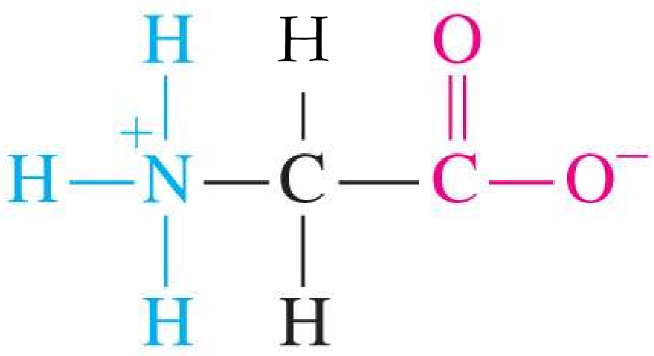
pI of Glycine using pH metry

Glycine (means 'sweet tasting' in Greek) is the simplest amino acid. In aqueous solution, glycine is amphoteric: at low pH it can be protonated and at high pH it loses a proton. Isoelectric point: pH at which a molecule or surface carries no net electrical charge.

Amino Acids as Zwitterions

- **Zwitterion** has equal number of positive and negative charged (net charge of zero)
- For amino acids, these charges are accumulated on $-NH_3^+$ and COO− groups
- The net charge is controlled by the pH of the solution

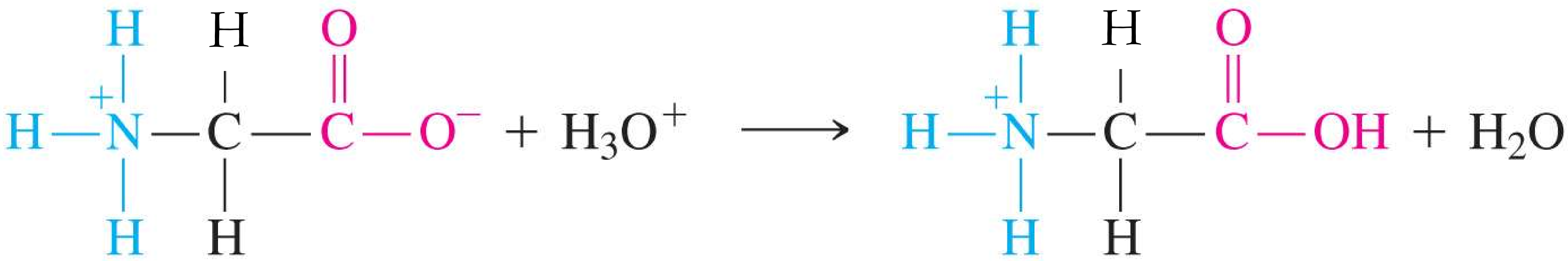


Zwitter ion of glycine (net charge = 0)

Zwitterions in Acidic Solutions

In solutions that are more acidic than the pI,

- the COO^- in the zwitterion accepts a proton
- the amino acid has a positive charge



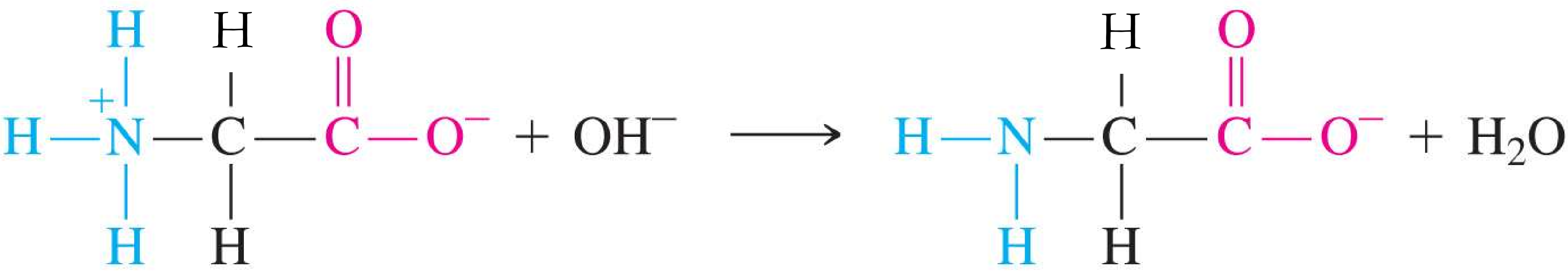
Zwitter ion of glycine at pH ~ 6 (net charge = 0)

