not give the desired pH for the specific type of reaction or the specific type of biochemical process that is under consideration.

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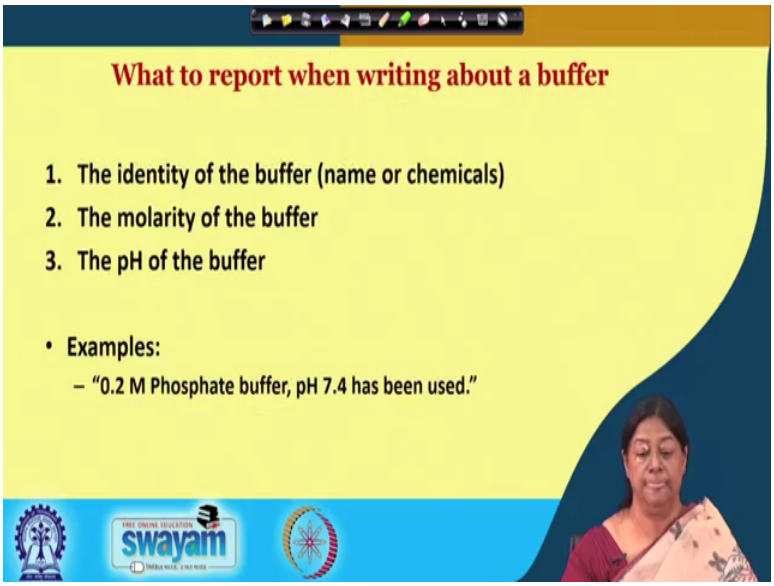

Now, when we report about how a buffer is used or what kind of a buffer is used; it is important to mention the identity of the buffer the name or the chemicals, the molarity of the buffer and the pH of the buffer, this is required. So, if the experiment has to be repeated it can be made reproducible from a knowledge of knowing that for example, 0.2 molar phosphate buffer has been used of a pH 7.4.

If this information is available then this gives us the identity of the buffer, it gives us the molarity of the buffer and it gives us the pH of the buffer. And with the knowledge of how to prepare phosphate buffers this specific buffer can be prepared in the laboratory.

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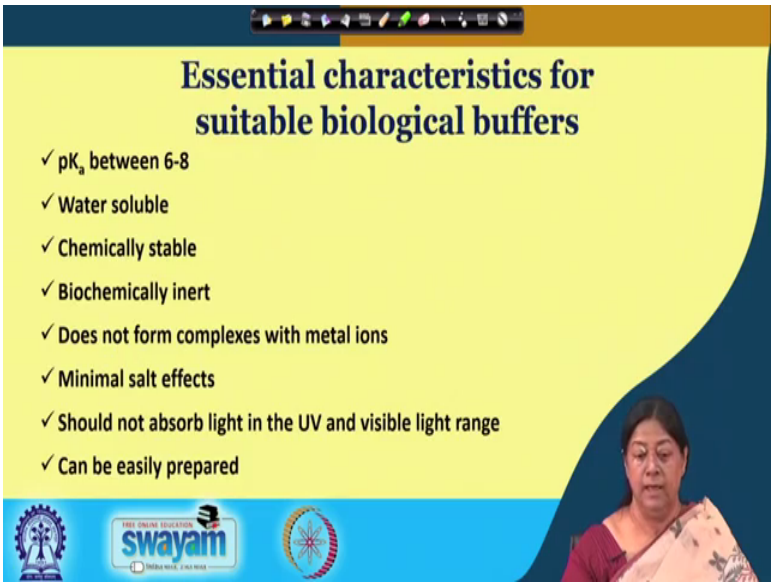

The essential characteristics of suitable biological buffers now, have to have a pK a between 6 to 8 this is desirable because the physiological pH is 7.4. So, any biochemical process normally occurs within this pH range. So, the pK a of the specific buffer that we would like to use would preferably be within this range.

It should be water soluble, chemically stable, biochemically inert and should not form complexes with metal ions, should have minimal salt effects. And should preferably not absorb light in the UV or the visible light range and should be able to be easily prepared.

