Experimental Biochemistry
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Lecture – 11
Summary

Hello. So, in this lab, in this week we learnt about amino acid titration and the determination of pI.

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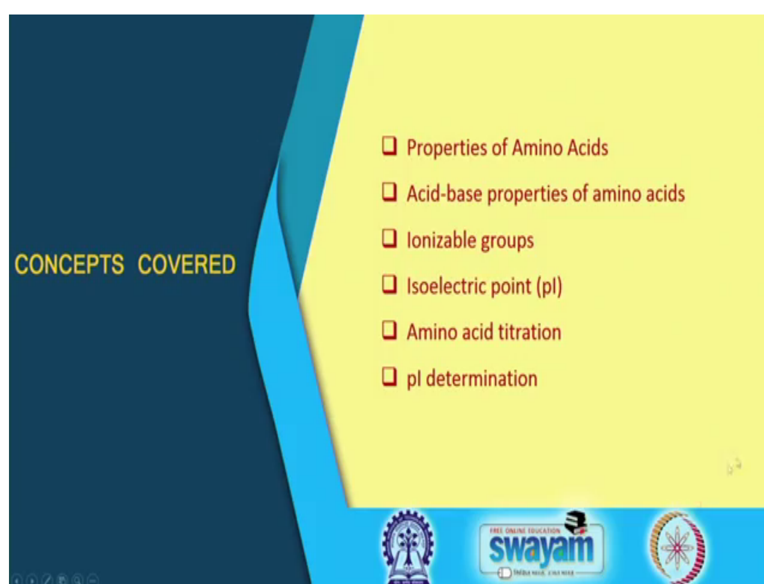

So, we learnt this the following topics where covered in this 2nd week. We learnt about the properties of amino acids, we learnt that amino acids have and amino group and a carboxylic group which gives it this acid base property. So, this amino and carboxyl groups are the ionizable groups. And we also learnt that there are some an amino acids which have additional ionizable groups in their side chain. For example, aspartic acid or glutamic acid, which have an acidic side chain or lysine arginine and histidine which have a basic side chain.

We learnt about the concept of isoelectric point for amino acids which means that a particular pH at which particular amino acid is neutral is refer to as its isoelectric point. We learnt that this pI isoelectric point can be determined using amino acid titration. And we saw two in lab experiments where we determine the pI of glycine and lysine.

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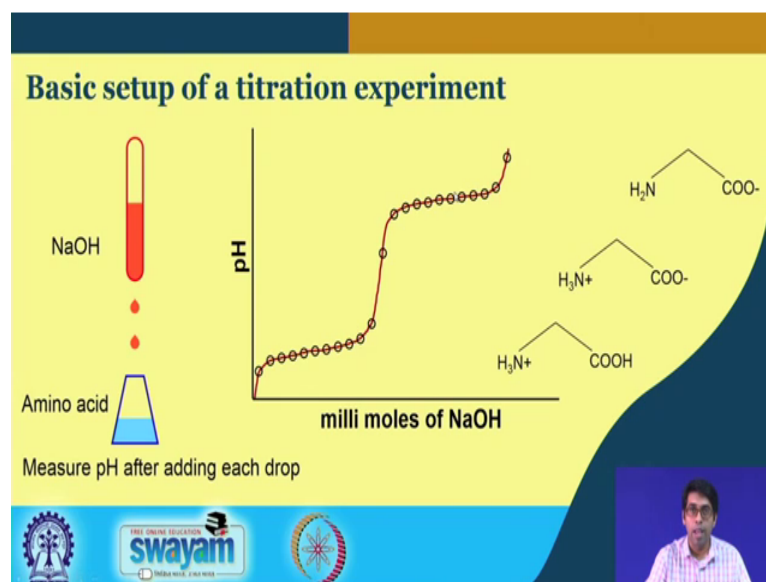

So, I will just quickly go over the concepts that we have learnt in this week and try to show you the important points.

So, here is a very basic set up of a titration experiment and we saw that being performed in the lab. So, what we wanted to do is with determine the pI of an amino acid. So, what we did was we made up solution of an amino acid for example, first we used glycine. So, a glycine solution point one molar was taken in a beaker and we titrated it with base we took sodium hydroxide which was roughly around 0.5 normal.

To start the titration experiment you want to span whole pH range. So, what we did was we drop the pH to a very low value to do that we used hydrochloric acid. So, we added hydrochloric acid to the glycine solution and drop the pH below 2 and then we started our titration experiment. So, the titration experiment was done by drop wise addition of sodium hydroxide to this amino acid, it was thoroughly mixed by stirring and the pH at each after addition of each drop was measured using a pH meter. So, we got two readings; one is the pH after addition of each drop and from the volume of each drop we also determined how much sodium hydroxide we are adding at each titration point.