Glycine at pH < 6 (net charge = +1)

Zwitterions in Basic Solutions

In solutions that are more basic than the pI,

- the NH_3^+ in the zwitterion loses a proton
- the amino acid has a negative charge



Zwitter ion of glycine
at pH ~ 6
(net charge = 0)

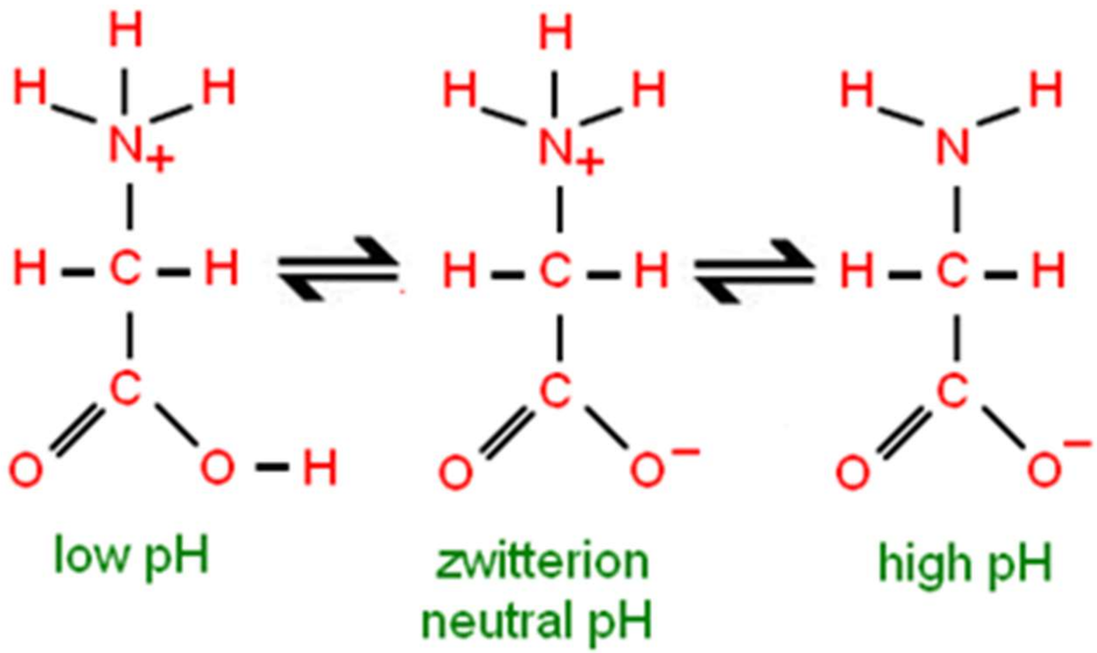
Glycine at pH > 6
(net charge = –1)

Isoelectric Point (pI)

The **isoelectric point (pI) is** the pH at which the amino acid has an overall zero charge (exists in the zwitterionic form)

The **pI** is also understood as the pH at which an amino acid does not migrate in an electric field

The **pI** is given by the average of the pK_a. K_a is the acid dissociation constant



Summary of pH, pI, and Ionization

TABLE 19.5 Ionized Forms of Nonpolar and Polar Neutral Amino Acids

Solution	pH < pI (acidic)	pH = pI	pH > pI (basic)
Change in H^+	$[H^+]$ increases	No change	$[H^+]$ decreases
Carboxylic Acid/Carboxylate	$-COOH$	$-COO^-$	$-COO^-$
Ammonium/Amino	$-NH_3^+$	$-NH_3^+$	$-NH_2$
Overall Charge	1+	0	1−

How to Determine pI?

