Experimental Biochemistry
Prof. Snigdha Maiti
Prof. Pritam Roy
School of Bioscience
Department of Chemistry
Indian Institute of Technology, Kharagpur

Lecture – 10
pI Determination of Lysine

(Refer Slide Time: 00:16)

Hello everyone. So, now we will begin with the titration of an acid and the basic amino acid, but before that we are using a separate form of the titrant that is NaOH out here. We are preparing another set of NaOH and for that we have to do the methods from the beginning like, first we have to prepare oxalic acid then we have to prepare fresh NaOH and then HCl and followed by proper standardization.

So, initially we have to prepare the primary standard that is oxalic acid, then NaOH, then we have to standardize the NaOH which is in nature secondary standard using the primary standard oxalic acid and after we standardize the NaOH we will standardize the HCl. And now NaOH is required for titration that is we will be adding NaOH drop wise to the acidic or basic amino acid as we have seen earlier in case of glycine.

And on the other hand, the amino acid initially if needed to be protonated for that, we will be using standard or rather of HCl which will be standardized using NaOH. Initially,

we will begin with weighing or oxalic acid and preparing the primary oxalic acid solution. The oxalic acid will prepare is 250 ml 0.2 normal oxalic acid, for that we will require 2.25 gram of oxalic acid. Now, this is the oxalic acid we will be using for preparation.

(Refer Slide Time: 01:45)

(Refer Slide Time: 01:56)

As we can see this is the digital balance. And here this is the butter paper, this butter paper here we will put the sample and we will weigh the amount of oxalic acid. Here we can see that, it is showing some amount of weight of the butter paper. So, we have to tear

this, every time you use any base like a butter paper or any a pin drop or anything or any vial, initially always do taring. The tare part of the instrument is very much essential.

(Refer Slide Time: 02:34)

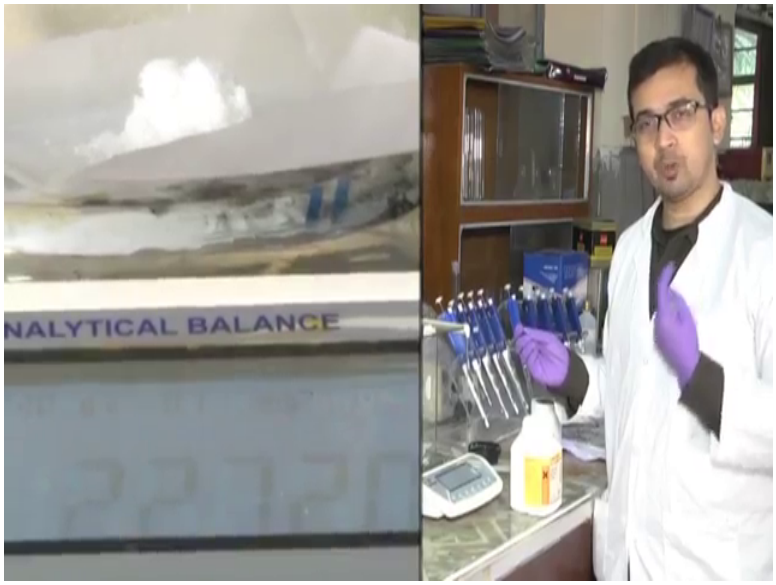
Now, I will be adding around 2.25 gram of oxalic acid. Here, we can see the value is actually 0, so once this value reaches 0, we will start adding the solid oxalic acid.

(Refer Slide Time: 02:52)

We are using a clean spatula, the spatula I am using has been washed with double distilled water and after that with acetone and dried and after that I am just cleaning with

clean tissue paper. Now, I will be adding 2.25 gram of oxalic acid. So, this is the oxalic acid. Add oxalic acid very carefully.

(Refer Slide Time: 03:20)



Here we can see the oxalic acid crystals, it is already 2 gram 1.9, it is around 2.1 and a pinch I will be adding out here it is 2.7. So, the amount of oxalic acid we require is 2.25. So, it is around 2.27, so we will keep it up till here as we all know that whatever the exact weight we require we have to take a small amount of an excess weight.

(Refer Slide Time: 04:32)

Now, we will take this spatula and we will again clean it, but before that we have to transfer this sample to the volumetric flask of 250 ml volume. Now, we will take the oxalic acid in the clean and dried volumetric flask and here we can see the volumetric flask has been dried and it is very much essential to keep it dried and free from any sort of moisture or water and similarly we should also use this funnel.