So, this can be very nicely plotted in a graph like this. So, the x axis is the amount of sodium hydroxide that is added. So, here I am writing it in terms of milli moles of sodium hydroxide and in the y axis we can plot the pH that we measure at each titration

point. So, if we do that it will look something like this. So, each of this points are the titration points.

And what you will see here is that during titration the pH was initially increasing in the linear fashion and suddenly here the pH jumps. So, it goes it increases a lot by just addition of 1 or 2 drops and then again it stabilizes and it increases linearly till we reach the end of the titration. So, we can join this points and get a nice titration curve like this. So, what are this different regions of a titration experiment?. So, if this was glycine, so this titration curve is actually shown for an amino acid which has only two immiscible groups. So, for example, let us say if this is glycine then one ionizable group is the amino group and then the other ionizable group is the carboxyl group.

At very low pH in this region glycine will be in this form where the carboxyl group is protonated and the amino group is also protonated, which means that the glycine amino acid will have a net positive charge. As we keep on increasing this the amount of sodium hydroxide the pH will increase and at this point we get an inflection. So, that inflection is due to the ionization of this carboxylic group ok. So, the carboxylic group loses the proton somewhere here and if you draw a straight line and take the midpoint of that straight line you will get the pKa value which will be the pH at this midpoint. So, that pKa value will be for the ionizable of this carboxylic group. So, that will be your pKa 1.

Then the pH suddenly jumps and again we have another straight line and here this second group is getting titrated it. So, it is losing its proton so that the amino group now becomes neutral. So, for this transition we get this straight line. So, if I draw straight line here and take that midpoint the corresponding pH will be pKa for this transition,

So, one pKa will be here, another pKa will be here and my pI will be somewhere in between that which will be the midpoint of this transition point. So, at that pH the species will be mostly in this form where we have a single positive ion and a single negative charge. So, positive charge negative charge gives you a neutral species. So, that will be the pI for glycine ok.

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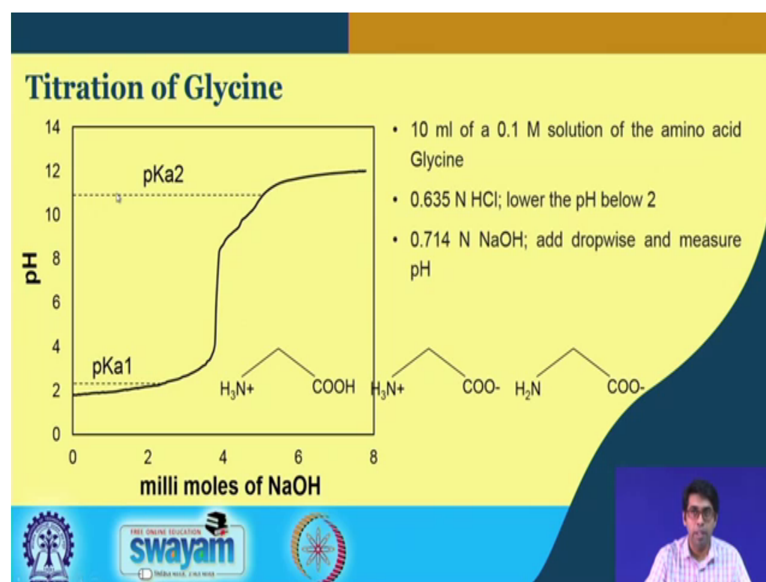

If we look at the actual data, so this is the data from the experiment that was performed in the lab and what I have done is have plotted the millimoles of NaOH and the pH that was recorded by Pritam when he demonstrated the titration of glycine.

So, to perform this experiment he used 10 ml of 0.1 molar solution of glycine he used 0.635 normal HCl to lower the pH below 2. So, we will see that we are starting somewhere just below 2, this was roughly around 1.8 and then he perform the titration using 0.714 normal sodium hydroxide, he added to drop wise and measure the pH. So, as he added sodium hydroxide drop wise the pH changed in this fashion. So, this is very similar to the titration curve that we saw in this slide except in this region there is something else is happening ok. So, this is something that you will notice for all experiments that they will not look exactly like the this idealized curves ok. So, it will deviate at some point and it depends on experiment to experiment but we can use this data.

So, if you can see that this is the first linear region and if we draw straight line here, we can determine the midpoint of the straight line somewhere here. So, that will be our pKa 1 and this is the second linear region and if we draw straight line here and get the midpoint our second pKa will be here. So, if I do that I will get pKa 1 and pKa 2 and this value roughly comes out around 2.3 and this is around 10.2. So, if I take an average of these two pKa values I will get the pI for the glycine from our titration experiment that

was performed in the lab. So, 2.3 and 10.2 you add them divide them by 2 you have your pI.