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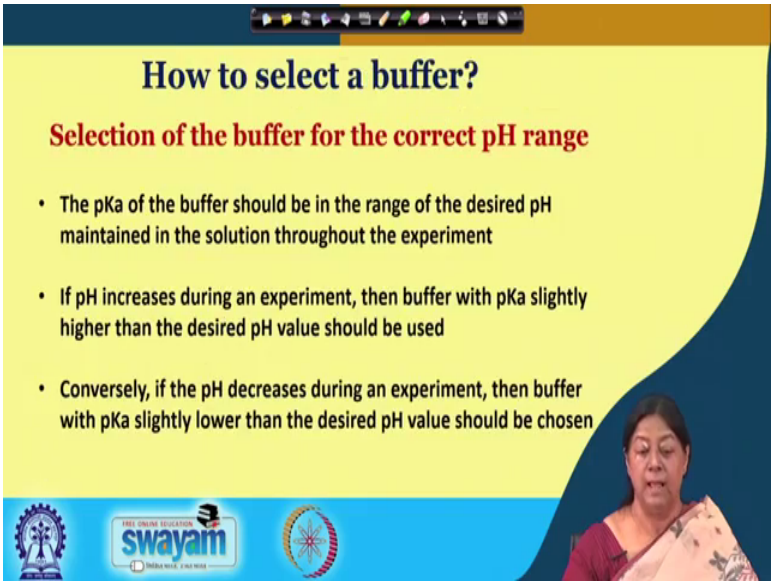

Now how would we go about selecting a specific buffer? First of all, the correct pH range at which we would want to do the experiment a knowledge of that is required. So, the pK a of the buffer should be in the range of the desired pH maintained in the solution that throughout the experiment.

For example, if the experiment releases H plus ions. For example, this means that during the experiment the pH is going to decrease. So, if the pH decreases during an experiment, then the buffer with pK a slightly lower than the desired pH value should be chosen. If the pH increases during an experiment, then the buffer with slightly pK a higher with than the desired pH value should be used.

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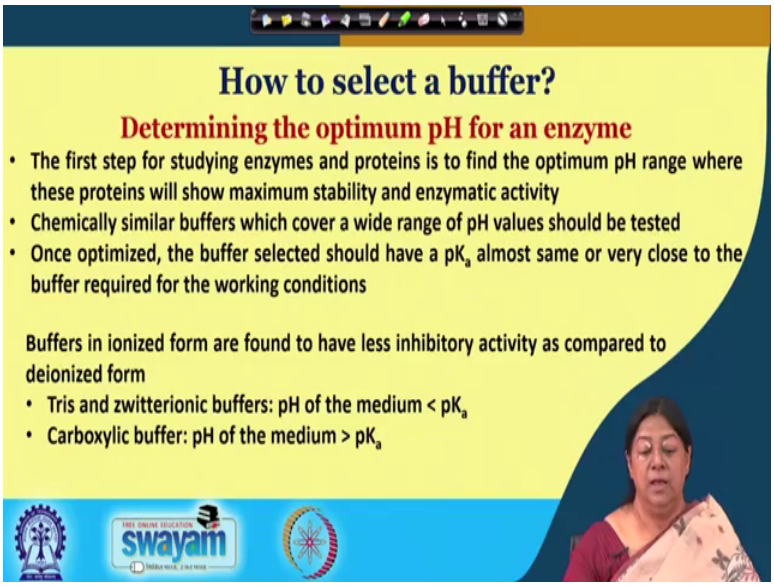

To determine the optimum pH for an enzyme, the if an enzyme activity is being acid or enzyme kinetics is being studied. It is necessary to know the optimum pH range of the activity of the enzyme; where it shows maximum stability and it is enzymatic or catalytic activity.

Chemically similar buffers covering a wide range of pH values should be tested. And once an optimum value or an optimum buffer solution is obtained, having the pK a as desired, then these can be used for the specific biochemical experiment to be conducted. The buffers in ionized form are found to have less inhibitory activity as compared to the deionized forms. There are different buffers mentioned here which we will talk about what we mean by TRIS and zwitterionic buffers and carboxylic buffers.

