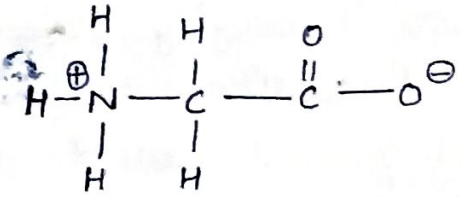
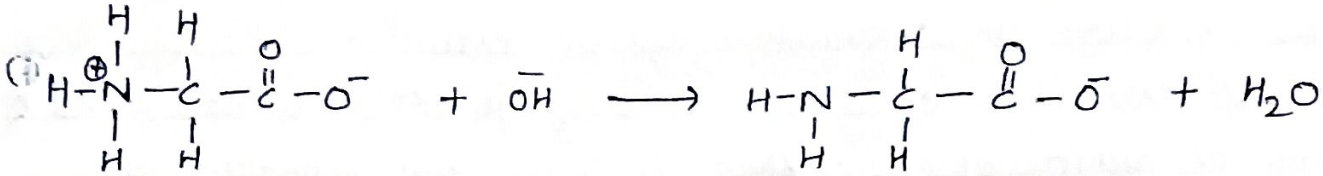
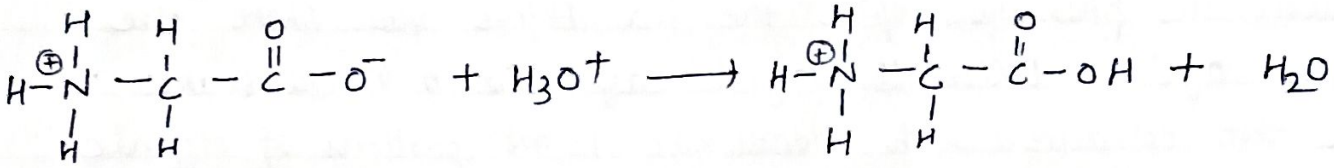
Zwitter Ion of glycine:



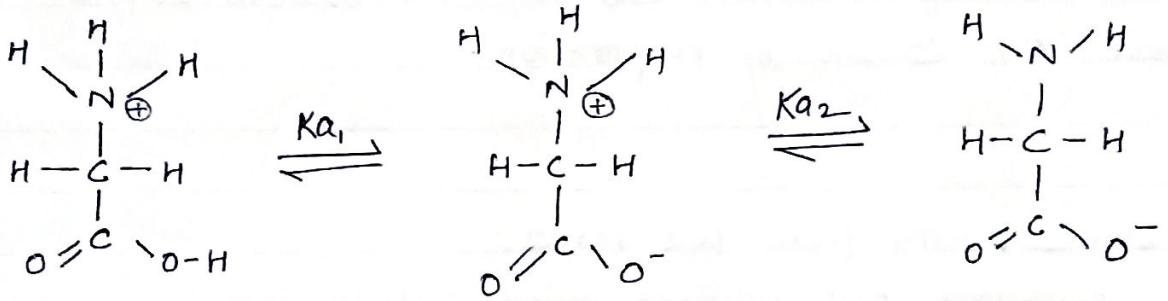
Net charge = 0.



Zwitter ion (at pI) (pH > pI) charge = -1



Zwitter ion (at pI) (pH < pI) charge = -1



low pH Zwitter ion high pH

Date 09.02.2022

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Aim: Determination of pI of Glycine using pH-metry.

Apparatus Required: pH-meter, 100 ml beaker, burette, pipette, flasks, glass rods.

Chemicals required: NaOH solution (0.1 ml), glycine, distilled water, dil. HCl

Principle: Potentiometry deals with the measurement of potential differences between two electrodes, which are combined to form an electrochemical cell. Glycine 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. Zwitter ions have equal number of positive and negative charged and are electrically neutral overall.

Isoelectric point: Isoelectric point (pI) is the pH at which the amino acid has an overall zero charge and exist in the zwitter ionic form. The pI can also be understood as the pH at which the amino acid doesn't migrate in an electric field. The pI is given by the average of the two pKas. where ka is the dissociation constant for loss of proton.

- In the solutions more acidic than pI;

(1) the COO^- in the zwitter ion accepts a proton.
(2) the amino acid has positive charge.

