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BIOL 4150 Laboratory: Biochemical Techniques 1

Experiment #3: The pH meter, amino acid titrations and buffers

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Abstract

In this experiment, the proton concentration, biomolecular interaction of ionic movement in Histidine was observed and determined using a pH titration curve and pH meter. Solutions added as a base and acid were mixed with histidine to provide increase or decrease of pH readings as more small amounts were added. It was found that the pKa values for the 3 equivalence points that were estimated in the graph are pKa1 as 2.12, pKa2 as 5.9 and pKa3 as 8.9 accordingly. It was found that the molar concentration led to a decreasing trend. At last, it was found that the difference in pH between the calculated and measured values, they were ±0.404, ±0.392, ±0.39, ±0.446 and ±0.458.

Introduction

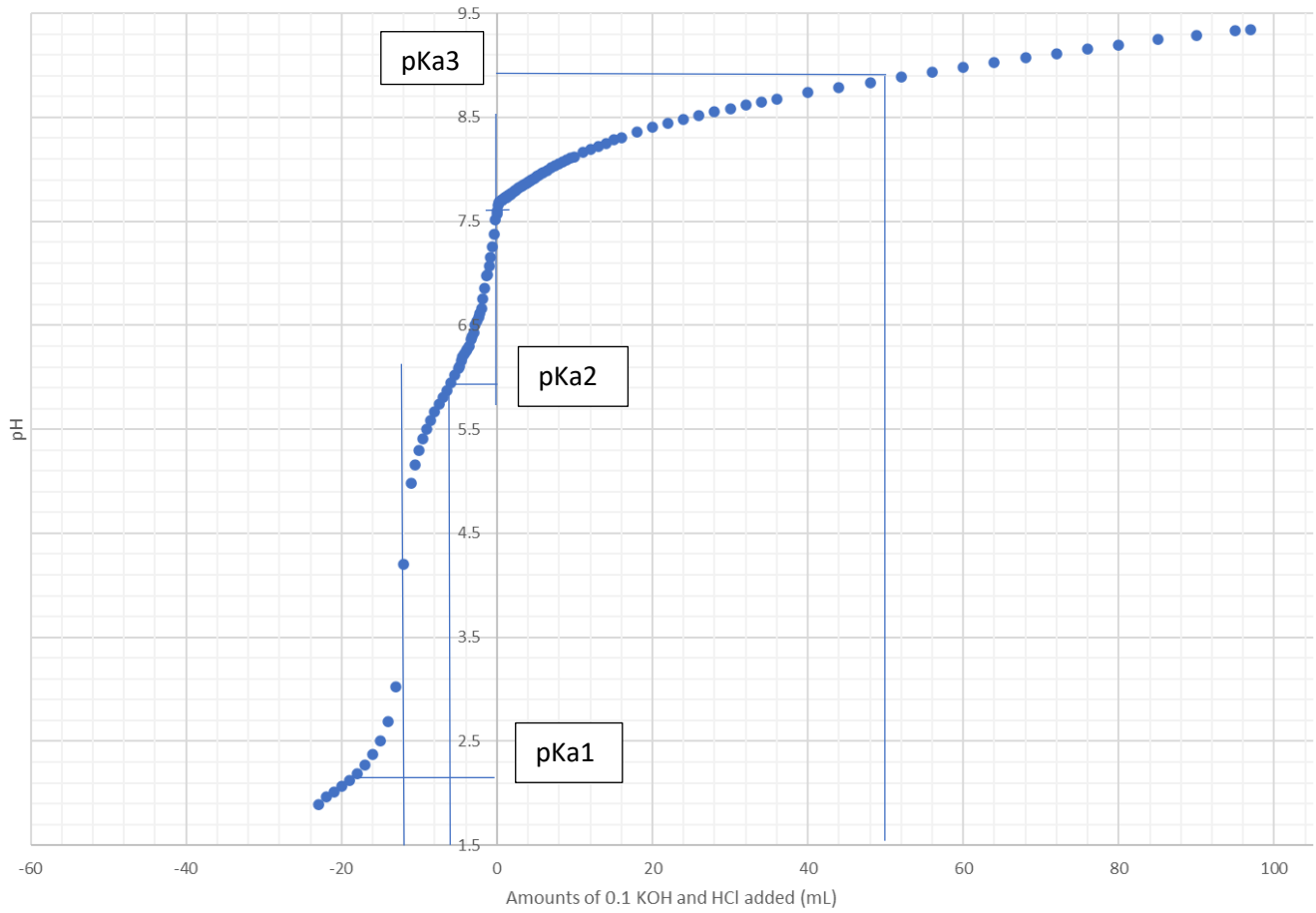
In the natural world, there are tons of applications in which acidity and basicity can be applied in everyday life. From bleach to stomach acid, in chemistry is very important to understand these things so that we can provide a proper understanding and measurement of different pH on certain substances. One very known method of applying the knowledge of biomolecule interactions and concentration of protons in an aqueous solution is the titration of acids against basic with the use of a pH meter.

