Experimental Biochemistry
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Lecture – 09
pI Determination of Glycine

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Now, we have taken oxalic acid in this conical flask for titration, we have to add phenolphthalein indicator two drops. Now this phenolphthalein indicator as we know is colorless in acidic solution and when base is added and in basic solution basically this turns pink. So, how to estimate a neutralization point? When the solution this colorless solution will turn pinkish, then we can know that the neutralization point has been achieved and our titration is complete.

Now, let us proceed for titration of oxalic acid with NAOH.

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So, let us start the titration experiment. Now we have taken the NAOH solution in burette and oxalic acid along with phenolphthalein indicator in the conical flask. Now we will be adding the solution from burette drop wise. Now how to operate this burette? You have to hold this burette by left hand, turn this knob like this slowly and we can see drop wise NAOH comes here and a faint pink color appears and upon stirring this color goes.

So, the experiment will go on like this, we have to add NAOH drop wise from left hand, we have to operate the knob and from the right hand we have to continuously stir this conical flask. Now you are adding NAOH gradually drop wise.

Here we can see we are almost close to the end point, since the intensity of the pink color if gradually increasing and here with one single drop, we can see we have reached this end point. This end point is marked by this pink color. The pink color shows that the entire oxalic acid has been neutralized with NAOH and from here we have to record the volume of NAOH required.

So, our titration is complete. Now for estimating the strength of sodium hydroxide, we need to find the volume of sodium hydroxide consumed in neutralizing oxalic acid solution.

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Now, the sodium hydroxide was initially in zero mark, and the final volume is comes to around 2.8. So, here we have to also consider the lower meniscus of the solution which touches the graduated mark here that is a 2.8. So, the volume of NAOH required is 2.8 from normal calculation we know that v 1 s 1equal to v 2 s 2.

Now, here the volume of oxalic acid taken was 10 ml, the concentration of oxalic acid was known and the volume of NAOH was also can be observed from this experiment and finally, we can estimate the concentration of NAOH. Now we will begin the second titration that is standardization of HCl using the previous standard NAOH solution.

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This HCl prepared has a rough strength of around 0.5 normal, now the titration process is pretty similar to the previous one. We will pip it out around 10 ml of HCl, we have to gradually adjust. So, that the lower meniscus of the solution touches the zero mark. The same concept we used in case of burette. Now we were transferring this into the conical. After that tap twice, again we will be adding two drops of phenolphthalein indicator and being an acid that is a HCl this again colorless and again we will be calculating this with sodium hydroxide.

Now, let us begin the second titration part, we have filled this burette entirely again with sodium hydroxide till zero mark.

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And let us add the slowly will open this knob using left hand and hold the conical in the right hand a drop wave and again stirred this one we can see a gradual appearance of pink color. What happens actually is that when NAOH is added when the one single drop falls out here the local concentration of NAOH is very high which shows this pink color.

However again on mixing and this is neutralized and the pink color disappears. Now when the pink color persist, we can be sure that there is no h plus and left which may neutralize the NAOH solution. And as a result the come solution is completely a neutralize or rather excess of OH minus is there and we have to stop this titration when the solution is pink. We have to gradually add those drop wise and stir it.

And one small thing out here is that if anytime you feel that one of the drop has been out there in the side of the conical. In fact, the conical you can use double distilled water anytime ok. And just clean the sides please note that if you can easily use water during titration, because up and using water only the volume changes the concentration may change, but the number of moles of the component remains same. So, titration is all about equivalence reaching the equivalence point; that is number of moles or equivalent present in the solution. So, adding water does not change anything in the titration process.

We can fit the pink color of their, it may be coming within one to two drops and here. Finally, pink color appears that is a solution of completely neutralize or rather a single; a

small amount of alkali is excess in the solution. Now we have to determine the strength of HCl solution again. So, we are done with the titration process the solution has been turn into pink.

