CH1202

Chemistry Laboratory II

Experiment Number - 01

Determination of the Isoelectric Point of an Amino Acid

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1 Aim

To determine the isoelectric point of an amino acid.

2 Apparatus Required

1. pH meter
2. Beaker
3. Burette
4. Pipette
5. Glass Rod
6. Spatula

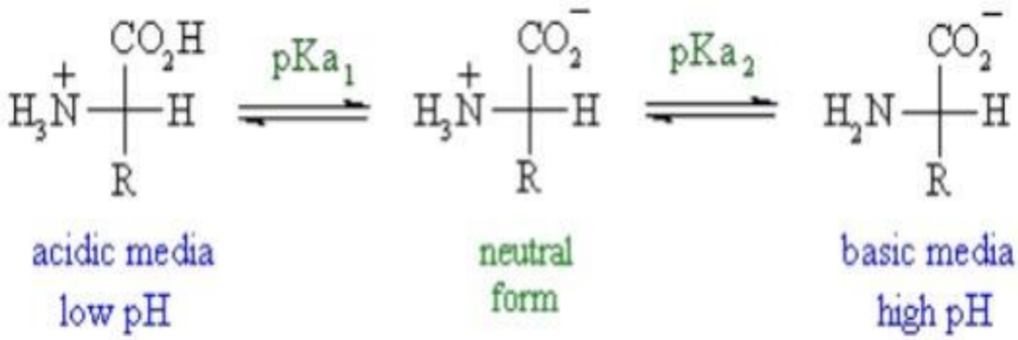
3 Reagents and Materials

1. Potassium Hydrogen Phthalate
2. Glycine
3. Alanine
4. Hydrochloric Acid
5. Sodium Hydroxide
6. Phenolphthalein

4 Equations

Henderson-Hasselbach Equation:-

$$pK_a = pH + \log \frac{[HA]}{[A^{-1}]}$$



To find pI (Isoelectric Point):-

$$pI = \frac{pK_{a_1} + pK_{a_2}}{2}$$

$$Moles\ of\ Acid = V_{Base}\ (at\ Equivalence\ Point) \times M_{Base}$$

5 Datasets

Room Temperature = $25°C$

Weight Taken (g)	Weight to be taken (g)	Strength of KHP solution
2.050	2.04222	20.5 gL^{-1}

Table 1: Preparation of 100 mL standard 0.1 N KHP solution.

Molecular weight of KHP = $204.222 gmol^{-1}$
Strength = $\frac{(mass\ of\ solute\ in\ g)}{Litre\ (L)} = \frac{2.05g}{0.1L} = 20.5\ gL^{-1}$

Sl. No.	Volume of KHP (mL)	Burette Reading (mL)			Average Volume (mL)	Strength of NaOH Solution
		Initial	Final	Difference		
1	10	0	10.1	10.1	10.167	3.949 gL^{-1}
2	10	0	10.2	10.2		
3	10	0	10.2	10.2		

Table 2: Standardization of NaOH solution using standard KHP solution

Molecular weight of NaOH = 39.997 $gmol^{-1}$

$$\frac{S_1 \cdot V_1}{M_1} = \frac{S_2 \cdot V_2}{M_2}$$

S1 = 20.5 gL^{-1}
V1 = 10 mL = 0.01 L
V2 = 10.167 mL = 0.010167 L

so, $\frac{20.5 \times 0.01}{204.222} = \frac{S_2 \times 0.010167}{39.997} \Rightarrow S2 = 3.949gL^{-1}$

Volume of amino acid = 25 mL

S.No.	Volume of NaOH (mL)	pH	ΔV (mL)	ΔpH	ΔpH/ΔV
1	0	1.41			
2	0.5	1.44	0.5	0.03	0.06
3	1	1.48	0.5	0.04	0.08
4	1.5	1.53	0.5	0.05	0.1