From this application, it will be shown that by changing the concentration of protons in a solution by the addition of acids or bases the interaction between the protons and other molecules will change accordingly. Luckily, in a pH titration curve, an important factor that looks at the functional group ability to interact with the movement of protons is the pKa. However, a pH meter scale is highly sensitive because it measures very accurate pH readings in every solution. Hence, the pH meter will be calibrated in different buffers of different standard pH of 4, 7 and 10. And for the pH readings for acid and bases, the pH meter must be calibrated once.

In this experiment, a pH titration curve will be performed to understand the proton interaction of molecules, the calculation of molar concentration and finding the pKa in Histidine Monohydrochloride from the use of Potassium hydroxide as the base and Hydrochloric acid as the acid.

Data and Results

Table 1. pH meter readings of KOH and HA volume added.				
mls KOH	pH meter reading			
0	7.602	0	7.57	
0.1	7.656	-0.2	7.517	
0.2	7.68	-0.4	7.378	
0.3	7.685	-0.6	7.255	
0.4	7.691	-0.8	7.156	
0.5	7.697	-1	7.066	
0.6	7.703	-1.2	6.99	
0.7	7.71	-1.4	6.98	
0.8	7.713	-1.6	6.858	
0.9	7.72	-1.8	6.751	
1	7.725	-2	6.659	
1.1	7.732	-2.2	6.617	
1.2	7.731	-2.4	6.576	
1.3	7.742	-2.6	6.539	
1.4	7.746	-2.8	6.503	
1.5	7.749	-3	6.43	
1.6	7.754	-3.2	6.394	
1.7	7.759	-3.4	6.367	
1.8	7.765	-3.6	6.303	
2	7.778	-3.8	6.275	
2.2	7.789	-4	6.249	
2.4	7.797	-4.2	6.222	
2.6	7.809	-4.4	6.194	
2.8	7.818	-4.6	6.165	
3	7.83	-4.8	6.108	
3.2	7.84	-5	6.082	
3.4	7.851	-5.5	6.018	
3.6	7.861	-6	5.947	
3.8	7.87	-6.5	5.876	
4	7.88	-7	5.812	
4.2	7.89	-7.5	5.742	
4.4	7.899	-8	5.671	
4.6	7.909	-8.5	5.584	
4.8	7.918	-9	5.502	
5.1	7.931	-9.5	5.409	
5.4	7.943	-10	5.296	
5.7	7.957	-10.5	5.158	
6	7.969	-11	4.98	
6.5	7.991	-12	4.206	
7	8.012	-13	3.024	
7.5	8.032	-14	2.69	
8	8.049	-15	2.502	
8.5	8.071	-16	2.371	
9	8.09	-17	2.271	
9.5	8.109	-18	2.186	
10	8.12	-19	2.124	
11	8.161	-20	2.065	
12	8.193	-21	2.017	
13	8.224	-22	1.966	
14	8.251	-23	1.896	
15	8.282			
16	8.308			
18	8.357			
20	8.403			
22	8.443			
24	8.482			
26	8.519			
28	8.554			
30	8.586			
32	8.617			
34	8.649			
36	8.676			
40	8.737			
44	8.783			
48	8.832			
52	8.887			
56	8.937			
60	8.982			
64	9.028			
68	9.071			
72	9.114			
76	9.156			
80	9.195			
85	9.247			
90	9.29			
95	9.336			
97	9.345			