So, what happens is that you cannot actually, take this sample and pour it directly through its mouth because some amount of sample might fall from this volumetric flask. So, it is better to always use a funnel out here. The funnel should also be clean and dry.

(Refer Slide Time: 05:23)

Now we are kept this funnel in this mouth of the volumetric flask. Here we have taken out this sample, here we can see the measured oxalic acid, we will carefully transfer this in the volumetric flask so, this funnel. What happens is that here we can see. the sample is out here in the funnel in the mouth of the funnel its stuck; so, how to transfer this sample out here, you can actually pour double distilled water through it.

(Refer Slide Time: 05:58)

So, here we have double distilled water, we are adding it in the funnel like this way. And, this is one of the very small and important method out here you have to just add water in a circular manner in the mouth of the funnel.

So, that whatever sample is stuck around this funnel will flow through it, always try to add water through this funnel because you never know whether some amount of sample is still stuck there in the side of the funnel or not. So, at least half of this volumetric flask should be filled with water, after adding it through this funnel. Now, we have added a portion of water double distilled water in this volumetric flask.

(Refer Slide Time: 06:57)

(Refer Slide Time: 07:26)

The next method is remove this funnel, and put this lid over it and gently stir this one. One important thing is that, do not fill up to this 250 ml mark out here and then start stirring because you basically cannot vibrate this entire solution freely in the, if it is completely filled. So, better to make it half filled with double distilled water whatever solvent you prefer and then gradually stir it. Entire sample may not be dissolved out here and again I will be adding doubled distilled water though the funnel.

So, this is the periodic step, we have to add water in portions and stir it gently and gradually you would see, that the crystals of oxalic acids starts to dissolve. If you see any time that, the solution the solute in the solution does not dissolve you can go for ultrasonic bath and sometimes people go for heating, but it is better not to heat samples because you may not know what might, what the reaction might take place if you start heating any sort of sample, without knowing its chemical properties. So, here we can see the solution is almost clean and oxalic acid is dissolved in this double distilled water. Now, here we have to fill it up to this mark, in every volumetric flask which we all used there is a mark for 100 ml there is one mark for 250 ml there is a mark, we will fill up to this mark, to make it 250 ml.

And one small thing to remember out here, while using the volumetric flask do not add 250 ml of water in through a measuring cylinder directly to it and then at sample or first at sample, then directly add 250 ml of water. Do not add entire volume of water to it because, due to volume error which you all study in our chemistry lab classes, due to volume error; the total volume on the solution might increase a bit. So, it is better to add sample, then add little bit of double distilled water and then you dissolve you, try to dissolve it as much as possible then, fill the volume totally with double distilled water up till the mark.

Now, I will pour double distilled water directly to it. Its taking a bit sometime, so you can add double distilled water directly, if you see that its taking a lot of time to fill this volume like for 250 ml or 500 ml you can avoid this nozzle and directly at double distilled water provided you are confident enough.

(Refer Slide Time: 11:32)

I will leave it till here, because this narrow neck it fills up very fast. So, I will prefer to pour water through this nozzle up to a mark here. So, this mark is very thin and it is not always clearly visible when you are seeing it from a distance. So, whenever you would be using any sort of volumetric flask you can easily see in the mark graduated out there.

(Refer Slide Time: 12:12)

So, we have prepared oxalic acid of 0.2 normal and this is the solution of oxalic acid. Now this is the primary solution of oxalic acid, we will now shift to NaOH solution. The NaOH we will prepared is actually 250 ml of 0.5 normal NaOH and this is actually the

rough concentration of NaOH. So, we have prepared oxalic acid which is the primary standard, now we will weigh NaOH we will weigh 5 gram of NaOH, which is actually the secondary standard and it will be estimated the exact strength of NaOH will be estimated using oxalic acid. So, we have kept this butter paper out here and we will tear this, keep in mind until unless it goes to 0 you have to just be patient, oh yeah it is 0 now. So, we have again clean this spatula and we will be adding NaOH.

Prefer not to use this NaOH beads using your hand, always take NaOH using a spatula. We will be taking around 5 gram of NaOH, it is 4 4 gram and 1 more is required 4.3. NaOH generally absorb moisture so, prefer not to keep this bottle open for long time and small bead, it will do 5.2 and a small amount. Because, the weight of NaOH bead is not exactly the weight it should be because it is already absorbed some amount of moisture. So, whatever is showing out here like 5.3, it may not be 5.3341 or rather much less than that the exact weight.

