CH1202: Lab Report

Determination of Isoelectric Point (pI) of Amino Acid

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§1 Equation

For this experiment of determination of isoelectric point of amino acid, as an amino acid, we use mainly two essential equations, namely

Proposition 1.1 (Henderson-Hasselbalch Equation)

Suppose, K_a be the dissociation constant of acid HA. A buffer solution is prepared with that acid having concentration [HA] and concentration of the corresponding conjugate base in that solution is $[A^-]$. Then the pH of the buffer solution is given by

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

Definition 1.2 (Isoelectric Point of an Amino Acid). Given an amino acid, it stays in the form of zwitterion, i.e., its neutral form. But since, the amine and the carboxyl group have different base and acid strengths, it does not necessarily stay neutral at $pH = 7$. Hence, we define the isoelectric point pI as the pH where the amino acid becomes neutral.

Proposition 1.3 (Isoelectric Point pI)

Deciphering the definition and some mathematical calculations yield

$$pI = \frac{pK_{a1} + pK_{a2}}{2}$$

The pK_{a1} and pK_{a2} are respectively the acid dissociation constants for the reactions given in the figure below.

Include figure ??

§2 Dataset

Preparation of 100 mL standard 0.1 N KHP solution

- Weight taken = 2.050 g
- Weight to be taken = 2.04222 g
- Strength of KHP solution = 20.5 g/L

Calculation

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Molecular weight of KHP = 204.222 g/mol
Strength = 2.05 g/0.1 L = 20.5 g/L
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Sl. No.	Volume of KHP (mL)	Burette Reading (mL)			Average Volume (mL)	Strength of NaOH Solution (g/L)
		Initial	Final	Difference		
1	10	0	10.1	10.1	10.17	3.93
2	10	0	10.2	10.2		
3	10	0	10.2	10.2		

Table 1: Standardization of NaOH solution using standard 0.1 N KHP solution

Calculation

Molecular weight of NaOH = 40 g/mol

$$N_1V_1 = N_2V_2$$

where, $N_1 = 20.5$ g/L; $V_1 = 10$ mL; $V_2 = 10.17$ mL. Hence,

$$N_2 \approx 3.93 \text{ g/L}$$

V	pH	ΔV	ΔpH	$\Delta pH/\Delta V$
0	1.41	0.5	0.03	0.06
0.5	1.44	0.5	0.04	0.08
1	1.48	0.5	0.05	0.1
1.5	1.53	0.5	0.05	0.1
2	1.58	0.5	0.06	0.12
2.5	1.64	0.5	0.06	0.12
3	1.7	0.5	0.05	0.1
3.5	1.75	0.5	0.07	0.14
4	1.82	0.5	0.07	0.14
4.5	1.89	0.5	0.06	0.12
5	1.95	0.5	0.08	0.16
5.5	2.03	0.5	0.09	0.18
6	2.12	0.5	0.09	0.18
6.5	2.21	0.5	0.1	0.2
7	2.31	0.5	0.11	0.22
7.5	2.42	0.5	0.15	0.3
8	2.57	0.2	0.08	0.4
8.2	2.65	0.2	0.07	0.35
8.4	2.72	0.2	0.08	0.4
8.6	2.8	0.2	0.09	0.45
8.8	2.89	0.2	0.09	0.45
9	2.98	0.2	0.16	0.8
9.2	3.14	0.2	0.13	0.65
9.4	3.27	0.1	0.11	1.1
9.5	3.38	0.2	0.33	1.65
9.7	3.71	0.1	0.12	1.2