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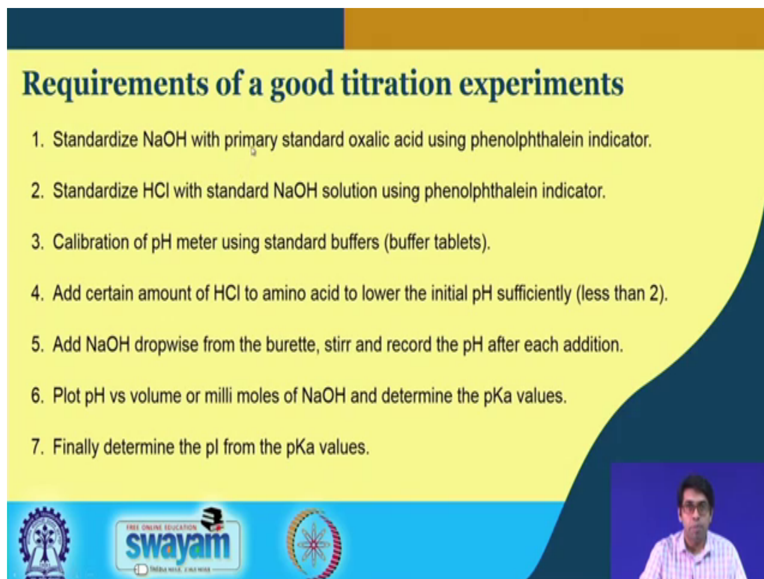

So, here this was again discussed in the lab, but I am just summarizing some of the important points. We did not make standard NaOH solution. We actually made us NaOH solution and then we titrated it with oxalic acid to determine the strength of the NaOH solution.

The reason we cannot make a standard NaOH solution is because sodium hydroxide is very hygroscopic it absorbs moisture. So, when you take out sodium hydroxide pellet us from your beaker or bottle wherever it is told and weigh it you do not know how much of that weight is actually sodium hydroxide and how much is moisture. So, you have to determine the actual amount of the number of moles of sodium hydroxide that you have in the solution by titaning it with some primary standard. So, in this case we used oxalic acid.

The reason oxalic acid is used is because it is non-hygroscopic, so it will not absorb moisture, it can be handled very easily, and it is also not very costly. So, we used as standard solution of oxalic acid to determine the strength of sodium hydroxide and phenolphthalein was used as the indicated. Once we know the strength of sodium hydroxide we use that to standardize the hydrochloric acid solution.

The pH meter were calibrated using standard buffers that was provided, again this is very important because if your pH meter is not calibrated, then again your reading will not be very reliable. We used this standardized HCl to lower the pH of or amino acid to a value which was less than two and then we used sodium hydroxide from the burette and it in drop wise stirred and recorded the pH at each titration point. I showed you the plot of the pH versus milli moles of sodium hydroxide and then I also showed you how we determine the pKa values and once we know the two pKa values for glycine we determine the pI from the pKa values.

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Preparing standard solutions

Preparation of standard Oxalic acid 250 mL 0.2 (N) oxalic acid
Mol. Weight. = 90 g/mole; Eqv. Wt = 45 g/mole
Weight of oxalic acid required: 2.25 g

Standardization of NaOH (~0.5 N)
Mol Wt = 40 g/mole; For 250 mL 0.5 N NaOH, wt. required = 5g
Fill the 25 mL burette with the prepared NaOH solution and check the lower meniscus
⇒ **Pipette 10 ml of standard oxalic acid solution** into a clean dry conical flask, add 2 drops of phenolphthalein indicator solution, and titrate with NaOH solution to the pink endpoint.
⇒ Record the final volume and calculate the strength of NaOH

Vol. of NaOH required= 2.8 mL
From $V_1S_1=V_2S_2$
V_1= 10 mL, S_1 = 0.2 N, V_2 = 2.8 mL, S_2 = ?

Conc. Of NaOH (S_2) = 0.714 N

This is the calculation that was used when the standard solutions where prepared. So, oxalic acid 250 ml 0.2 normal oxalic acid was prepared. And here the molecular weight is given, so based on these volume that you need and the amount and the strength that you want to make we can calculate only 2.5 grams of oxalic acid was required. So, it was without resolved in 250 ml of water to make the standard solution and then that standard solution was used to standardize sodium hydroxide.

Our aim was to make 0.5 normal sodium hydroxide, molecular weight of sodium hydroxide is forty grams and we want to make 250 ml of sodium hydroxide. So, if we weigh out 40 grams then that will be enough to make one normal 1000 ml ok. So, this is one-forth of 1000 ml, so 40 by 4 will become 10 grams, but then that will give us 1 normal this is 0.5 normal. So, we again divided by 2, so 10 by 2 is 5 gram. So, you need

only 5 grams of sodium hydroxide. So, 5 gram of sodium hydroxide was dissolved in water and then that was used to titrate with oxalic acid. So, this is the final result.