So, sometimes what happens is that, whenever this bottle this NaOH bottle is open and closed it absorb moisture from time to time. So, it's exact weight actually increases. Now here we have again taken this volumetric flask, it is a 250 ml volumetric flask and I have kept again a clean funnel, now I will transfer this NaOH beads. So, here we will be adding double distilled water in a similar fashion through this funnel, try to avoid your bare hand from here because whenever water is added to NaOH the reaction is highly exothermic so, heat is generated.

A small amount of NaOH is still here in the mouth of this funnel. So, you have to add a few amount of water. Now here we can see, this volumetric flask is almost half filled with double distilled water and this funnel is almost a clean like more or less; most of the NaOH has been passed has been dissolved in double distilled water and still we will be adding small amount of double stilled water again through this funnel like the previous way, will be closing this lid and again stirring it, NaOH dissolves very easily in water.

So, here we can see that, the solution is clear out here. Now, we can add the remaining of this volumetric flask the double distilled water. I will be carefully adding double distilled water to it without the nozzle, it is better to add double distilled water without this nozzle till this part of this round region of the volumetric flask and this make of this volumetric flask always use this nozzle.

So, you can you can add double distilled water without this nozzle up till this mark the round part of this volumetric flask, but prefer to add water using this funnel. So, that this level, that is the mark which is there in the volumetric flask does not cross. So, it is safe to use this nozzle because once you reach this neck, it fills the volumetric flask fills at a very fast rate. So, here we are adding very slowly, slowly slowly and now it has reached this graduated mark and we are done with this NaOH solution.

So, we have completely filled this volumetric flask with double distilled water this is the NaOH solution, up strength around 0.5 normal. So, we will measure the exact strength of the solution, using titration method. Now we will use this NaOH to carry out titration of basic and acidic amino acid. And the acetic amino acid will be using today is glutamic acid and the basic amino acid will be using today is glycine. So, we will measure each of this individual amino acid. One interesting thing to note out; note down here is that, people may ask you, how many beads of NaOH have you taken while measuring 5 gram of NaOH?

Actually, you do not basically count the number of beads while measuring any chemicals or something like that, but generally what happens is that, it is a very common question, you know weight of each bead of NaOH. Basically it is 0.15 gram for each half bead and one full bead that is, one half, one half becomes 0.3 gram. So, it is exactly not the same, but it is close to one another like, if you do not have any weighing balance and you have to measure NaOH of 5 gram. So, what you can do? You can actually, measure the number of beads required for actually for measuring the 5 gram of NaOH.

(Refer Slide Time: 21:22)

Now, let us see: what is the weight of half bead of NaOH. Now, we will take one half bead of NaOH. So, what is meant by half bead? We have all seen NaOH beads, it is basically half sphere. So, we can see a sphere it is around and this is half sphere is basically one half bead of NaOH.

Now, this here we can see one half bead of NaOH, we have taken it in a spatula. We have kept one butter paper, we will tear this one, it is 0. Now, we will place we will tear once, it is 0, we will place this half bead here in this butter paper. Here we can see, it is actually 0.1660 gram. So, it is not exactly 0.15 gram it is basically, 0.1660 grams. So, it is bit more than that.

(Refer Slide Time: 22:07)

Now, let us take another half bead, so we have taken another half bead and we are putting another half bead out here, here we can see the weight changes to 0.3276 grams. So, it is bead more than 0.3 gram. So, what is full bead, here inside we can see above this butter paper, on the butter paper here we can see one half a bead and one half bead, one half sphere and another half sphere over it.

So, it is basically a sphere and two half beads make one full bid. So, basically the question is, how many full beads make; how many full beads are there in 5 gram of NaOH or how many half beads are there in 5 gram of NaOH, solve it and write down the answer, I will not tell it correctly.

(Refer Slide Time: 23:25)

Now, we will wait l lysine out here, we can say it is l lysine it is actually l lysine hydrochloride. Here, we have to always note down before measuring. So, we will measure actually 100 ml and 0.1 molar lysine solution and for that we will require around 1.5 gram of lysine. So, we have taken again this butter paper out here, we will tear this one, again I have cleaned it this spatula and I will be adding lysine of around 1.5 gram.

Here we are preparing 0.1 molar solution, you can actually vary this amount, you may not prepare 0.1, you may prepare lower than that like this, 0.1 molar becomes 100 millimolar you can prepare like 10 millimolar or something like that, whichever is convenient for your work. Sometimes it better to use a spatula, which is bigger than this one. So, it depends upon your convenience, but spatula with this small mouth is generally good for your measurements for like very critical measurements like in a decimal places or for that, a smaller spatula is preferred.

