Exp. 6

Aim: Determination of pI of Glycine.

Apparatus required: pH meter, burette, pipette, beaker, distilled water, tissue paper, measuring cylinder

Chemicals Required: solution mixture of Glycine & HCl, NaOH (0.1M), reference buffer (de-ionized water) solution,

Principle: Glycine (means 'sweet tasting' in Greek) is simplest amino acid. In aqueous solution, it is amphoteric: at low pH it can be protonated and at high pH it loses a proton. As it can be noted that at low pH glycine has net +ve charge and at high pH, it has net -ve charge, so in between at an intermediate pH, Glycine should carry a net 0 charge, & this special pH is known as pI or Isoelectric point at which the molecule or surface carries no net electrical charge.

At this pH i.e. pI, it becomes Zwitterion (i.e. having equal +ve & -ve charge but net charge 0). Not for only Glycine it happens with all Amino acids, and charges are accumulated on $-NH_3^+$ and COO^- groups. Also this experiment is valid for all AA.

Interesting fact is that net charge is controlled by pH of the solution. pI is also understood as the pH at which amino acid does not migrate in an electric field.

pI is given by average of the pK_a of molecule, K_a is acid dissociation constant.

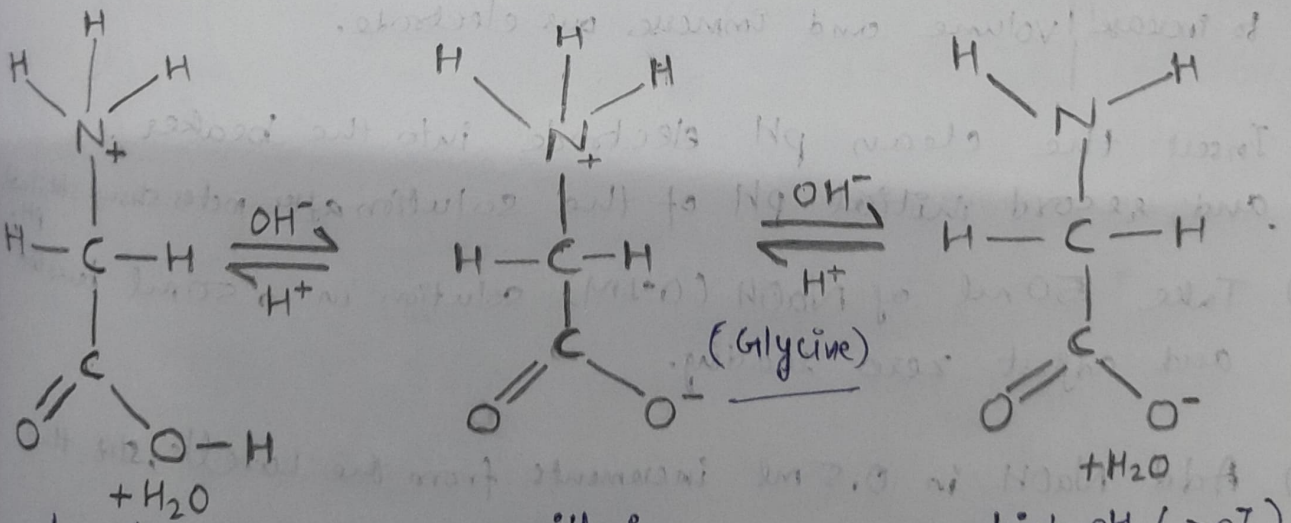
In the experiment, we use pH meter which consists of potentiometer and combined electrode which have in it a working and a reference electrode, which basically measures the Hydrogen activity as a potential difference of the solution of our interest, and this potential difference is measured by machine

and displays pH directly.

A Buffer solution (as we know solution having constant and stable pH, not affected much by external triggers) is used to calibrate the pH meter. As we already know the pH of buffer solution taken, we do put electrode of pH meter into buffer solution and check that it is showing same value or not.

Now for determining pI, we take glycine + acid mixture and add distilled water to increase volume and put electrode of pH meter after taking it out from buffer and wiping with tissue paper. Then NaOH is added via burette and pH and volume of NaOH used are noted continuously till solution becomes very basic say (pH = 12), after which this experiment also not works well. Two plateau regions of graph of pH vs vol. of NaOH depicts $pH = pka_1$ & pka_2. And as said earlier pI is average of pka_1 & pka_2. So we get pI by making graph plotting of data.

Chemical Reactions & Structures:



low pH (<pI)
(net charge = +1)

Zwitterion
(pI)
(net charge = 0)

(Glycine)

high pH (>pI)
(net charge = -1)

Procedure :

(A) Calibaration of pH meter

1) Take reference buffer in a beaker.
2) Put combined electrode in the buffer solution.
3) Take reading of pH and match it with pH of buffer.