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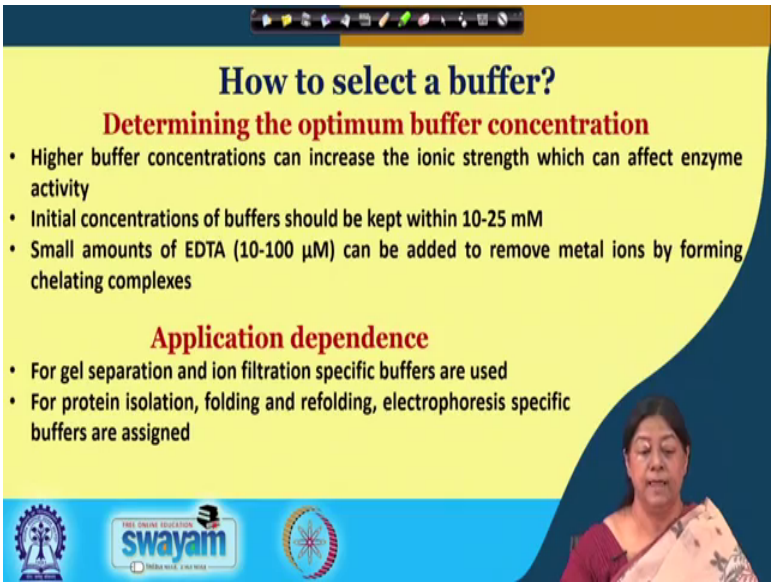

The higher buffer concentrations can sometimes increase the ionic strength which can also have an effect on the enzyme activity.

So, the initial concentrations are of the buffer should be preferably be kept between 10 to 25 mill molar and small amounts of EDTA added to the solution can are a measure to prevent any interference from metal ions, where EDTA will be able to complex these metal ions.

Then the pH or the buffer chosen has to also depend on the experiment being conducted, whether it is an enzyme activity assay, whether it is gel separation ion filtration or any technique specific biochemical technique that is going to be considered the optimum buffer selection also depends upon the experiment to be conducted. For example, gel electrophoresis, chromatographic methods we will be looking at specific experiments as we conduct this course, and you will realize the importance of having the correct buffer, the correct pH and what implications this can have on biochemical activity.

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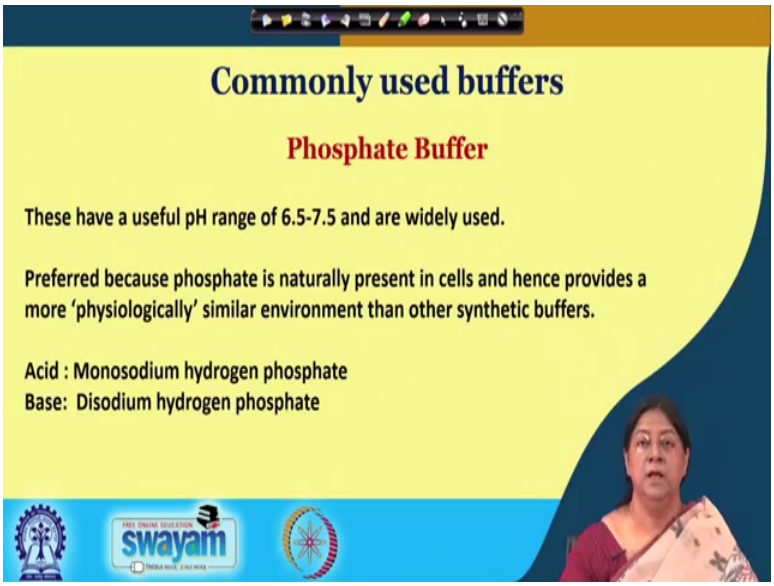

The most commonly used buffer is probably the phosphate buffer. The useful nature of the phosphate buffer is it is pH range from 6.5 around to 6.5; 6.5 to 7.5, and it is preferably used because phosphate is naturally present in cells. And hence provides a more physiologically similar environment than other synthetically used buffers. Here the acid is mono sodium hydrogen phosphate the base is disodium hydrogen phosphate.