5	2	1.58	0.5	0.05	0.1
6	2.5	1.64	0.5	0.06	0.12
7	3	1.7	0.5	0.06	0.12
8	3.5	1.75	0.5	0.05	0.1
9	4	1.82	0.5	0.07	0.14
10	4.5	1.85	0.5	0.03	0.06
11	5	1.95	0.5	0.1	0.2
12	5.5	2.03	0.5	0.08	0.16
13	6	2.12	0.5	0.09	0.18
14	6.5	2.21	0.5	0.09	0.18
15	7	2.31	0.5	0.1	0.2
16	7.5	2.42	0.5	0.11	0.22
17	8	2.57	0.5	0.15	0.3
18	8.2	2.65	0.2	0.08	0.4
19	8.4	2.72	0.2	0.07	0.35
20	8.6	2.8	0.2	0.08	0.4
21	8.8	2.89	0.2	0.09	0.45
22	9	2.98	0.2	0.09	0.45
23	9.2	3.14	0.2	0.16	0.8
24	9.4	3.27	0.2	0.13	0.65
25	9.5	3.38	0.1	0.11	1.1
26	9.7	3.71	0.2	0.33	1.65
27	9.8	3.83	0.1	0.12	1.2
28	9.85	4	0.05	0.17	3.4
29	9.9	4.27	0.05	0.27	5.4

30	9.95	4.8	0.05	0.53	10.6
31	10	5.99	0.05	1.19	23.8
32	10.05	7	0.05	1.01	20.2
33	10.1	7.44	0.05	0.44	8.8
34	10.15	7.68	0.05	0.24	4.8
35	10.2	7.86	0.05	0.18	3.6
36	10.25	7.98	0.05	0.12	2.4
37	10.3	8.08	0.05	0.1	2
38	10.4	8.24	0.1	0.16	1.6
39	10.5	8.37	0.1	0.13	1.3
40	10.6	8.46	0.1	0.09	0.9
41	10.8	8.62	0.2	0.16	0.8
42	11	8.79	0.2	0.17	0.85
43	11.3	8.95	0.3	0.16	0.53
44	11.8	9.07	0.5	0.12	0.24

45	12	9.14	0.2	0.07	0.35
46	12.5	9.28	0.5	0.14	0.28
47	13	9.39	0.5	0.11	0.22
48	13.5	9.5	0.5	0.11	0.22
49	14	9.6	0.5	0.1	0.2
50	14.5	9.7	0.5	0.1	0.2
51	15	9.79	0.5	0.09	0.18
52	15.5	9.88	0.5	0.09	0.18
53	16	10	0.5	0.12	0.24
54	16.5	10.09	0.5	0.09	0.18

55	17.5	10.25	1	0.16	0.16
56	18.5	10.39	1	0.14	0.14
57	19	10.52	0.5	0.13	0.26
58	19.5	10.67	0.5	0.15	0.3
59	20	10.87	0.5	0.2	0.4
60	20.2	10.95	0.2	0.08	0.4
61	20.4	11.03	0.2	0.08	0.4
62	20.5	11.07	0.1	0.04	0.4
63	20.6	11.11	0.1	0.04	0.4
64	20.7	11.15	0.1	0.04	0.4
65	20.9	11.23	0.2	0.08	0.4
66	21.1	11.31	0.2	0.08	0.4
67	21.3	11.38	0.2	0.07	0.35
68	21.5	11.44	0.2	0.06	0.3
69	22	11.59	0.5	0.15	0.3
70	22.5	11.7	0.5	0.11	0.22
71	23	11.79	0.5	0.09	0.18
72	23.5	11.86	0.5	0.07	0.14
73	24	11.96	0.5	0.1	0.2
74	24.5	12	0.5	0.04	0.08
75	25	12.06	0.50	0.06	0.12