Graph 1. pH titration curve of KOH and acid solutions of 0.1 M added to histidine

From the equivalence points made on Graph 1, the 3 pKa values found are:

pKa1: 2.12

pKa2: 5.9

pKa3: 8.9

According to Graph 1, at halfway of the equivalence point:

pH=pKa

Hence, [Molar]=10^{-pH}.

Calculations

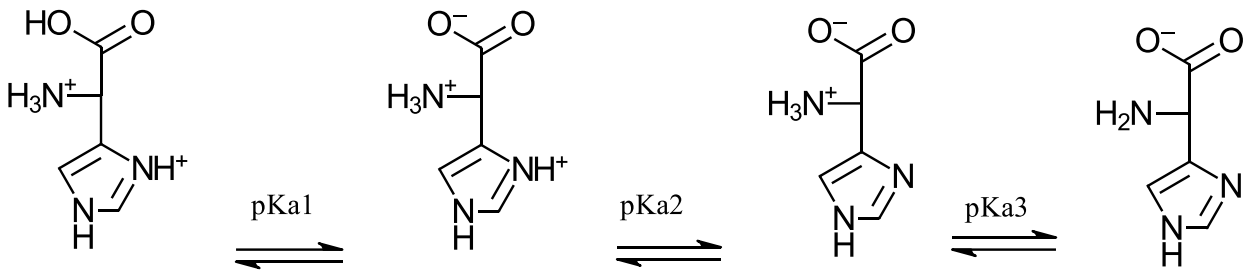
Using the previous formula, we got:

$$[Molar1] = 10^{-2.12} = 0.0076\ M$$

$$[Molar2] = 10^{-5.9} = 0.0000013\ M$$

$$[Molar3] = 10^{-8.9} = 0.000000001\ M$$

The ionic structures of Histidine in each pKa state are drawn as follows:



1 sample calculation of concentration ratio of phosphate buffers. First buffer:

$$Con.ratio\frac{[base]}{[acid]} = \frac{24.0}{15.0}mL = 1.6$$

$$Log\ 1.6 = 0.204$$

Since we are only dealing with $KH_2PO_4\ and\ Na_2PO_4$, the Ka dissociation constant will be:

$$K2 = 6.2\ x10^{-8}$$

To convert this value to pKa:

$$pKa = -LogKa$$

$$pKa = -Log(6.2x10^{-8})$$

$$pKa = 7.21$$

For the phosphate buffers, we will use the Henderson Hasselbalch equation:

$$pH = pKa + Log\ \frac{[unprotonated]}{[protonated]} = 7.21 + Log\ 1.6 = 7.414$$

Experimental procedure

Calibration of the pH meter:

- The pH probe got in hold over a beaker. Followed by a rinse with distilled or DI water.