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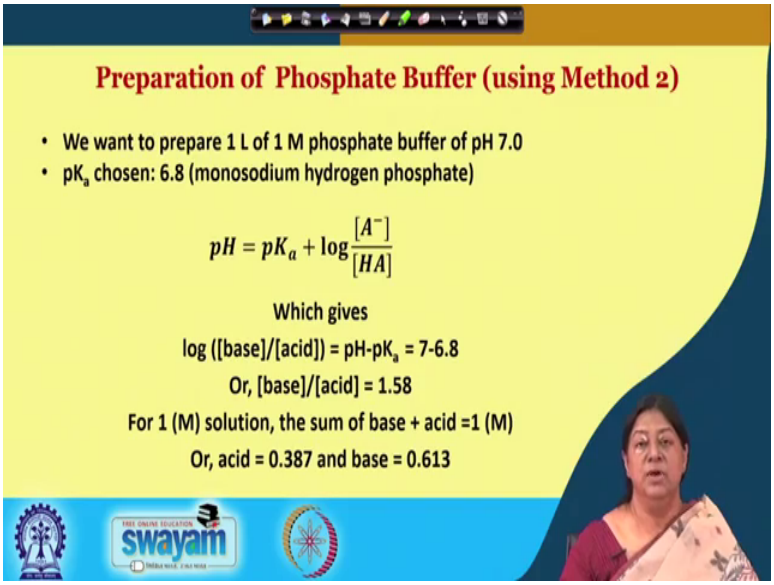

So, if we want to prepare 1 liter of a 1 molar phosphate buffer of pH 7. The pK a chosen in this case is 6.8 corresponding to the mono sodium hydrogen phosphate. From our

equation pH is equal to pK a plus log salt by acid which gives us a log base by acid ratio equal to the pH minus the pK a 7 minus 6.8. So, we get a ratio that tells us this is the molarity of the acid required and this is the molarity of the base require. Based on an understanding of the monosodium phosphate molecular weight, we can determine the width required for a specific volume of solution.

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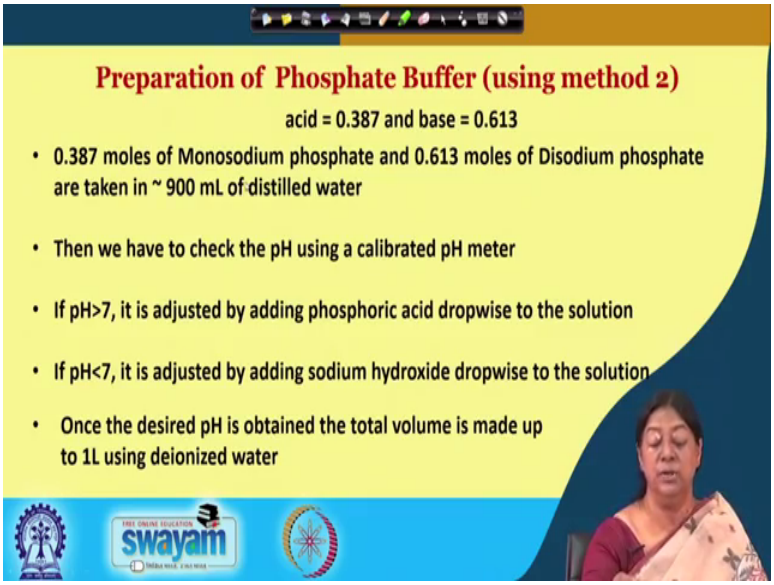

So, 0.387 moles of monosodium phosphate, 0.613 moles of disodium phosphate are taken in approximately 900 milliliters of distilled water if 1 liter is to be prepared. The pH has to be checked with a calibrated pH meter we saw in the first lecture how a pH meter is required to be calibrated. If the pH is greater than 7 it is adjusted by adding phosphoric acid drop twice to the solution, if the pH is less than 7, it is adjusted by adding sodium hydroxide drop wise to the solution. It is important to note here that the ions that are being added to the solution are already present.

So, because we are using a phosphate buffer to decrease the pH we are adding phosphoric acid to ink. We are using a mono sodium phosphate and a disodium phosphate to increase the pH of the pH is found to be less than 7 we add sodium hydroxide thereby not adding any additional ions to the buffer solution under preparation. Once the desired pH is obtained the total volume can be made up to 1 liter using deionized water which makes a preparation of 1 molar, 1 liter of pH 7 of the phosphate buffer which can be used for further biochemical experiments.