9.8	3.83	0.05	0.17	3.4
9.85	4	0.05	0.27	5.4
9.9	4.27	0.05	0.53	10.6
9.95	4.8	0.05	1.19	23.8
10	5.99	0.05	1.01	20.2
10.05	7	0.05	0.44	8.8
10.1	7.44	0.05	0.24	4.8
10.15	7.68	0.05	0.18	3.6
10.2	7.86	0.05	0.12	2.4
10.25	7.98	0.05	0.1	2
10.3	8.08	0.1	0.16	1.6
10.4	8.24	0.1	0.13	1.3
10.5	8.37	0.1	0.09	0.9
10.6	8.46	0.2	0.16	0.8
10.8	8.62	0.2	0.17	0.85
11	8.79	0.3	0.16	0.53
11.3	8.95	0.5	0.12	0.24
11.8	9.07	0.2	0.07	0.35
12	9.14	0.5	0.14	0.28
12.5	9.28	0.5	0.11	0.22
13	9.39	0.5	0.11	0.22
13.5	9.5	0.5	0.1	0.2
14	9.6	0.5	0.1	0.2
14.5	9.7	0.5	0.09	0.18
15	9.79	0.5	0.09	0.18
15.5	9.88	0.5	0.12	0.24
16	10	0.5	0.09	0.18
16.5	10.09	1	0.16	0.16
17.5	10.25	1	0.14	0.14
18.5	10.39	0.5	0.13	0.26
19	10.52	0.5	0.15	0.3
19.5	10.67	0.5	0.2	0.4
20	10.87	0.2	0.08	0.4
20.2	10.95	0.2	0.08	0.4
20.4	11.03	0.1	0.04	0.4
20.5	11.07	0.1	0.04	0.4
20.6	11.11	0.1	0.04	0.4
20.7	11.15	0.2	0.08	0.4
20.9	11.23	0.2	0.08	0.4
21.1	11.31	0.2	0.07	0.35
21.3	11.38	0.2	0.06	0.3

21.5	11.44	0.5	0.15	0.3
22	11.59	0.5	0.11	0.22
22.5	11.7	0.5	0.09	0.18
23	11.79	0.5	0.07	0.14
23.5	11.86	0.5	0.1	0.2
24	11.96	0.5	0.04	0.08
24.5	12	0.5	0.06	0.12
25	12.06			