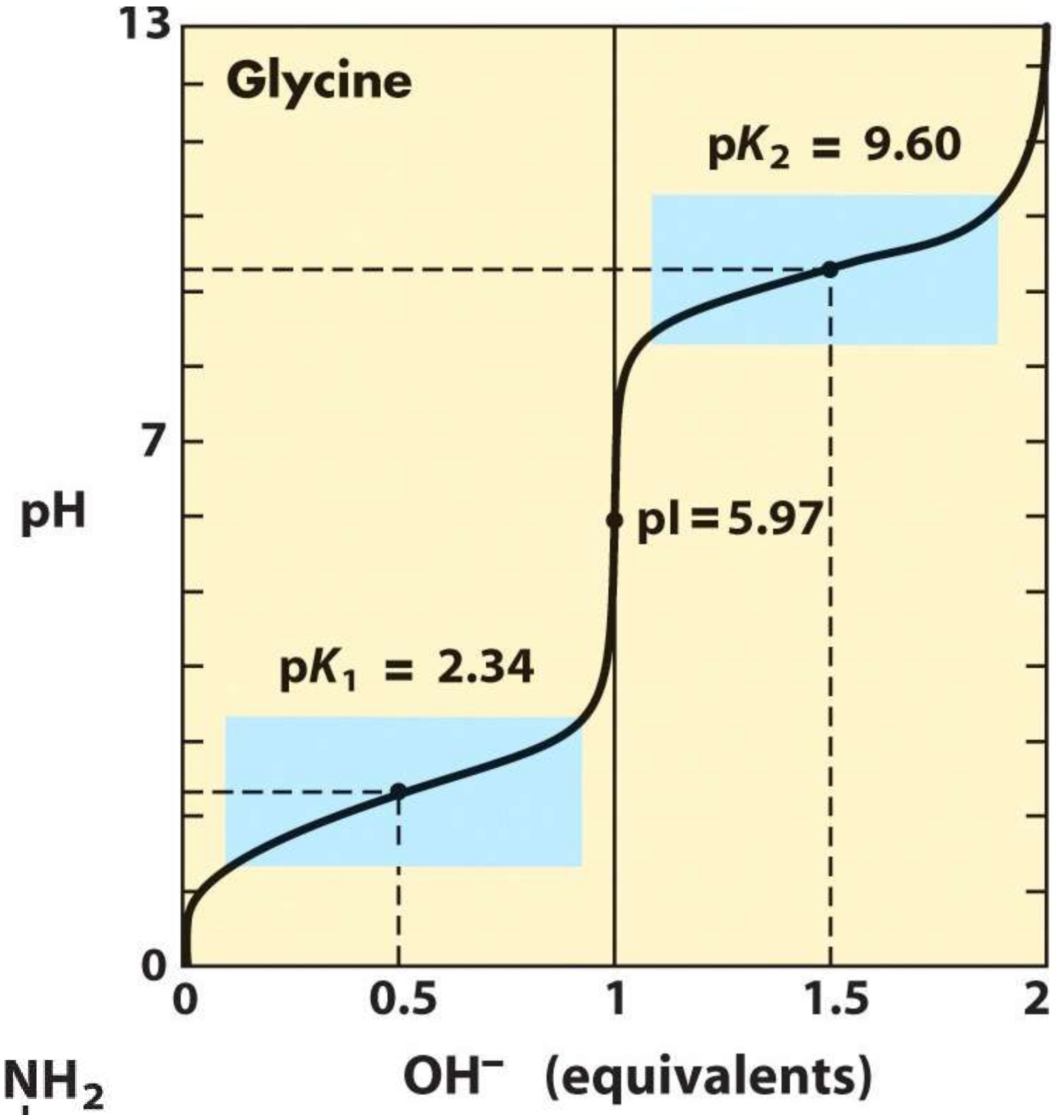
Glycine acid-base titration

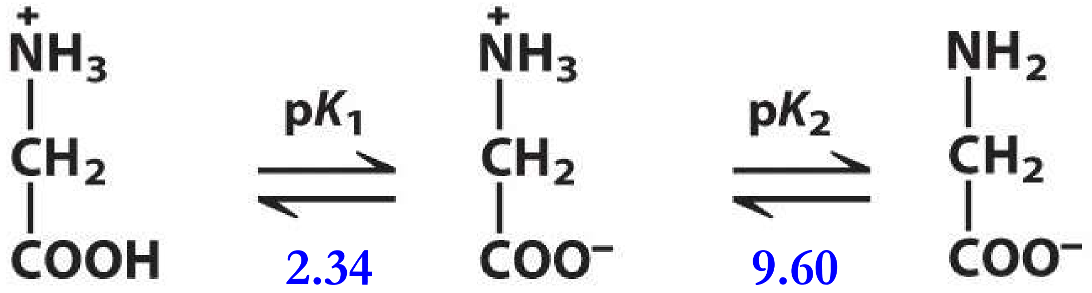
Plot of pH vs amount of base (OH)