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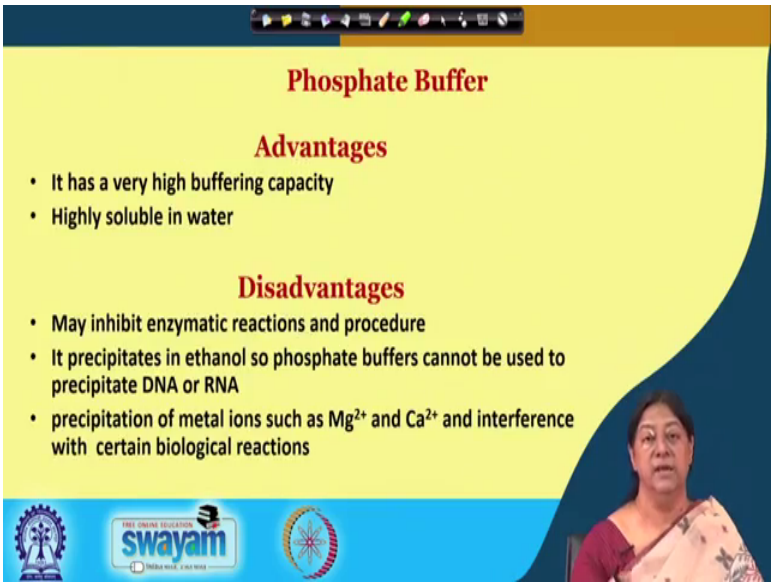

The advantages of using a phosphate buffer is that it has very high buffering capacity it is highly soluble in water. The disadvantage in case of phosphate buffer is it might inhibit some enzymatic procedures and enzymatic reactions. It precipitates in ethanol, so the phosphate buffers cannot be used to precipitate DNA or RNA and there is a possibility of it is interaction with magnesium and calcium ions and interference with some biological reactions. In this case the phosphate buffer cannot be used.

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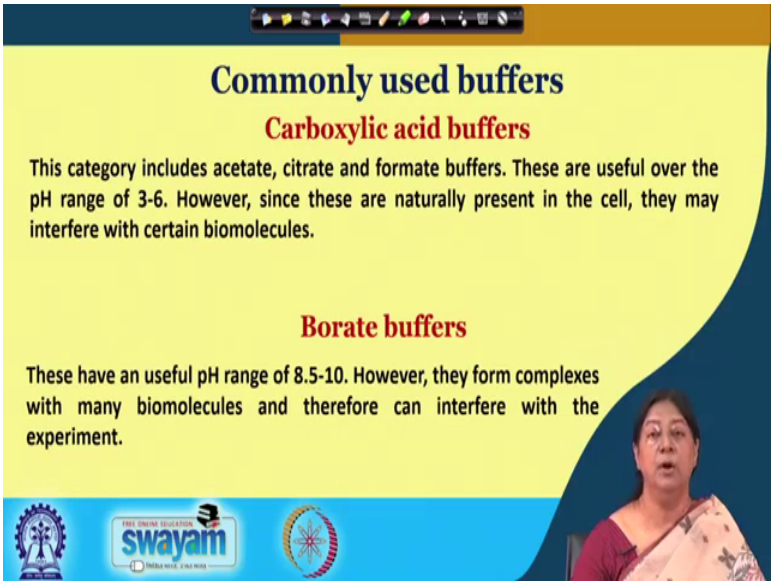

Other commonly used buffers are carboxylic acid buffers, this includes acetate citrate and formate buffers.

These are useful over the pH range of 3 to 6 considering their acidic properties; however, since these are naturally present in the cell they sometimes interfere with certain biomolecules. Borate buffers are also used and they are useful pH ranges around 8.5 to 10, but the problem of using borate buffers is that they form complexes with many biomolecules and sometimes interferes with the experiment being conducted.