- The probe got dried and blotted with the use of a KimWipe tissue paper.
- pH was turned on and waited to load up until a main menu dashboard appeared.
- The first standard buffer of pH 7.0 was dipped on the probe for a few seconds. A notification on the screen of the pH meter indicated that the pH calibration for pH 7.0 was ready.
- The probe was in hold again over the beaker and rinsed again with distilled water or DI. Followed by a blot off on the tip of the probe with KimWipe tissue paper.
- Now, the standard buffer of pH 4.0 was dipped into the probe. A notification in the screen of the pH meter indicated that the calibration was ready.
- Again, the probe got rinsed over the beaker with distilled or DI water. Followed by a blot off with KimWipe tissue paper.
- The same procedure was followed with pH 7.0 again, and then finally with pH buffer 10.0

Titration of Histidine with base:

- The results sheet section labelled as "mls KOH added" and "pH meter reading", was used to record the values using it in the respective space.
- An initial volume of 25 mL 0.05 M histidine monohydrochloride was poured in a 100 mL Erlenmeyer flask. Then this amount was measured and recorded from the pH meter.
- While the probe was removed and placed in the solution, it was not needed to rinse the probe after first measuring the first pH reading.
- Now, small amounts of 0.1 mL 0.01 M KOH were added to the solution while each amount the probe was placed in the solution to record each pH value. Through the course of looking at significant jumps of pH reading, the small amounts should be increased to 0.2 or 0.5 and so on.
- The probe was left unrinsed with distilled water or DI.
- The probe was calibrated with pH 7.0
- More small amounts of 0.01 M KOH were poured until a pH has reached 12.0. When that happened, after the last pH reading the probe was thoroughly washed with distilled or DI water.

Titration of Histidine with acid

- The pH meter was recalibrated with the acid range pH standard buffers: pH at 4.0 and 7.0.
- The section "mls HCl added" and "pH meter readings" were used from the Results data sheet in the lab report to record the measurements of the pH in this solution.
- An initial volume of 25 mL 0.05 M solution of histidine monohydrochloride was pipetted in a 100 mL flask while the pH was measured and recorded at that time.
- The probe was removed and placed in the solution while each amount of 0.2 mL of 0.1 M HCl was pipetted and mixed to measure the pH while the probe wasn't needed to be washed.

- If there are any significant changes of pH between each small amount, accommodate the volume amounts accordingly until the pH has fallen below 2.0.

Preparation of phosphate buffers

- The following amounts of 0.1 M KH_2PO_4 and Na_2HPO_4 were added in the following table:

Table 1. Volumes of 0.1 M phosphate in 5 different concentration buffers.

	Buffer 1	Buffer 2	Buffer 3	Buffer 4	Buffer 5
0.10 M KH_2PO_4	15.0 ml	7.5 ml	3.5 ml	1.5 ml	1.0 ml
0.10 M Na_2HPO_4	24.0 ml	30.0 ml	35.0 ml	37.5 ml	40.0 ml

- The above amounts from the table were added in five 50 mL volumetric flasks.
- After the amounts were poured in all volumetric flasks, an amount of 1.00 mL Phenol Red indicator (0.25 g/L) was added.
- Dilute to mark the 50 mL flask limit with distilled water. A color change between the flasks is expected to be seen.
- The buffers were poured in 50 mL tubes followed by a pH reading on each buffer to record it in the Results data sheet.
- The buffers were kept in a 600 mL beaker and given to the instructor to keep them in a safe place until next week to continue.

Discussion

In this experiment, the biomolecular interaction of the proton concentration in amino acids was observed in a pH titration curve. The volume amounts were located in the x-axis while the pH readings were located on the y-axis. In the recollection of data, the titration of 0.1 M hydrochloric acid was decided to use the small volumes used as negative values. This was done so that the trend could go increasing and therefore estimating the pKa and molar proton concentration values accordingly in the graph. However, it was expected that all titration points should be located at the positive quadrant of the graph. In addition, different volume patterns were used accordingly to show the significant jumps in the pH readings. From the join of all titrations, the points showed an increasing trend, and the equivalence points were easily determined. From the 3 equivalent points, in the midpoint that's where the pKa values between the ionic transition of histidine were determined. They were to be found as pKa1 for 2.12, pKa2 as 5.9 and pKa3 as 8.9. According to the lab manual, the pKa values of histidine are 1.8, 9.2 and 6.0. It was expected to be close to one another.