$pk_a1 = 2.34$ (for carboxyl H)

$pk_a2 = 9.60$ (for amino group H)

$pI = (pk_a1 + pK_a2)/2$





Experimental Protocol

1. Take 50 mL of NaOH (0.1 M) solution in a 50 mL burette and adjust zero reading.
2. Pipette out 25 mL of the given solution of amino acid in a 250 mL plastic beaker and add 25 mL of distilled water to the amino acid solution using a pipette.
3. Insert the cleaned pH electrode into the beaker solution and record the initial pH of the solution.
4. Do not remove the electrode from the beaker till the end of the experiment.
5. Add NaOH in 0.5 mL increments from the burette. Stir the solution and mix it well.
6. 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.
7. After reaching pH 8, continue adding 0.5 mL increments of NaOH till you reach pH 11.
8. Plot the graph of pH vs volume of NaOH solution.
9. The two almost horizontal parts of the graph give the values of pKa1 and pKa2 for glycine. Use mid-points of these regions to get the values.
10. The average of these values (pKa1 and pKa2) gives the pI of glycine.

Observations

Given Concentration of NaOH solution = 0.1 M

Vol. NaOH (mL)	pH
0	1.86
0.5	1.88
1	1.89
1.5	1.91
2	1.93
2.5	1.95
3	1.97
3.5	1.99
4	2.01
4.5	2.03
5	2.05
5.5	2.07
6	2.1
6.5	2.1
7	2.14
7.5	2.16
8	2.18
8.5	2.2
9	2.22
9.5	2.25
10	2.27
10.5	2.3
11	2.32
11.5	2.34
12	2.37
12.5	2.39
13	2.42
13.5	2.44
14	2.47
14.5	2.49
15	2.52
15.5	2.54
16	2.57
16.5	2.6
17	2.63
17.5	2.66
18	2.69
18.5	2.72
19	2.75
19.5	2.79