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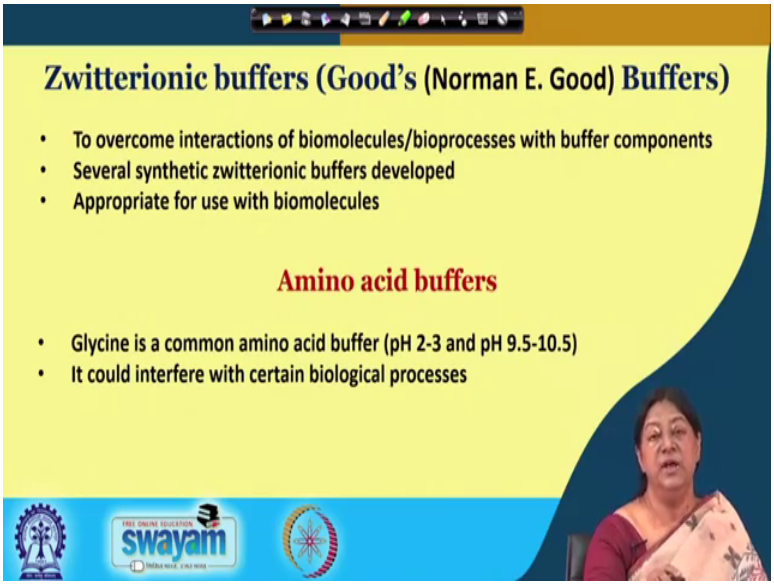

Zwitterionic buffers or these are goods buffers, that was determined by Norman e good that all to overcome these interactions of biomolecules or by processes with the buffer components. A set of synthetic zwitterionic buffers were developed that were appropriate to use with biomolecules. For example, we will consider when we study the pH or the amino acid base amphoteric properties in the next class we will see how amino acid buffering capacities can also be used. For example, glycine is a common amino acid buffer and it has 2 ranges where it can work on where we have pH 2 to 3 and pH 9.5 to 10.5.

However, this can also interfere with certain biological processes.

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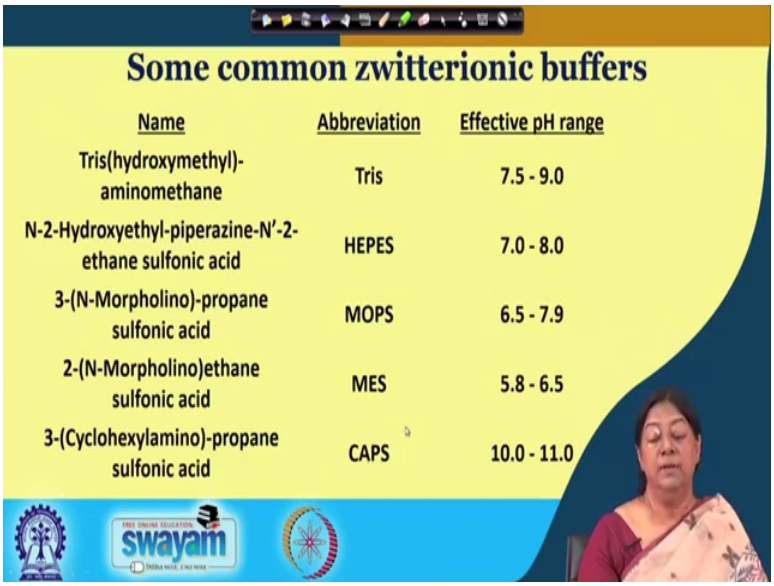

Some commonly used zwitterionic buffers are the TRIS buffer, the HEPES buffer, the MOPS the MES and the CAPS buffer, and the effective pH range is given in this table here.