B) pHmetry (i.e. pH measurement) & Glycine acid-base titration

1) Use pipette with a pump on its upper mouth to take known volume (25ml) of Glycine + Acid solution.
2) We take slightly larger amount than 25 ml and then pipette out extra amount, to make measurement correct, while pipetting out make ensure that there is no air bubble inside and pipette out till lower meniscus touches graduation of 25 ml.
3) pipette out this solution in a 250 ml beaker and add 25 ml of distilled water using measuring cylinder to increase volume and immerse our electrode.
4) Insert the clean pH electrode into the beaker and record initial pH of the solution after meter shows stabilized pH.
5) Take 50 ml of NaOH (0.1M) solution in a 50 ml burette and adjust zero reading.
6) Add NaOH in 0.5 ml increments from the burette, stir the solution and mix it well.
7) Do not remove the electrode from the beaker till the end of experiment.

8) Record the corresponding pH values until the pH starts increasing drastically. At this time, add 0.1 ml increments of NaOH till the pH stabilizes around 8.

9) After reaching pH 8 continue adding 0.5 ml increments of NaOH till you reach pH 11.

10) Remember that each time, when you add NaOH, shake solution and wait for next increment till pH stabilizes (reading).

11) plot the graph of pH vs volume of NaOH solution.

12) The two almost horizontal parts of the graph give the values of pka_1 & pka_2 for glycine. Use midpoints of these region to get the values.

13) Average of these values gives pI of glycine.

Observations & Calculations :

- Initially change in pH is relatively lower due to addition of NaOH, but as we reach towards pI, drastic change in pH can be seen and after the region of pI is gone again changes in pH would be relatively lower than at pI.

From graph : (on next page)