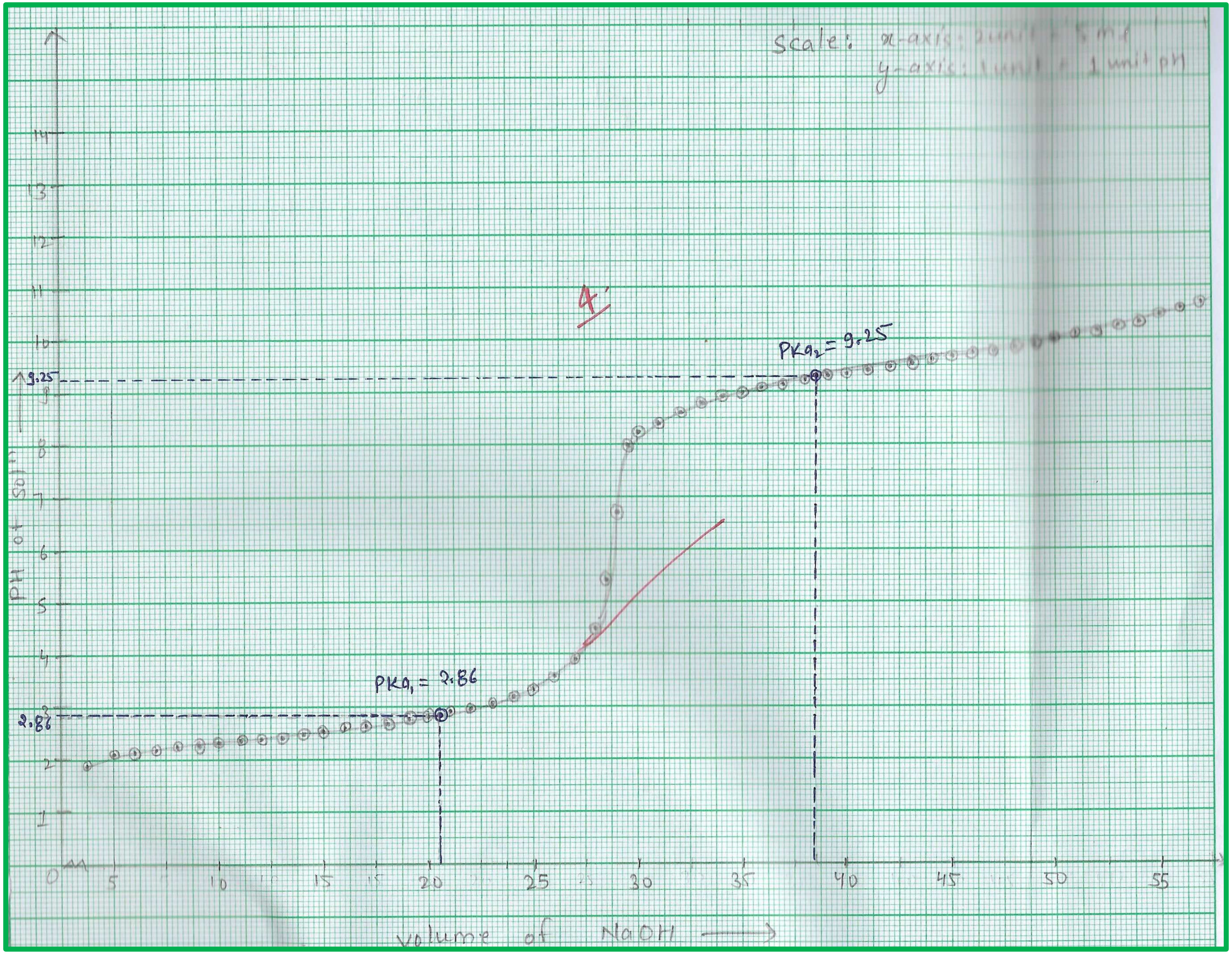
Vol. NaOH (mL)	pH
20	2.82
20.5	2.86
21	2.91
21.5	2.94
22	2.99
22.5	3.04
23	3.09
23.5	3.15
24	3.21
24.5	3.28
25	3.36
25.5	3.48
26	3.61
26.5	3.79
26.6	3.81
26.7	3.84
26.8	3.87
26.9	3.9
27	3.94
27.1	3.98
27.2	4.02
27.3	4.06
27.4	4.12
27.5	4.18
27.6	4.25
27.7	4.27
27.8	4.31
27.9	4.38
28	4.49
28.1	4.51
28.2	4.66
28.3	4.9
28.4	5.16
28.5	5.4
28.6	5.62
28.7	5.85
28.8	6.15
28.9	6.42
29	6.72
29.1	6.99

Vol. NaOH (mL)	pH
29.2	7.2
29.3	7.47
29.4	7.73
29.5	8
30	8.24
30.5	8.36
31	8.48
31.5	8.57
32	8.65
32.5	8.73
33	8.79
33.5	8.85
34	8.89
34.5	8.93
35	8.98
35.5	9.03
36	9.07
36.5	9.11
37	9.14
37.5	9.18
38	9.21
38.5	9.25
39	9.28
39.5	9.31
40	9.35
40.5	9.38
41	9.41
41.5	9.46
42	9.48
42.5	9.5
43	9.53
43.5	9.57
44	9.6
44.5	9.63
45	9.65
45.5	9.69
46	9.72
46.5	9.76
47	9.78
47.5	9.82

Vol. NaOH (mL)	pH
48	9.85
48.5	9.89
49	9.92
49.5	9.95
50	9.99
50.5	10.03
51	10.07
51.5	10.11
52	10.15
52.5	10.2
53	10.25
53.5	10.3
54	10.35
54.5	10.42
55	10.48
55.5	10.54
56	10.61
56.5	10.67
57	10.74
57.5	10.81
58	10.87
58.5	10.94
59	10.99

Observations

Image of a graph pH vs volume of NaOH



Observations and Calculations

From graph;

$pK_{a1} = 2.86$
$pK_{a2} = 9.25$
$pI = (pK_{a1} + pK_{a2}) / 2 = (2.86 + 9.25) / 2 = 6.055$

pI of glycine was determined using pH Metry

pI of Glycine = 6.055