department of biology and biochemistry.

Bioc. 331Lab **Exp. date:**sep 27, 2021

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Experiment No: 2

Experiment title: Titration Curves of Amino Acids.

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Objective:

to evaluate zwitterions by the process of titration by adding a base to neutral amino acids. Then, measuring the Pka values and comparing them to the literature values in a given table. overall, recognizing the difference between ionizable and non-ionizable amino acids.

introduction:

The development of current titration theory and its application to analytical procedures has substantially expanded the scope of the volumetric technique, as well as significantly improved its accuracy. Both basic and acidic groups in amphipathic electrolytes can be estimated using a variety of acidimetry and alkalimetry methods, and experimental data will be presented to show that when titrations are monitored by the circumstances requested by theoretical study, substantial accuracy is obtained. (1)

A zwitterion is a molecule having two functional groups, one of which has a positive electrical charge and the other has a negative electrical charge. The whole molecule has a net charge of zero. The most well-known zwitterions are amino acids. A carboxylic and an amine (basic) group are present (acidic). Because it is the stronger base, the -NH2 group absorbs H+ from the -COOH group, forming a zwitterion. (2)

Materials:

- Sodium hydroxide (100 mM)

- Amino acids (100 mM glycine, alanine, histidine, and lysine; 50 mM glutamic acid, pH < 2)
- pH meters
- Burettes (10 ml)
- Pipette

Methods

1.10 ml of the amino acid solution was pipetted into a 100 ml beaker.

2. The pH meter was standardized and the pH of the solution was determined.

3. a burette was filled with 100 mM NaOH and was placed on top of the amino acid solution on a stand.

4. known volumes of the NaOH solution were added from the burette (small amounts at first) and the pH was determined after each addition. The pH and volume added to the Pka was recorded.

5. The base was continuously added until the pH rises to about 11 or a little more.

6. The pK values from the curves were determined and were compared with the literature values.

Data:

image 1 titration curve (NaOH vs pH of alanine)

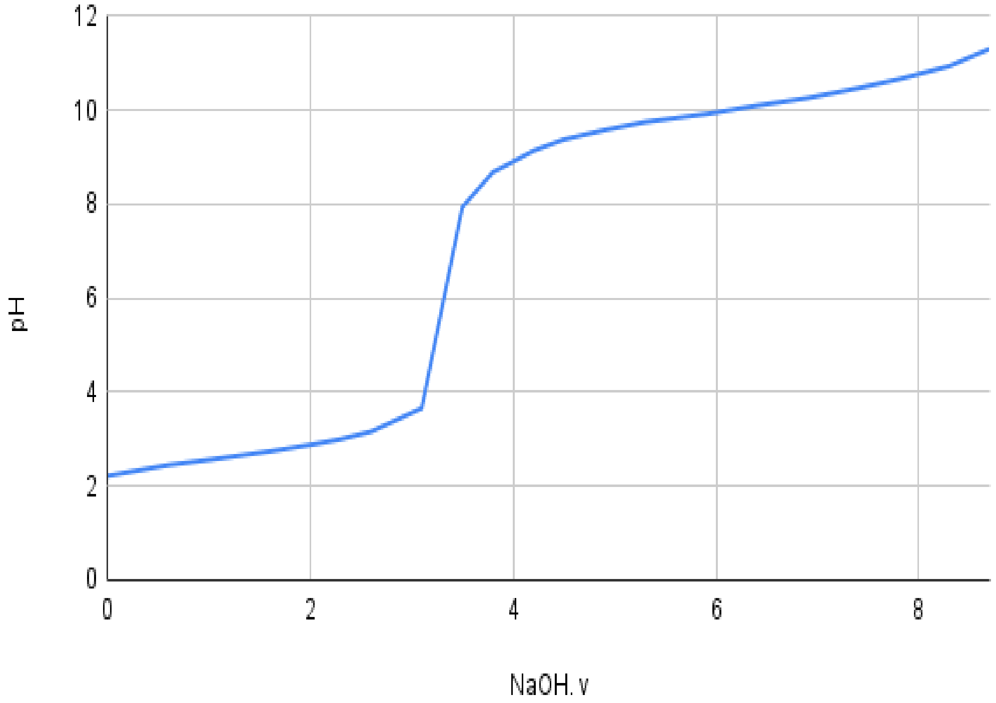


image 2 titration curve (NaOH vs pH of glycine)