The volume of sodium hydroxide that was required was 2.8 ml, 10 ml of oxalic acid was used and the strength of oxalic acid was 0.2 normal. So, using this volume multiplied by strength of one species equals to volume multiplied by strength of the other species we can figure out the strength of sodium hydroxide.

So, V 1 is 10 ml, S 1 is 0.2, V 2 is 2.8 so, we can solve for S 2 and it turned out to be 0.714 normal.

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Preparing standard solutions

Standardization of HCl solution (~0.5 N)

Concentrated HCl ~ 12 M

For 250 mL 0.5 N HCl, take ~10 mL HCl in a clean volumetric and make up the volume with double distilled water.

⇒ Pipette exactly 10 ml of the HCl solution into a clean conical flask, add 2 drops of phenolphthalein indicator solution, and titrate to the pink endpoint with standard NaOH.

⇒ Vol. of NaOH required= 8.9 mL

From $V_1S_1=V_2S_2$

⇒ V_1= 8.9 mL, S_1 = 0.714 N, V_2 = 10 mL, S_2 = ?

⇒ **Conc. Of HCl (S_2) = 0.635 (N)**

Similarly, using this sodium hydroxide solution we determined the strength of the hydrochloric acid. So, we prepared 0.635 normal of hydrochloric acid which was used to drop the pH of the amino acid to less than 2.

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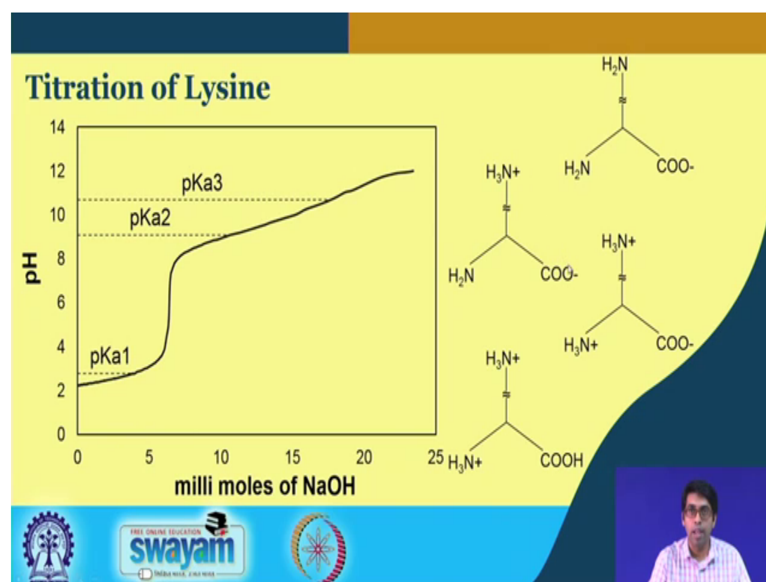

This is the titration curve of glycine. So, again you can see that lysine has 3 ionizable groups; the amino group and the carboxyl group and then again another amino group in the side chain. So, instead of drawing all the carbons I have just represented it like this.

When the pH was very low you see the pH was again close to 2 or may be slightly less than 2, lysine will be in this form where the carboxyl group is protonated and both the amino groups are protonated. So, this species has a net charge of plus 2. The first titration occurs for the carboxylic group with where it loses its proton and it becomes negatively charged.

So, now this is this has a net one positive charge because plus 1 plus 1 minus 1, so the net charge is plus 1. And this transition is given by this straight line. So, a straight here midpoint of the straight line will be pKa 1 which corresponds to the first transition or the first iteration ok. The next species is where this back bone NH 2 back bone amino group loses its proton. So, this NH 3 plus becomes NH 2. And this transition is given by a straight line from here to somewhere here ok. So, if I take a straight line from here to here if I take the midpoint then by pKa for this transition is somewhere here and that comes out close to 9 between 8 and 10.

The third titration is this one where the side chain loses its amino proton ok and that is given by this other part of the straight line, and if I take the midpoint it will come

somewhere here ok. So, if I draw point out this 3 pKa values, pKa 1 is for the carboxylic acid, pKa 2 is for the amino group and pKa 3 is for the side chain amino group right.

Now, the neutral species is this one, because it has one positive charge and one negative charge. So, we get this neutral group by titration from here to here and there to here. So, this is pKa 2 and this is pKa 3. So, our pI for lysine will be an average of pKa 2 and pKa 3. We determine pKa 2 as roughly 9 and pKa 3 as somewhere around 10.2. So, the pI of lysine will be average of 9 and 10.2 ok. So, that is all for week 2.

Thank you. We will see you in the next week.