In this experiment, the Henderson-Hasselbalch equation was used to explain that pKa=pH. From this theory, the pKa values estimated from the graph are the same as their pH values. Now, the $[Molar] = 10^{-pH}$ was used to determine the molar concentration of protons in each of the pKa values. They were found to be 0.0076 M, 0.0000013 M and 0.000000001 M accordingly.

On the other side, the phosphate buffers were measured on the pH meter. They were found to be 7.010, 7.420, 7.820, 8.164 and 8.352. The phosphate buffers were calculated. They were found to be 7.41, 7.81, 8.21, 8.61 and 8.81. The difference of pH between each phosphate buffer was found to be ±0.404, ±0.392, ±0.39, ±0.446 and ±0.458. The discrepancies for these changes in pH could possibly be the miscalibration of the pH meter, the misreading amounts of phosphate in each buffer or the rinsing process of the pH probe. In addition, a source of error for the pH titration curve with

Histidine was the avoidance of using more open volumes so that the points could have been separated across the trend.

Conclusion

In this experiment, the titration curve of amino acids was performed using a pH meter. In addition, the calibration of pH was done so that the pH readings could be recorded accurately. The pKa values of Histidine in the titration curve were found to be pKa1 for 2.12, pKa2 as 5.9 and pKa3 as 8.9. From these values, according to the Henderson Hasselbalch equation, pKa is equal to the pH. Now that the pH values were given, the molar concentration of protons were found to be 0.0076 M, 0.0000013 M and 0.000000001 M. On the other side, the phosphate buffer pH values were measured by the use of the pH meter. They were found to be 7.010, 7.420, 7.820, 8.164 and 8.352.

Additional questions

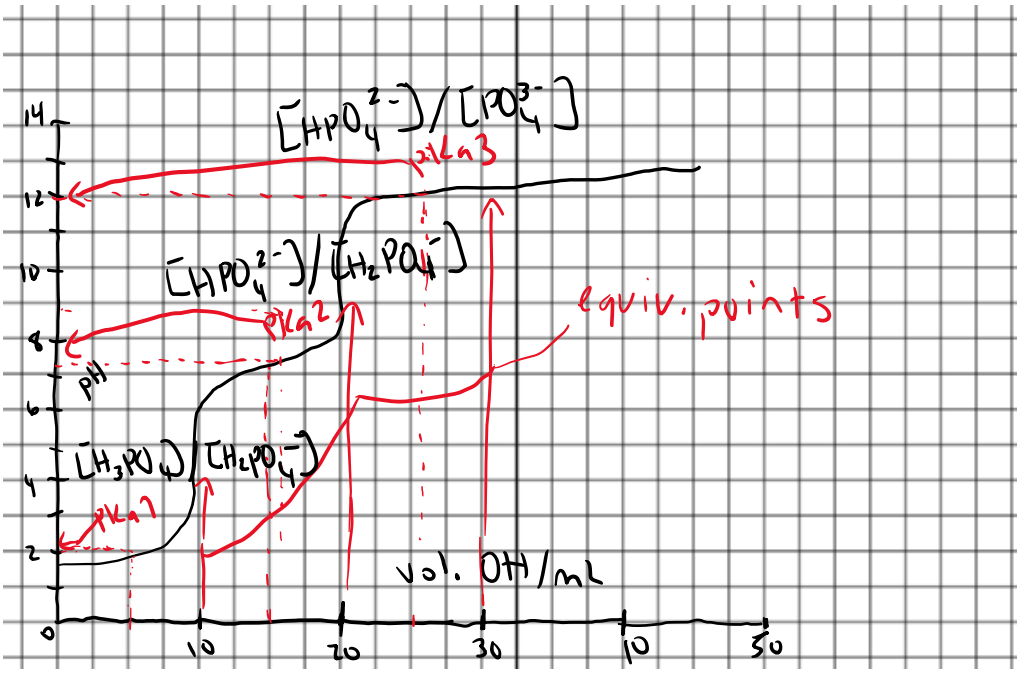
1. What is the purpose of washing and blotting the probe?