$pka_1 = 2.34$

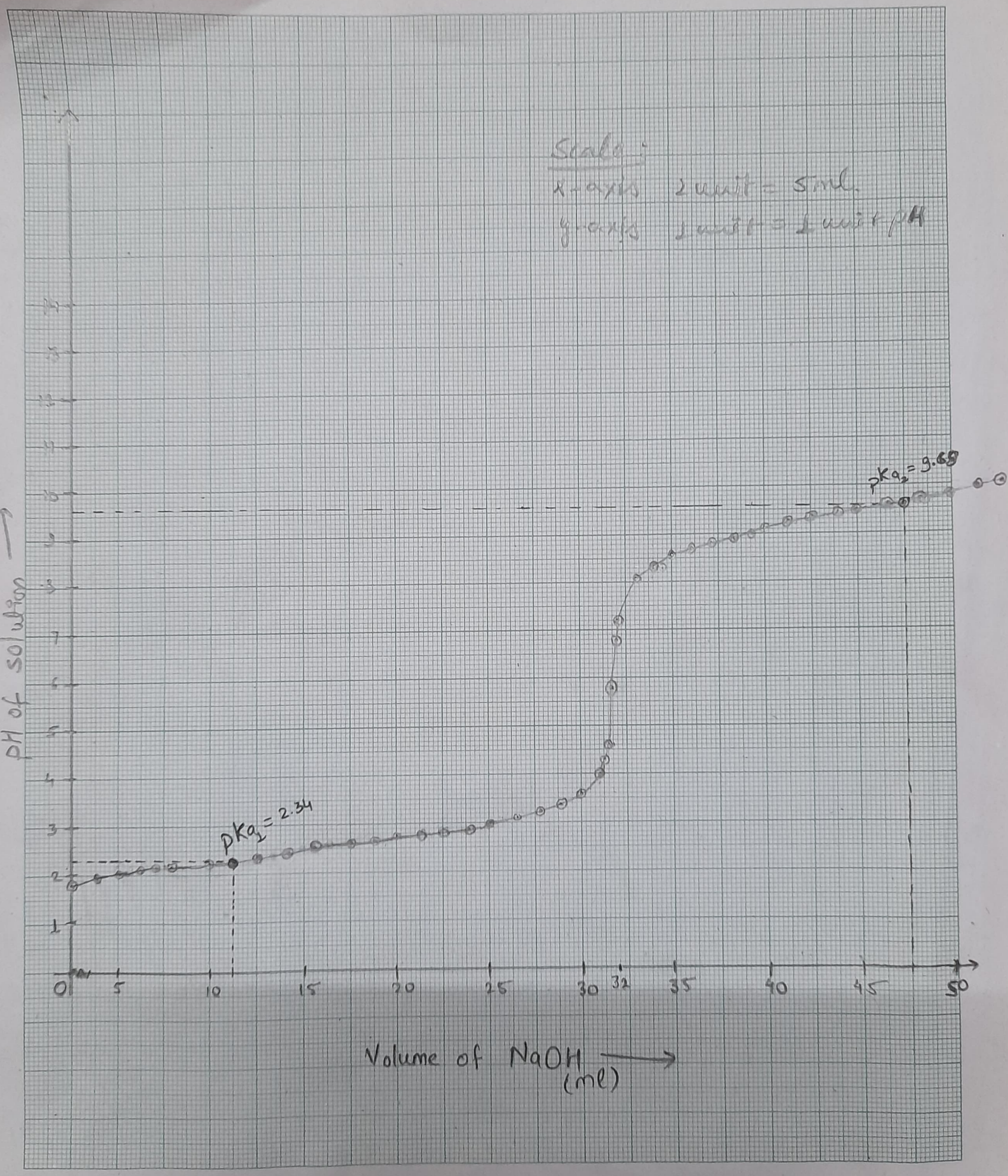
$pka_2 = 9.68$

$pI = (pka_1 + pka_2)/2 = (2.34 + 9.68)/2 = 6.01$

Observation table —:

given concentration of NaOH solution = 0.1 M or 0.1 N

Vol NaOH (ml)	pH	Vol: NaOH (ml)	pH	Vol: NaOH (ml)	pH	Vol. NaOH (ml)	pH
0	1.86	20	2.66	31.1	4.07	45.5	9.44
0.5	1.86	20.5	2.69	31.3	4.21	46	9.45
1	1.87	21	2.73	31.4	4.35	46.5	9.53
1.5	1.89	21.5	2.75	31.5	4.41	47	9.56
2	1.91	22	2.78	31.6	4.68	47.5	9.57
2.5	1.93	22.5	2.81	31.7	5.75	48	9.59
3	1.95	23	2.85	31.8	6.98	48.5	9.62
3.5	1.96	23.5	2.89	32	7.21	49	9.65
4	1.98	24	2.9	32.1	7.52	49.5	9.7
4.5	2	24.5	2.93	32.2	7.71	50	9.72
5	2.01	25	2.97	32.3	7.8	50.5	9.74
5.5	2.03	25.5	3.01	32.4	7.89	51	9.75
6	2.04	25.6	3.02	32.5	7.95	51.5	9.79
6.5	2.06	25.7	3.02	32.6	8.04	52	9.82
7	2.08	25.8	3.04	33	8.1	52.5	9.86
7.5	2.1	25.9	3.05	33.5	8.2	53	9.89
8	2.11	26	3.06	34	8.36	53.5	9.91
8.5	2.13	26.5	3.1	34.5	8.49	54	9.93
9	2.15	27	3.15	35	8.59	54.5	9.97
9.5	2.16	27.5	3.21	35.6	8.66	55	10
10	2.19	27.6	3.22	36	8.77	55.5	10.04
10.5	2.21	27.8	3.24	36.5	8.79	56	10.07
11	2.23	27.9	3.25	37	8.86	56.5	10.1
11.5	2.25	28	3.27	37.5	8.91	57	10.13
12	2.27	28.5	3.34	38	8.96	57.5	10.16
12.5	2.3	28.6	3.36	38.5	9	58	10.19
13	2.31	28.8	3.38	39	9.04	58.5	10.23
13.5	2.37	28.9	3.4	39.5	9.08	59	10.25
14	2.4	29	3.51	40	9.11	59.5	10.3
14.5	2.43	29.5	3.53	40.5	9.15	60	10.36
15	2.45	29.6	3.56	41	9.2	60.5	10.4
15.5	2.5	29.7	3.58	41.5	9.23	61	10.44
16	2.51	29.8	3.6	42	9.27	61.5	10.5
16.5	2.53	29.9	3.64	42.5	9.29	62	10.55
17	2.55	30	3.67	43	9.32	62.5	10.6
17.5	2.57	30.1	3.78	43.5	9.36	63	10.67
18	2.6	30.5	3.82	44	9.38	63.5	10.71
18.5	2.63	30.9	3.93	44.5	9.4	64	10.76
19	2.65	31	3.99			64.5	10.83
19.5						65	10.87
						65.5	10.94
						66	10.99


Scale:
x-axis 2 unit = 5 ml
y-axis 1 unit = 1 unit of pH
pH of solution
$pKa_1 = 2.34$
$pKa_2 = 9.68$
0
5
10
15
20
25
30
32
35
40
45
50
Volume of NaOH (ml)

Result: 1) pI of glycine was determined using pH meter

pI of Glycine = 6.01

Precautions: 1) pH meter calibaration must be done correctly.

2) Sensitivity of electrode must be taken care.

3) When pipetting out ensure no air bubble inside.

4) Take increments of 0.1 ml near pI to have more data points.