Let us see the concentration of HCl required, for this we have to note the volume of NAOH that is utilized in this process. Here we can say this if it is zero mark and this is the graduated mark out here where the lower meniscus touches is 8.9. So, the volume of NAOH required for titrating this HCl is 8.9 ml. Now let us calculate the squint of HCl using v 1 s 1 v 2 s 2 method like the previous way. So, we have calculated and seen that the concentration of HCl that has been titrated is 0.635. Again the concentration which you have prepared 0.5 is not exact being a secondary stranded the concentration of HCl that has been determined using titration method if 0.635 normal.

This HCl will be used for protonation of the amino acid used for titration, now let us proceed for the estimation of pI of amino acids.

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Now, for calibration first we will calibrate it using pH 7 standard buffer. Now this pH 7 standard buffer has been transferred into this beaker and the pH electrode is immersed into this beaker.

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Now, here we can see that the pH is not exactly 7, rather the pH is a bit lower than 7 now we have to calibrate it in order to reach 7. We will gradually calibrate this solution it is increasing ok. Now we can see it is showing pH 7 that is the pH electrode has been calibrated with the solution to pH 7.

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Similarly, we will calibrate it using pH 4 buffer this one is another buffer pH 4 standard buffer will calibrate it using this one and after that, we will also calibrate it using pH 9

buffer that is pH this is the pH 9.2, now we will calibrate it using pH 4 and pH 9.2 respectively.

Now, let us begin with the pI estimation; for this we have taken this glycine solution this is 0.1 molar glycine and we will take around 10 ml of glycine in this beaker now let us pip it out 10 ml glycine.

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Now, the solution lower meniscus just touch the 0 mark, the pip it out 10 ml of glycine. You carefully taking this in this beaker is 100 ml beaker again tap twice.

Now, this will be used for pI estimation. We have taken this glycine solution in the beaker this 10 ml glass in solution. Now before beginning this with this experiment, we have to completely protonate this glycine for this we have to add HCl solution the standardized HCl solution. Now what amount of HCl should be add? The HCl should be sufficient enough to completely protonate in the glycine, for this we have to calculate the number of moles of glycine prevent here.

That is we had taken 0.1 molar 10 ml glycine. Now we have to we can calculate from v 1 s 1 the x then amount of the moles of glycine prevent out here; now we have taken 10 ml of 0.1 molar glycine solution from this we can calculate in the number of moles of glycine prevent here. We know the concentration of HCl that is standardized in our just previous experiment. The from this standard HCl we have to add the fixed amount of

HCl the fixed volume of HCl, which will make up the total number of moles of HCl equal to a number of moles of glycine prevent.

That will protonate the number of NH 2 that is the equal moles of NH 2 present in glycine. So, let us start the protonation process and let us add this HCl. So, before beginning with that pH titration method, so, what we have to do? We have to just add distilled water to. So, before beginning we have to add distilled water to this glycine solution. So, as we know adding water does not basically change it a number of moles and it is required since we need to deep this pH electrode completely into the solution.

So, we are adding the amount of water with the sufficient enough to emerge the electrode completely into the solution. Now let us proceed for adding the HCl solution. Now for calculating the amount of HCl that is required to be added here now we have to find the number of moles of the amino acid.

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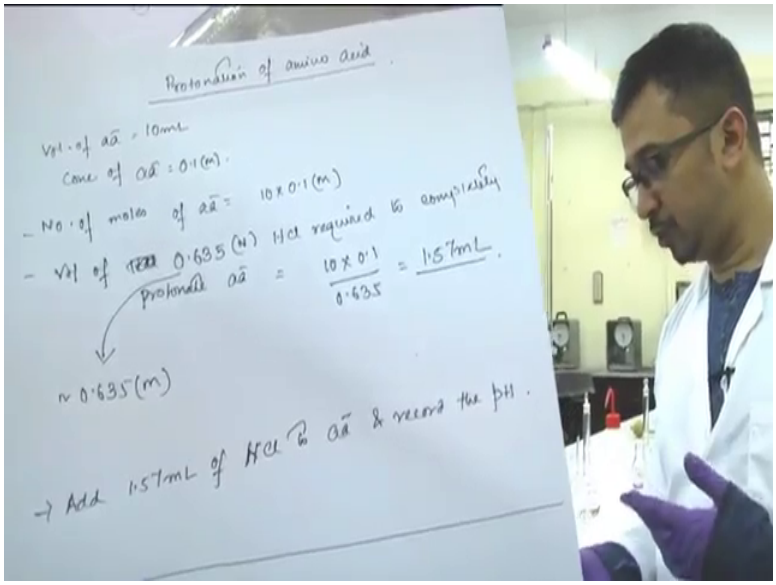