So, it is always better to use a small spatula, but you can go for largest spatula if your weight is around 4 gram, 5 gram or around 10 gram. It is almost 4; 4.6 it is 5.2. So, again I am taking a small amount of excess lysine out here, this is the 100 ml volumetric flask which you have cleaned and dried and again this is the clean funnel, we will take out this lysine carefully out here and again we will put this through this funnel. Always we can see a small amount of this compound whether it lysine or anything is stuck in this butter

paper. So, it is better to use excess a bit excess amount of the sample which we are measuring each and every time.

Now, here you can see I have taken this lysine out here, I will be adding double distilled water through this funnel. Although, we can see the more or less all the lysine have passed through this funnel into this volumetric flask, still its prefer to add water through this funnel. Sometimes, the solute might stick there in this mouth of the funnel. So, add water this way and stir it gently, more or less the solution is clear only, only thing we can see a small lump of this lysine still there in the solution and we will be adding a bit of double distilled water once more to this funnel.

Do not put directly, just encircle in this way like you are revolving around this mouth of the volumetric flask. Almost half of this volumetric flask is filled with double stilled water and here we can see the solution is almost clean or some of the residues are still there. We have to dissolve it completely. Now, for 100 ml volumetric flask prefer not to add water directly without this nozzle, here the there is a mark, very faint mark is there, I will put this slowly till this mark. Now, we are done with this lysine solution, it is basically 0.1 molar lysine of 100 ml, now we will measure glutamic acid.

(Refer Slide Time: 30:26)

And next thing we will measuring is L glutamic acid. Now, the concentration of glutamic acid is again 0.1 molar is similar to lysine and 100 ml for that we require around 1.3 gram of this glutamic acid. So, we will tear this one, I have cleaned this spatula, now I

will be adding 1; 3 gram of glutamic acid. So, it is 1.1 gram or now it is just below 1.1 and now it is 1.1 gram, we will be taking 1.3 gram a bit excess.

So, what basically happens is that, we will transfer this glutamic acid again in a 100 ml volumetric flask fill the volume again double distilled water till the mark and we will dissolve it. We might see at times it happen that, glutamic acid is quite difficult to dissolve for that we will go to ultrasonication method in which we will keep it in the ultrasonic bath and let it dissolve for some time and then proceed for the experiment.

(Refer Slide Time: 32:17)

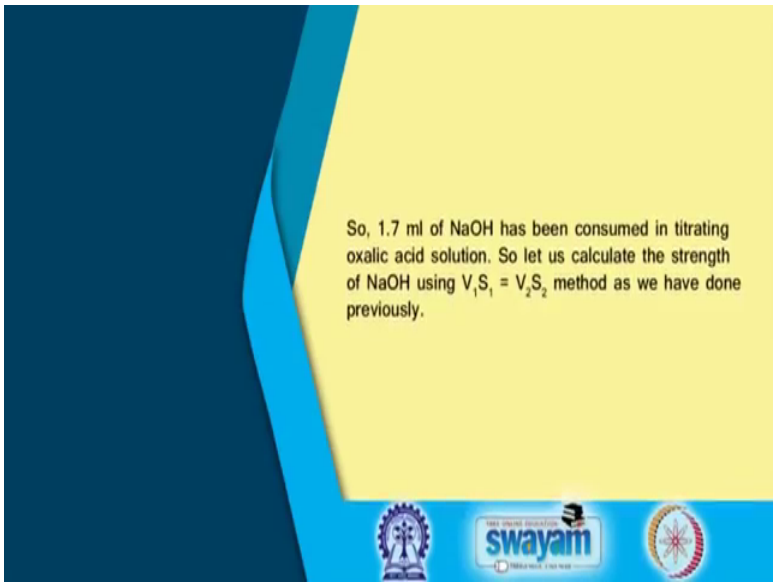
So, we have taken sodium hydroxide solution in this burette and oxalic acid is there in the volumetric flask and we will take 10 ml of oxalic acid has previously we have seen using this pipette. So, this pipette has been cleaned properly and we will gradually take this solution yeah, a 0 meniscus has been reached a small amount, yeah done. Now, I will transfer this 1 in this clean and conical flask, after that add 1 or 2 drops of phenolphthalein indicator.