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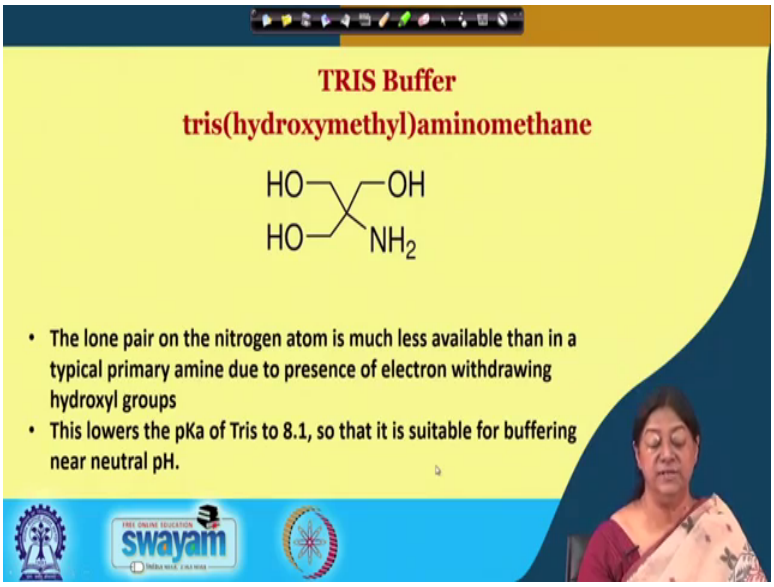

We will just go through some of them for showing their uses for example the TRIS buffer. In this case the lone pair on the nitrogen atom is much less available than in a typical primary amine due to the presence of the hydroxyl ions present, which are electron withdrawing in nature.

So, this lowers the pK a of the TRIS buffer to around 8.1. So, it is useful for buffering near neutral pH.

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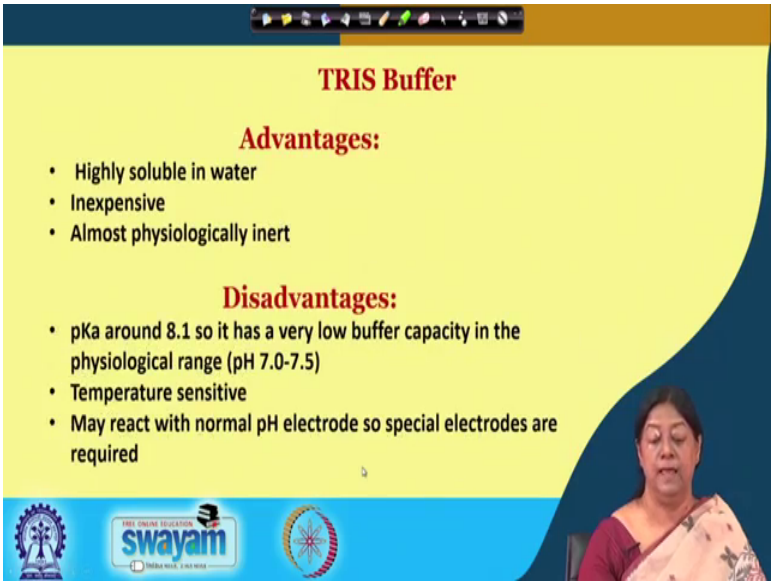

The advantages of using the TRIS buffer is that it is highly soluble in water inexpensive. And it is almost physiologically inert in nature. The pK a is around 8.1 so, it has very low buffer capacity in the physiological range that is 7 to 7.5, it is temperature sensitive, and may react with the normal pH electrode; so special electrodes are required.

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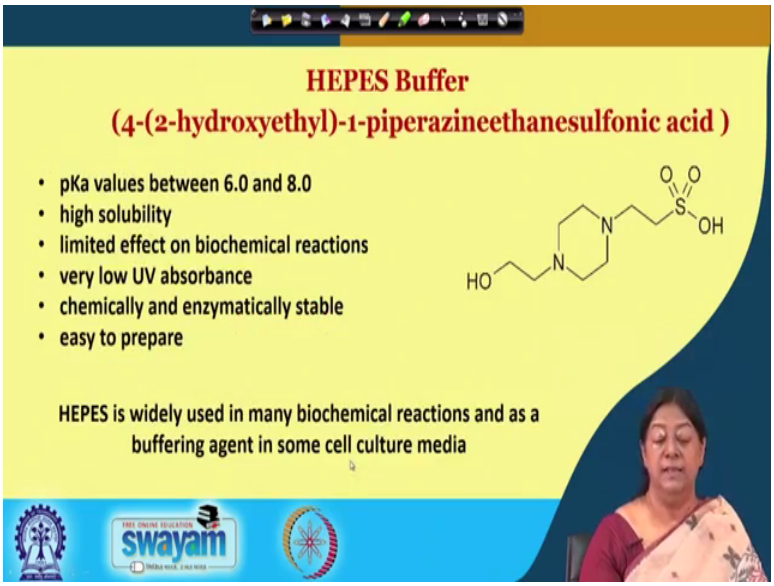

The HEPES buffer is another commonly used buffer. The pK a value in this case ranges between 6 and 8, it has high solubility, it has limited effect on biochemical reactions which is what is required for a suitable biological buffer. It has very low UV absorbance, it is chemically and enzymatically stable and also easy to prepare. Because of the several advantages of the specific buffer, HEPES is widely used in many biochemical reactions and as a buffering agent as well in some cell culture media.