- In the solutions more basic than pI,

(1) the NH_3^+ in the zwitter ion loses a proton.
(2) the amino acid has a negative charge.

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Observations:

Concentration of NaOH = 0.1N.

Volume is in (ml)

Vol NaOH	pH	Vol NaOH	pH	Vol NaOH	pH	Vol NaOH	pH
0	1.86	20	2.66	31.1	4.07	46.5	9.53
0.5	1.86	20.5	2.69	31.3	4.21	47	9.56
1	1.87	21	2.73	31.4	4.35	47.5	9.57
1.5	1.89	21.5	2.75	31.5	4.41	48	9.59
2	1.91	22	2.78	31.6	4.68	48.5	9.62
2.5	1.93	22.5	2.81	31.7	5.75	49	9.65
3	1.95	23	2.85	31.9	6.98	49.5	9.7
3.5	1.96	23.5	2.89	32	7.21	50	9.72
4	1.98	24	2.9	32.1	7.52	50.5	9.74
4.5	2	24.5	2.93	32.2	7.71	51	9.75
5	2.01	25	2.97	32.3	7.8	51.5	9.79
5.5	2.03	25.5	3.02	32.4	7.89	52	9.83
6	2.04	25.6	3.01	32.5	7.95	52.5	9.86
6.5	2.06	25.7	3.02	32.6	8.04	53	9.89
7	2.08	25.8	3.04	33	8.1	53.5	9.91
7.5	2.1	25.9	3.05	33.5	8.2	54	9.93
8	2.11	26	3.06	34	8.36	54.5	9.97
8.5	2.13	26.5	3.1	34.5	8.49	55	10
9	2.15	27	3.15	35	8.59	55.5	10.04
9.5	2.16	27.5	3.21	35.6	8.66	56	10.07
10	2.19	27.6	3.22	36	8.77	56.5	10.1
10.5	2.21	27.7	3.24	36.5	8.79	57	10.13
11	2.23	27.8	3.24	37	8.86	57.5	10.16
11.5	2.25	27.9	3.25	37.5	8.91	58	10.19
12	2.27	28	3.27	38	8.96	58.5	10.23
12.5	2.3	28.5	3.34	38.5	9	59	10.25

13	2·33	28·6	3·36	39	9·04	59·5	10·33
13·5	2·34	28·8	3·38	39·5	9·08	60	10·36
14	2·37	28·9	3·4	40	9·11	60·5	10·4
14·5	2·4	29	3·51	40·5	9·15	61	10·44
15	2·43	29·5	3·53	41	9·2	61·5	10·5
15·5	2·45	29·6	3·56	41·5	9·23	62	10·55
16	2·5	29·7	3·58	42	9·27	62·5	10·6
16·5	2·51	29·8	3·6	42·5	9·29	63	10·67
17	2·53	29·9	3·64	43	9·32	63·5	10·71
17·5	2·55	30	3·67	43·5	9·36	64	10·76
18	2·57	30·1	3·78	44	9·38	64·5	10·83
18·5	2·6	30·5	3·82	44·5	9·4	65	10·87
19	2·63	30·9	3·93	45	9·44	65·5	10·94
19·5	2·65	31	3·99	45·5	9·45	66	10·99

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Procedure :

1) Take 50ml of NaOH (0.1M) solution in a 50ml burette and adjust zero reading.
2) Pipette out 25ml of the given solution of amino acid in a 250ml plastic beaker and add 25ml 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) Don't remove the electrode from the beaker till the end of experiment.
5) Add NaOH in 0.5ml increments from the burette and 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 stabilises 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 pKa_1 and pKa_2 of glycine. Use mid points of these regions to get the values.
10) The average of these values (pKa_1 and pKa_2) gives the pI of glycine.

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From the graph:

$pKa_1 = 2.45$

$pKa_2 = 9.65$

$pI = \frac{1}{2}(pKa_1 + pKa_2) = \frac{1}{2}(2.45 + 9.65) = \frac{12.10}{2} = \underline{\underline{6.05}}$.

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Result:

1) pI of glycine was determined using pH-metry.
2) pI of glycine = 6.05.

Precautions:

1) Handle the glassware carefully.
2) pH meter should be properly callibrated.
3) Sensitivity of electrode should be taken care of.

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Scale: 1cm = 5ml on X axis
1cm = 1 unit pH on Y Axis