(Refer Slide Time: 32:47)

(Refer Slide Time: 33:24)

Now, we will titrate this with sodium hydroxide solution; we will slowly titrate this oxalic acid with NaOH, we will be adding NaOH drop wise and stir it gently, a faint pink colour appears in the solution and once we stir it fades actually. Here we can see, the faint pink colour becomes completely permanent, leave it for some time stir it. If it fades away once again, then you will understand the end point is not here, but out here in this case we can easily conclude that the end point has been reached because this pink colour is persistent.

(Refer Slide Time: 34:35)



Now, we will note down the volume from here and the volume that has been consumed of NaOH is around 1.7. So, 1.7 ml of NaOH has been consumed in titrating oxalic acid solution. So, let us calculate the strength of NaOH using V1 S1 equal to V2 S2 method as we have done previously. We have calculated the concentration of sodium hydroxides, the concentration of sodium hydroxide is 1.11 normal. So, using this sodium hydroxide solution, we will measure the concentration of HCl solution, we have prepared this HCl solution which is a dilute HCl solution. So, we will again take 10 ml of HCl and transfer it in this conical flask.

I am using again a new conical flask, this conical flask has been cleaned properly and after that, we will give 1 to 2 drops of phenolphthalein indicator, as you know phenolphthalein is colorless in acidic solution and pink in basic solution, one small thing to note down here is that, why we do not use NaOH in the conical flask and acetic in the burette? So, either lots of reasons out there, one reason which you can basically thing at this moment is that, if you add phenolphthalein in this conical flask continuing NaOH, it will completely turn pink.

And, once you start adding HCl solution, the pink colour will gradually fade, but sometime what is happened due to persistent of vision you cannot actually discriminate when the pink colour completely fades from pink to colorless, that will it come dark pink, light pink, very light pink, very very light pink and then colorless and that very

very light pink or what we can serves, slight light tinge of pink colour and colorless solution is not very easy to detect with our eyes.

So, it is better to use acidic solution or acid in this conical flask add phenolphthalein and you can very well distinct from colorless to pink rather from pink to colorless. So, it is better to take acidic solution here in this conical and base in the burette. So, let us proceed for titration. Once you have done our first titration, we can do two things, once one is that, we can fill this burette again up to 0 mark with NaOH and the second thing is that we can mark this 1 that is 1.8 ml and we mark this as V initial Vi and we will note the V final that is the point where the pink colour appears ok.

So, this one for my case V i is actually 1.8 so let us start with the titration, we will again slowly add NaOH, you can go on adding the solution; you can go on adding the base out here freely, but once the pink colour appears try to add drop wise. A faint pink colour has started appearing and but again it disappears. So, the end point has not yet reached, here we shall approach the end point shown whereas, in 1 or 2 drops, k 1 drop, 2drops here. Here we can seen, 2 drops we have reached this pink colour.

So, this is the end of our titration and now we will note the volume that is the final volume, that is V f. V f from here is around 9.3 our Vi is actually, 1.8 and that is the volume initial volume and the final volume is 9. 3. So, it is better to you see the level of this graduation at your eye level like this way; do not look at in this way like in this or in this or it is, better to always take this meniscus in your at your eye level ok, it is 9.3. So, we will do the calculation and measure the concentration of HCl solution.

We have calculated it using the V1 S1 equal to V2 S2 part and the concentration of HCl we calculated out here is 0.832 normal. So, we have initially calculated a standardized NaOH using pre standard to primary standard oxalic acid solution. The secondary standard NaOH has been estimated and using this standardized NaOH we have measured the concentration of HCl.

Now, we will go for titration of lysine and glutamic acid. Now, we have taken lysine solution in this 100 ml beaker and we will be adding HCl to it, why we will be adding HCl? As we have seen in case of glycine, we have to first protonate completely this amino acid. For lysine we have taken protonated for this (Refer Time: 41:35) protonated with using HCl, the HCl we have standardized just a few minutes ago.

So, what is the concentration of HCl? It is around 0.832normal and what is the volume of HCl required, for that we have to actually calculate the number of moles of this amino acid out here. In case of 0.1 molar 100 ml lysine there are number of moles of lysine present and a number of moles of HCl required to protonated. In this case, we will be adding initially 12 ml of HCl 0.832 normal HCl and let us check out the pH after that.

Now, this is a 10 ml pipette. Initially, we will pipette out first 10 ml, add it here. Now again, we will pipette out 10 ml, but here we will add only 2 ml because total volume we have to do is 12 ml; carefully add 2 ml out here, done. So, we have prepared this lysine solution, protonated lysine solution and we will check its pH value.