Table 2: Titration of amino acid using NaOH solution

§3 Plots

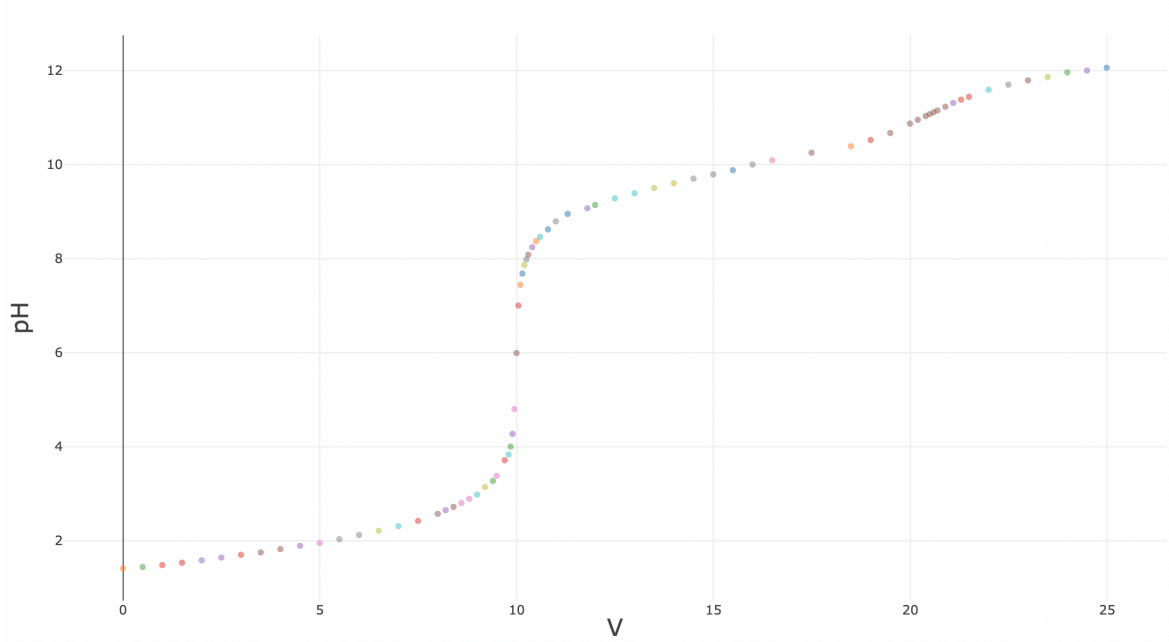

Figure 1: pH vs. V

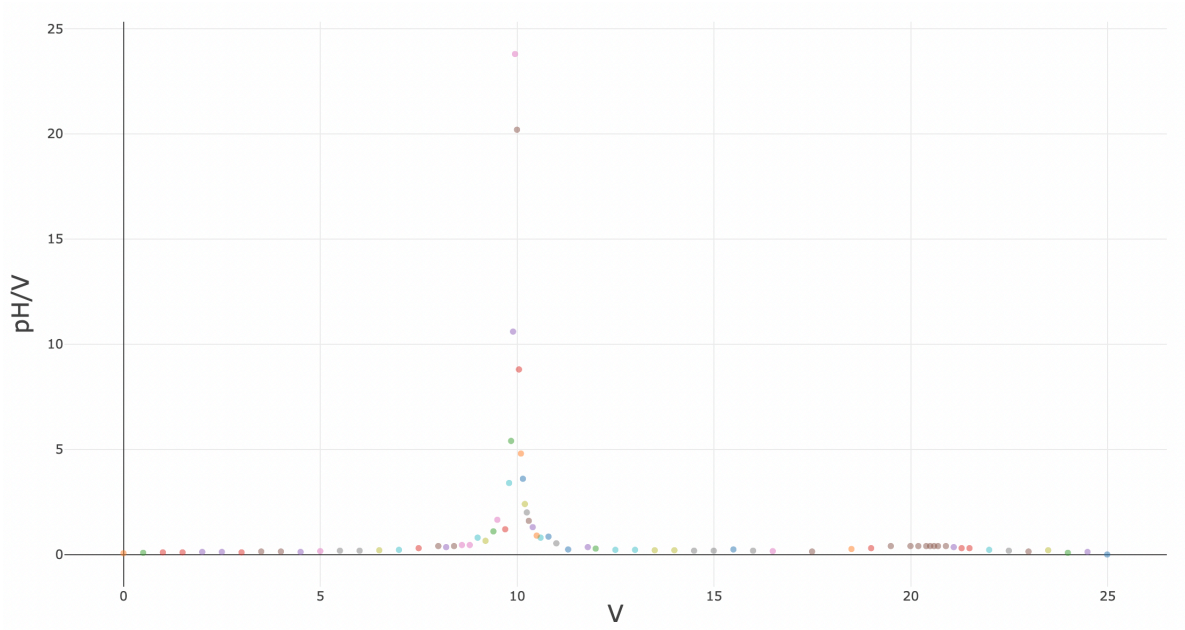

Figure 2: $\Delta pH/\Delta V$ vs. V

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§4 Results

First equivalence point = 9.95 mL (The big peak). So, half the volume at first equivalence point would give $pK_{a1} \approx 1.95$ at volume $9.95/2 = 4.975$ mL. And, second equivalence point = 20.4 mL (The tiny peak). So, half the volume from the first peak to second peak is $20.4 - 9.95/2 = 5.225$ mL. Hence, the $pK_{a2} \approx 9.79$ at volume $9.95 + 5.225 = 15.175$ mL. Therefore, we found the isoelectric point

$$pI = \frac{1.95 + 9.79}{2} = \boxed{5.87}$$

§5 Acknowledgements

Thanks to our instructors for exposing to this nice experiment.