   It eliminates the excess water that is adhered to the surface of the probe. Washing eliminates any impurities or contaminant between substances and hence provide an accurate pH reading.

2. Why should you not wash and blot the probe during the titrations?

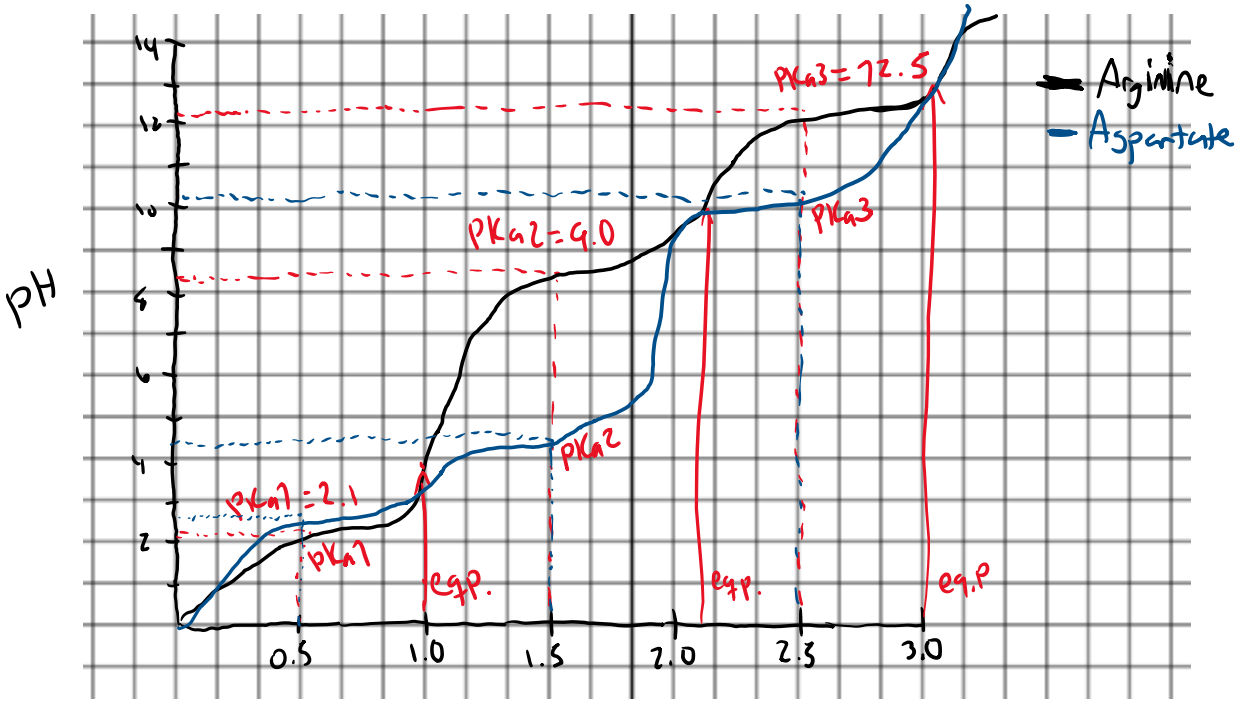
   A big difference of results might appear in the data sheet. The concentration of protons should be maintained between the addition of small amounts in the solution that is being titrated. Washing and blotting the probe in the titration will reset the reading and need to recalibrate again.