(Refer Slide Time: 43:20)

We will be titrating the lysine solution with sodium hydroxide and we will counting a number of drops and we will see the change in ph, but while during the graph plot, we will be plotting pH versus the volume of NaOH. For that, we need to count in the number of drops corresponding to a particular volume of NaOH. How to proceed for that? Initially we have to know, the volume corresponding to one single drop and for that we will do this type method.

For that, what we will do? We will take this sodium hydroxide in this burette, here we have filled this burette. She will be counting the number of drops out here and I will be monitoring the change in volume. So, the change in volume from 0 to 1 ml, the number

of drops associated with one ml we will calculate and then we will see, one drop corresponds to how much volume ok. So, are you ready?

Yes sir.

(Refer Slide Time: 44:22)

So, let us start 1 drop, the volume is decreasing and it is gradually approaching 1 ml and I am slowing down it and when we discuss, done.

Is around 20.

20, right.

So, generally it is the standard number of drops. So, 1 ml corresponds to 20 drops. Now, we have taken one reading, we will again repeat it. I will again go from 1 to 2 ml and kindly count the number of drops ok. So, let us start we will see again from 1 to 2 ml. So, we can see again it is going from this meniscus is going from 1 to 2 ml, it is going and above two stop this one done.

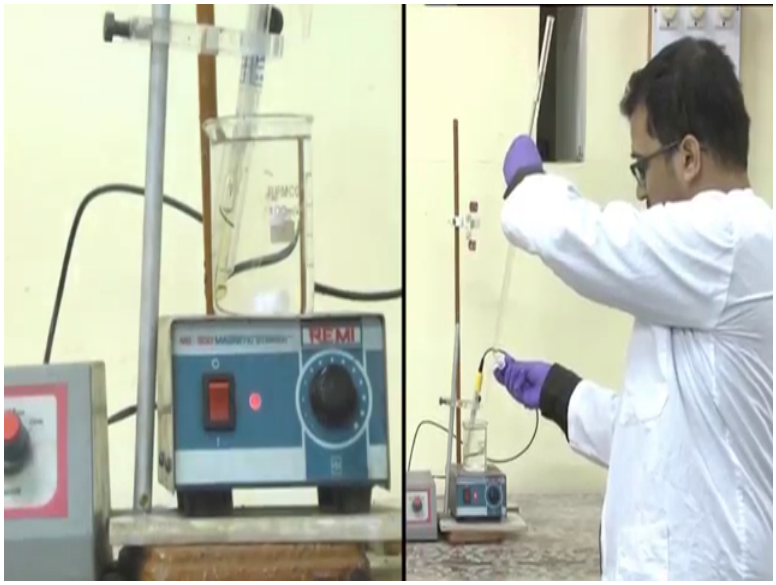
18.

18. So, in one reading, it is showing 20 drops, in the other one it is 18. So, we can take the average, we can also repeat this one. So, we can take the average from 18 and 20 and see the number of drops corresponding to 1 ml or other number of, what is the volume corresponding to a single drop.

Now, we will proceed for the pH metric titration of lysine solution. We have taken lysine in a beaker, we have kept it over a magnetic stirrer out here and dip the pH electrode into it and we should be careful with the magnetic bar of that it that do not touches the pH bulb.

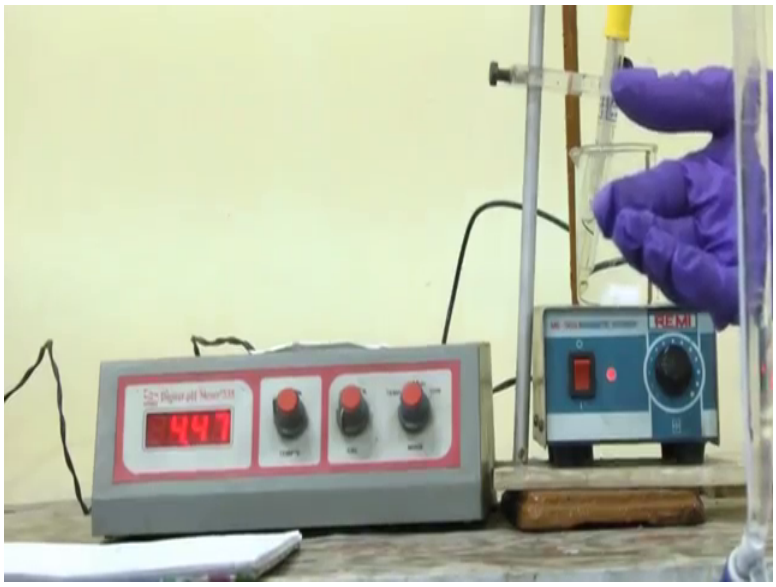
Now, we can see the pH of this solution basically the protonated solution is around 2.27. This is the NaOH, we will be adding NaOH drop wise to it and we will record the pH corresponding to number of drops we are adding, should help me with noting down the pH values. Generally, it is convenient for 2 partners to carry out this experiment because you should be careful in adding these drops in this solution and one should minutely note down the pH change during this experiment. So, let us start with this experiment.