Number of moles of amino acid is equal to 10 into 0.1 and 10 ml of amino acid was required in to 0.1 molar. And here we can see the volume of HCl required is equal to in the symbol v 1 f 1 v 2 f 2 method is around 1.57 ml.

So, it is not the exact one. So, around 1.5 several to 1.5 ml of each fill can be added gradually and we can see a lowering in the pH value and we will start the experiment once we have added this HCl.

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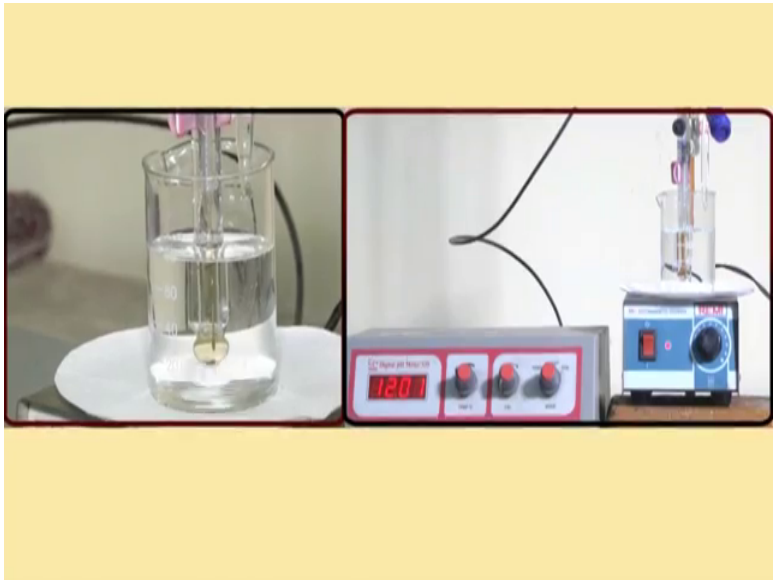

So, let us add this HCl solution we have taken this HCl the amount of HCl to be added we have seen, it is around 1.5 ml. So, we have taken this glycine solution and in a beaker and put it in a magnetic stirrer, we have put a magnetic bar to it, we have to make sure that the bar is completely clean and free of any sort of impurities covered over it.

So, let us start with this. So, before that we have to add HCl solution, pip it out HCl. We have require around 1.5 I am completely making this level up to 0 so, that I can just pip it out 1.5 out here. Now we will be slowly adding this one HCl and once you start the magnetic stirrer here we can see there is a gradual decrease in the pH value.

Now, we will slowly go on adding the required amount of HCl, we will be slowly adding this HCl out here and we can see the pH decreases. Here we have added up to 1 ml 1.4 and now 1.5. However, we can see that the pH is around 2.64, in order to check whether complete protonation has taken place or not we may add a small amount of we may add a small amount of h plus if required just a single drop.

Here we can see there is no major changes in the pH its around 2.6 to 2.5. So, from here we can say that there is completely almost protonated and we can carry out with our experiment. Now let us start with this pH metric calculation method. To in determine the pi we have to plot pH versus volume of NAOH solution. Now for taking this reading we have to note the volume and the amount of volume that of NAOH that is added there were change in pH we have to record the change in pH now we will be recording

simultaneously change in volume and change in pH. However, for recording change in volume it is quite difficult because one time we have to see the pH and the next time we have to jump into the burette and see the volume.

In other two minimize such. So, this might lead to some error in order to minimize that error what we need to do? We have to need to count in the number of drops in order to estimate the volume in relation to the drops we have to note what we have to note down the number of drops corresponding to 1 ml. Now the number of drops what we have done earlier is that the number of drops corresponding to 1 ml is equal to 20 drops that is 20 drops of NAOH is equal to 1 ml.