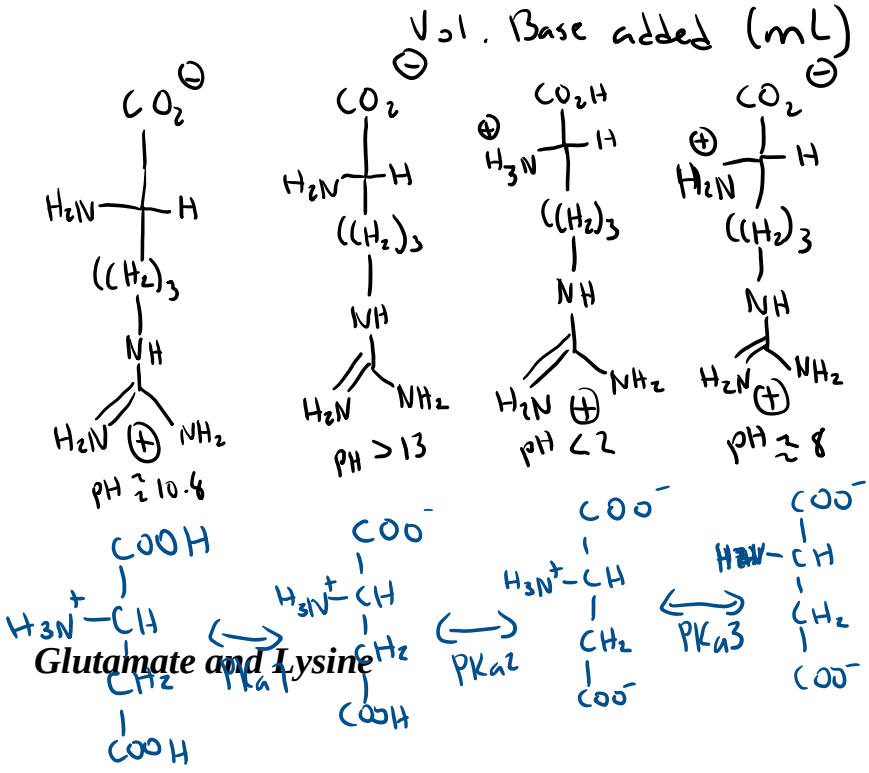
3. Draw freehand a titration curve of phosphoric acid versus hydroxide, showing equivalent points, three pKa points, and the ionic forms present at all of these.



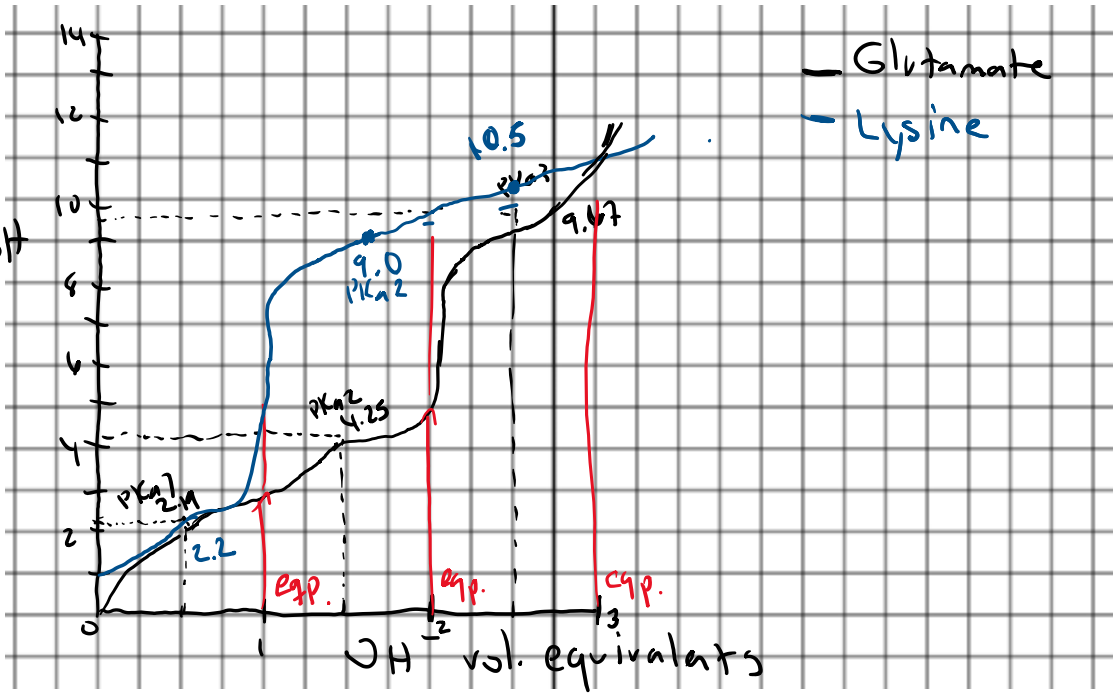
4. Use graph paper to draw a freehand sketch of the titration curves of the α-amino acids aspartate & arginine, on one graph, and glutamate and lysine on another graph.