(Refer Slide Time: 47:05)



(Refer Slide Time: 47:19)

(Refer Slide Time: 47:27)



Now, now we will be adding 2 drops of NaOH. And the pH changes a bit it is 2.25 now, again 2 drops that is total 4 drops and the pH changes not that much out at the pH did not change here, it is same. Now, I am added another 2 drops that is 6 drops.

So, here you can basically go on adding or you can actually add 2 drops and wait for the solution to be homogenous, another 2 drop the pH changes very slow in this case, we should wait in order to so that the solution gets stabilized reading. Here we can see the increase in pH is not that much, the change in volume of NaOH is 2.29; again 2 drops I am adding 2.29 2.30 and 29 it is a 30, 131 32.

Now, I will be adding 4 drops, 1 2 3 and 4, is 2.33 again 4 drops, 2.34; 35, 4 drops, 2.37, it is 2.40. Again, I will be adding 4 drops, this one is basically a buffer zone, that is why the pH change is very negligible out here, 2 drops 52, again 4 drops; 1486 drops. Now, again 4 drops 2.54, now I am adding 2 drops ok, 4 drops, again 4 drops 2.5859, 2.61, 4 drops 66 2.66. Here we can see the change in pH comes to around 2.69 from 2 point around 2.2, 234 2.71, 234; 2.75 and for it is 2.79. So, drops 84. Again, 4 drops 2.91, it is 3.30.

Student: Sir, (Refer Time: 55:42).

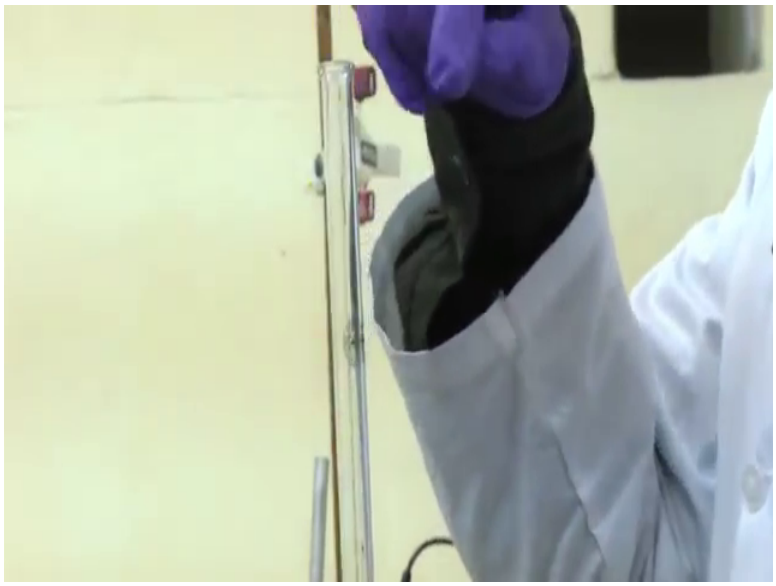
So, here we can see a straight jump from 3.17 to 3.24 3, I am adding 2 drops, 3.2 26 again 4 drops, 2drops 3.46 2drops 3.54; 2drops 3.55, again 4 drops and give.

After adding like 2 or 4 drops we should leave the solution to stir for some time, in order to get homogenous because once we can see the increase in pH is very abrupt and again it decreases in certain cases. Now, 2 drops 3.90, we can gradually see a straight jump from 3.76 value, I mean again adding 2 drops 4.12, again 2 drops.

So, here we can see the fluctuation or the change from 4.1 to 4 point almost 45, 46, it will vary, we have to keep it give it some time for stabilization it is 4.45 or 44. Again I am giving 2 drops. So, here the inflection point, somewhere around here we can see it has attained something around 6 range now it is decreasing. So, the pH in the region of the bulb actually changes in the first stage, but; however, after homogenization the pH again decreases, it is 5.11 and 10. Again 2drops, now again a rapid jump from 5.1 to around 6.9 and again it decreases.