Now, if we count the number of drops write it down suppose for one drop the change in pH about some x unit regard down the pH value and the number of drops and later on we convert that number of drops into volume. So, let us start this experiment. So, before beginning in the beginning we have to note down this pH that is 1.80 pH 1.80 a number of drops added here is 0. So, we write down a number of drops 0 and pH 1.80. Now we add carefully a single drop of NAOH out here and again close it.

And gradually increase this magnetic stirrer, we can see at around you know one drop whenever one drop there is no spatial change in pH value and it remains around 1.8 to 1.81. So, it is around 1.81. Now let us add two drops; one drop two drop. So, real number of drops out here is 3 because we have added two drops now and we can see that there is no significant change in the pH.

So, let us add another two drops, we can see the pH gradually increases slightly. Now let us add another two drops and now three drops total number of drops comes out to 10 1.84. So, we can see at that that increasing pH is very slow. So, what we can do we can carry out with around2 to 3 drops at a time it is around 1.85 let us add again two drops.

1.86 again two drops, here we can see when we are adding NAOH the pH change is very small that is the change in pH is not that much. The low change in pH is due to the fact thus this region is basically the buffer zone. As we have studied in the theoretical part as we have seen in the theory classes that this is a buffer zone where there change in pH is very less because as we know the buffer resist the change in pH due to buffer zone here the change in pH is very small. Now once this inflection point is reach here we can see there will be a sudden jump in the pH value. So, let us go on adding.

Now, gradually we can fit at the pH of achieve around 3.2 to 3.23. So, here we will have to be very careful about the addition of NAOH since the inflection a 4.2 4.19. So, it is jumped again around 4.19 again we add one drop. Here we can see the inflection point around it has jump to it has jumped around 6.3 to 6.2. So, it will get stabilized gradually.

So, it is around 6.02 now it has sharp a jump to 8.2 to 8.29. So, here we have just crossed inflection point. So, we can sharp jump from pH 6 to pH 8. So, the first inflection point. So, we have just cross the first inflection point where there is a sharp change in the pH. So, this indicates that the comp in the first inflection point that is a sharp change in the pH value, which indicates that the COOH that is which was completely protonated come converted into COO minus in glycine.

And which gives another form where only NH 3 plus is protonated and co minus I mean the carboxylic this gives another form of glycine where the NH 2 plus is protonated to give NH 3 plus, and this carboxylate is de protonated andCOO minus that is the carboxylic acid has come to carboxylate group and again it has reach to a second buffer region where we will see again as gradual or slow rise in the pH value. So, while when gradually we are adding NAOH after the first inflection point, when the value of the pH which is around 9 to 10 the second inflection point occurs.

However what happens is that this inflection point the change in pH out here is not that sharp like the previous one. So, we cannot basically I mean distinguish out here just by we cannot actually distinguish out here just by observing the pH value what the inflection point occurs, for that we need to plot the graph. Now one thing it is recommended that kindly add the burette from the kindly add NAOH drop wise one to two drops as recommended ok. It is recommended to add NAOH one to two drops each and every time it may take quite a long time for this titration to occur. However, if you add suppose 4 to 5 drops at a time what may happen is that, the inflection point you might cross the inflection point.

And up in crossing inflection point the graph which we will be plotting out there will not be accurate and the proper pi value cannot be determined from that graph. It better its it is better to add NAOH drop wise one to two drops at a time, in order to get a proper inflection point from this titration. We will go on adding NAOH till the pH around 12 we have reached. Once the pH around 12 has been achieved out here in the pH meter we

will stop this pH meter titration now we have almost reached pH 12 will be able slowly adding one two drops.

However in this region as we can see the change in pH is very slow. So, when this is completely a buffer region, again let us are two drops.

So, here we can see that the pH is just above 12 or almost 12 here we will stop this titration part. And we will go now we will shift to the calculation part where we will plot pH versus number of drops which will be converted into the volume of NAOH and from there we can calculate the pI. Now we are done with this glycine titration we will next move into titration of lies in with NAOH.