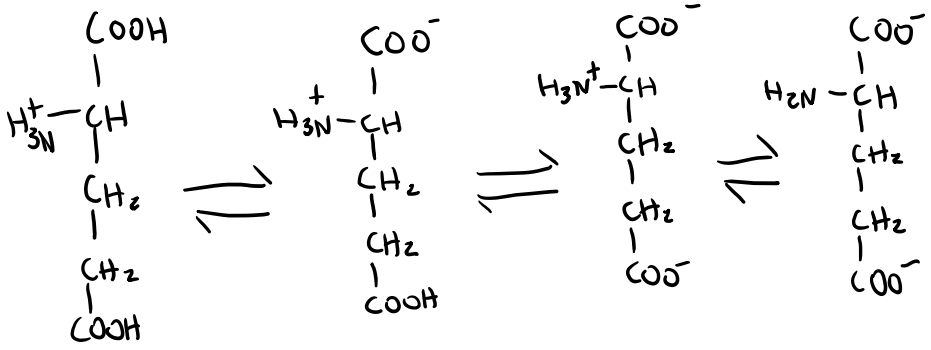
Glutamate and Aspartate

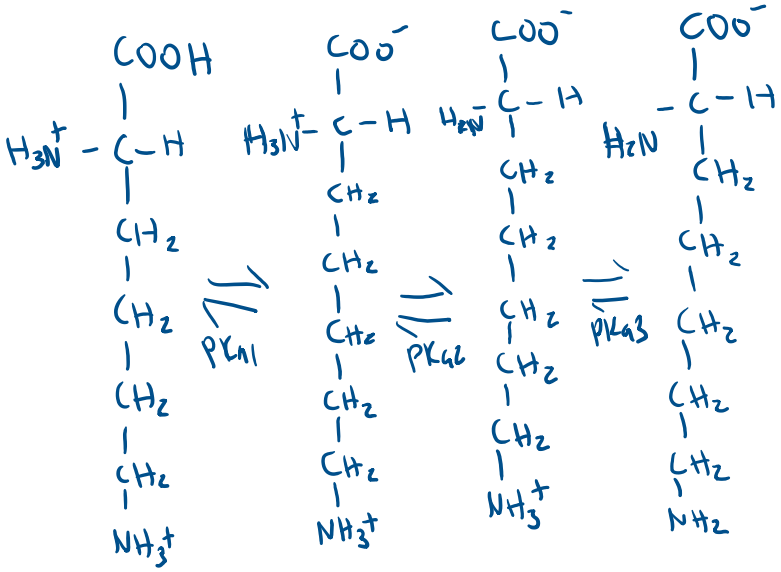




Glutamate and Lysine


Glutamate
Lysine
pH
14
12
10
8
6
4
2
0
10.5
9.0
pKa2
9.67
pKa2
4.25
pKa1
2.2
eqp.
aqp.
cqp.
1
2
3
OH⁻ vol. equivalents




pKa1
pKa2
pKa3

5. Define ‘buffer’ and list common buffers used in biochemistry.

   Buffer stands for a solution that can withstand significant changes of pH when an acid or base is applied. Most common ones are hydrochloric acid buffer, acid phthalate buffer, Phosphate buffer, etc.

Reference

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