6 Plots

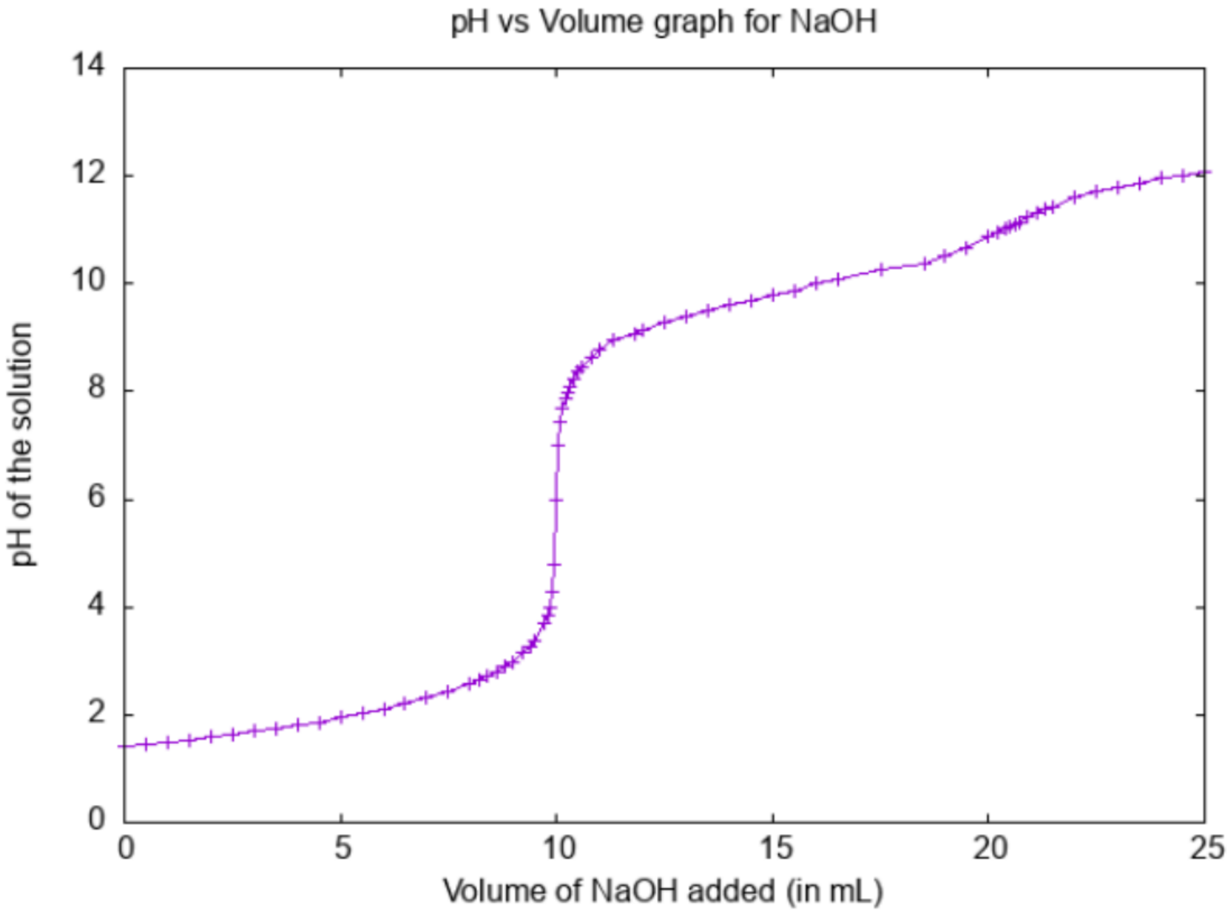


Figure 1: pH vs Volume graph for NaOH

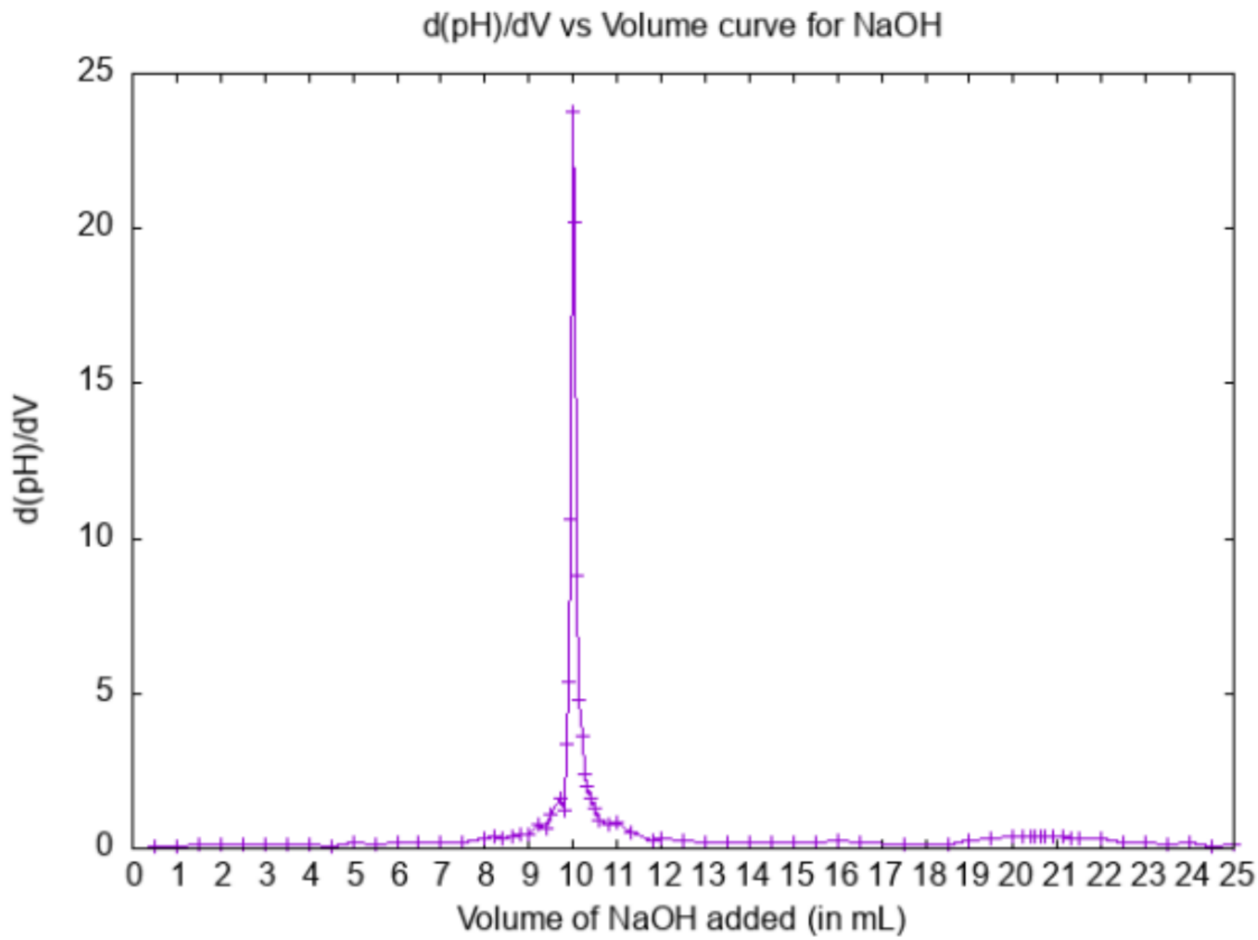


Figure 2: $\frac{\Delta pH}{\Delta V}$ vs Volume curve

7 Results and Data Interpretation

From the second plot above, it is clear that there are two equivalence points (since there are two peaks : first between 9 mL and 11 mL and second between 20 mL and 21 mL).

<u>*First Equivalence Point :*</u>

The first equivalence point corresponds to volume = 10 mL (shown in RED in Table 3) So, half equivalence point = 5 mL From Table 3, pK_a value corresponding to first half equivalence point, $pK_{a_1} = 1.95$

<u>*Second Equivalence Point :*</u>

For the second equivalence point there is no single point peak. Instead 8 different volume readings correspond to the local maximum value of pK_a (shown in BLUE in Table 3)

So, we take the average of the 8 volume readings to find the 'Second Equivalence Point' as below:

$$\text{Volume at second equivalence point} = \frac{20 + 20.2 + 20.4 + 20.5 + 20.6 + 20.7 + 20.9 + 21.1}{8} = 20.55mL$$

$$\text{So, the half equivalence point} = \frac{First\ Eq.\ Point\ +\ Second\ Eq.\ Point}{2} = \frac{10 + 20.55}{2} = 15.275mL$$

From the Table, pK_a value nearest to second half equivalence point, $= 9.88$

Now, we know that,

$$\text{Equivalence Point (pI)} = \frac{pK_{a_1} + pK_{a_2}}{2} = \frac{1.95 + 9.88}{2} = 5.915$$

Thus, pI $= 5.915$

8 Conclusion

The Isoelectric Point of the given Amino Acid (Glycine) is 5.915.[1].

[1] All the graphs were plotted using *gnuplot*