Basically, what happens is that, if this is the bulb of the pH meter here locally NaOH accumulates in this case, here NaOH accumulates locally near the bulb for the pH meter, that is why at initial cases it detects like one's we have added NaOH out here, it reaches the bulb of the pH, it detects pH value of around certain from 5.5 to 7.5.

(Refer Slide Time: 59:54)



But, after that once this NaOH molecules are homogenous within the solution. So, the concentration NaOH near the bulb decreases again; and again we can see a gradual lowering of the pH values. So, it is better do not note down the pH values immediately after adding this, give it some time for stabilization once we have reaches a fixed pH then note it down.

So, it is 6.76, we will be adding again 2 drops, if you wish we can go on adding 1 drop in this case also, it is 7.22, let us add 1 drop here ok. So, I am adding 1 single drop, but almost we have almost passed the inflection point I guess. we have added 1 drop out here, 7.36 and again 1 drop I am adding 7, 7.44 talking about, 7.44 pH as you can all will it this is the physiological pH basically; so the in this experiment we can say we have reached a very interesting point out here ph 7.4.

Anyways we have to go a bit ahead from here, I am adding 2 drops 7, 7.58; again 2 drops 7.71. Now, we are again adding 2 drops 7.8, after that it will again reach a buffer region where the change in pH will be very slow and it is gradually heading there, again 2 drops. We have seen initially, the change in pH was very slow in the range of 0.01 or 02 and again it went in the range of 0.5 and again in the range of 1 value; 1 unit and those are the inflection points. Now again, it is changing in the range of 0.1 point 1.5. Again 2 drops, now here we can see the increases like 0.5 units from here which was initially in during the inflection point it was around 0.8 0.9.

Again 2 drops, now here we can see it is again, slowed down out here and in 2 drop, we have to take this to a value nearly of pH 12. Now, I am adding 4 drops. So, we are at a buffer range, so we can increase the number of drops I have added 4 drops, 8.25. Again four drops, 8.30; again I am adding 4 drops.

So, here the change in pH is basically in the range of 0.05 or 06 units, again four drops, 4 and 46 and 4 drops ok. Now, I am increasing the number of drops, I am adding 6 drops 1, 2, 3, 4, 5, 6. So, here we can see that operating 6 drops also the number of the change in the pH value is still not that much, 2 3 4 5 6. Slowly, it has reached the value 8.8.

So, here we can see the change in pH of very slow, in the again in the buffer region, again 6 drops; 6 drops, 8 drops 9.06 ok; now 8 drops, 9.12 I will be adding 4 drops, 9.15, 9.63, again 10 drops, it is now 10.12 and we have to take it up to 12; 10 drops, 10 it is 10.25, now I will be adding 20 drops. So, after 20 drops the pH change is around from 10.25 to 10.40. Again 20 drops, 10.58 is 10.7, 10.80 it is 20 again, 11.02; it is 10, 11.10 again 20, 11.30 and we are close to 12 now, again 20, 10.5 and again 20 near 12 will go it last 20 drops.

Actually the first pk, even in the range of around 2 to 3 whereas, in the second one is in the range of around 10. So, after reaching 10 or 10.8 we can gradually move up to 11.5 or 12. Now, 10 drops so, 11.96 and final 10 drops; so it is 11.99 and it is 12 basically. So, we will end this titration till here and we will now plot the change in pH against, the volume which you can calculate from the number of drops and we can find the pk1 pk2and the pi for lysine.

So, we have seen the preparation of sodium hydroxide NaOH that is sodium hydroxide then, oxalic acid and how sodium hydroxide was standardized as an oxalic acid and that standard NaOH was used to again standardize HCl. And now, the lysine solution was protonated using HCl and then gradually, we added NaOH and titrate it and we have seen the two infection ranges.

We have notice the first one, but somehow we have could not actually monitor the second one or the other inflection points cannot be monitored using this pH because they are very small. And we have carried out from pH range 2 to around 12 and from here we will plot our pH metric curve from which will be the pH in the y axis and the volume of NaOH in the x axis.

And from there similar graph like this so, we can obtain for lysine and from there we can calculate the pk1 pk2 pk r and from just three we have to calculate pi for lysine. In a similar way, we can carry out for glutamic acid or anything or aspartic acid, what was the acidic amino acid maybe and in that case also we will get another p1, pk1, pk2, pkr for glutamic and aspartic acid also.

In a similar way, now we will get another pk1, pk2 and pkr for aspartic acid or glutamic acid also and we can calculates its pi from that values. So, in this experiment we can see, how to calculate the pi of amino acid using NaOH in a pH metric titration method.