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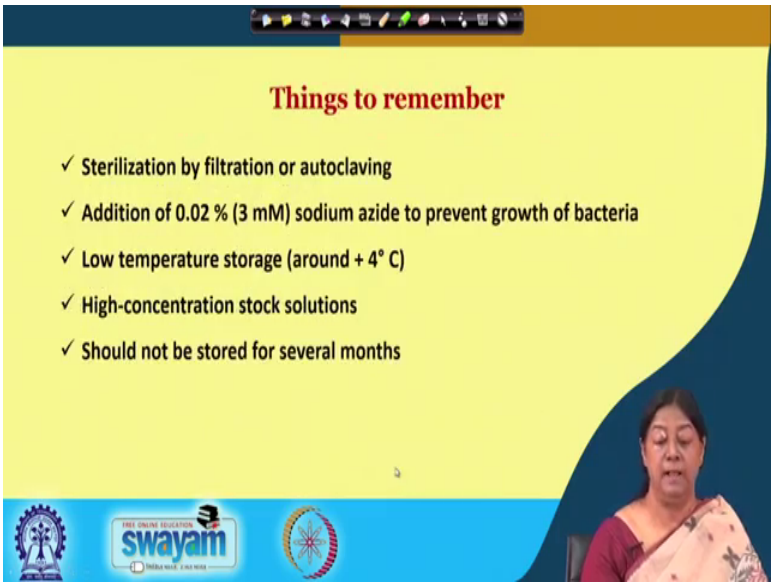

So, there are certain things that we need to remember. The sterilization of our vessels our reagents and our volumetric flasks and all the apparatus that we use is extremely essential. The addition of around 0.02 percent of sodium azide is a very commonly used procedure to prevent the growth of bacteria.

Low temperature storage of buffers, high concentration of stock solutions that can be used with proper dilution; however, the storage should not be for more than several months.

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The references that we will be considering are hydrogen ion buffers for biological research. There are methods in enzymology with that tell us what buffers are to be used and in as I spoke about the different specific and publication tables that are available the options that are present that can make us use the specific buffers with a specific desired pH.

So, to conclude the buffer preparations can be accurately carried out by using the Henderson-Hasselbach equation. In this case we need the knowledge that is required is a knowledge of the pK a value. A knowledge of the pH of the desired solution, if we want to determine now what our biochemical reaction is going to undergo or what enzymatic reaction is going to take place. It is always useful to get an optimum pH idea before prep the preparation of the buffer. And this can be prepared or this can be accurately determined by the Henderson-Hasselbach equation using a knowledge of the pK a value of the acid combination that is desired.

The buffers used should be stable and chemically inert in the reaction medium. The metal ions present or any components of the specific biochemical reaction being undergone should not interfere with the components present in the buffer. For example, as was mentioned a while ago when we are using the phosphate buffer, it is very essential to adjust the pH by either adding phosphoric acid or sodium hydroxide so, that no additional ions are present in the solution. When we are considering any enzymes or

proteins or amino acids, they have a very high interaction because of the different moieties present. They have a high interaction probability with the components present in solution.

So, it is extremely important that this is taken care of. So, the choice of buffer depends on the desired pH of the required medium and also the pK a of the components. The specific buffers that are used for biological systems; like, proteins and cellular systems or certain biochemical reactions are the common ones that we mentioned. Phosphate buffered saline is another very commonly used buffer that has to it added sodium chloride; which mimics the physiological pH, the physiological condition the physiological solution, so this is another buffer that is very commonly used apart from the other ones that were mentioned.

Thank you.