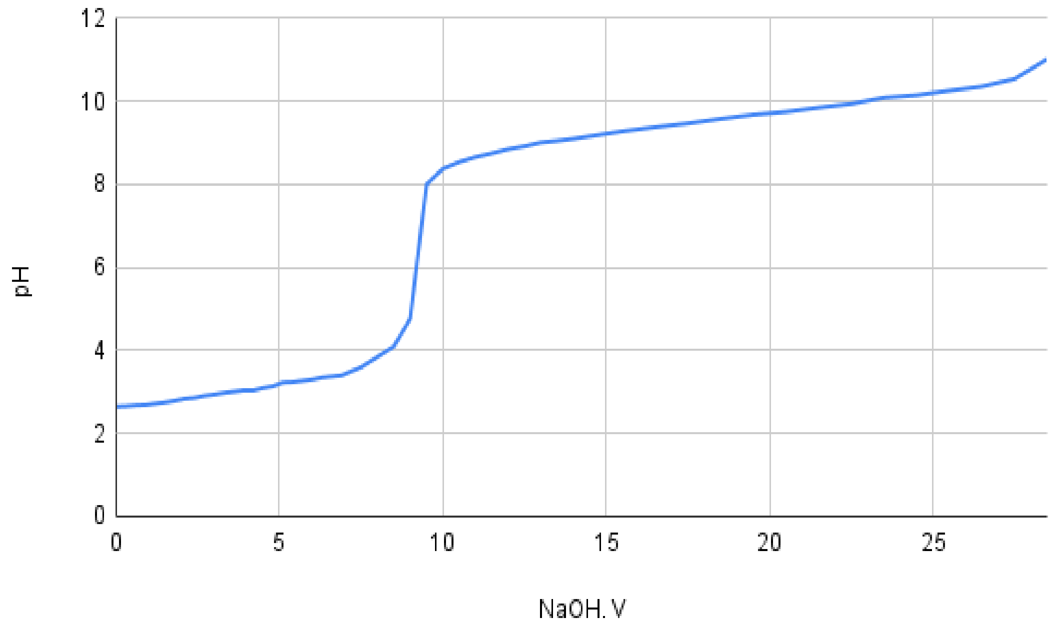


image 3 titration curve (NaOH vs pH of histidine)

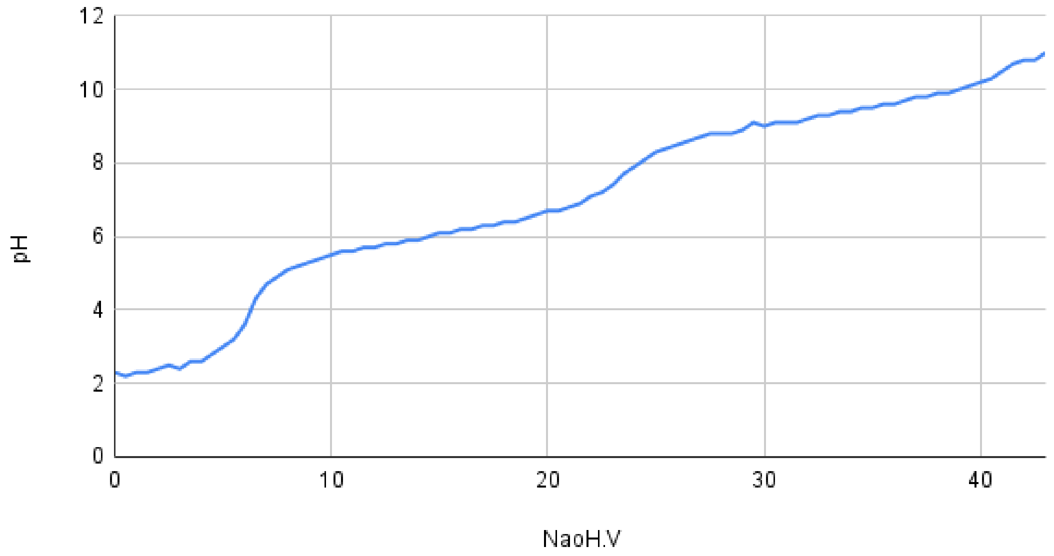


image 4 titration curve (NaOH vs pH of lysine)

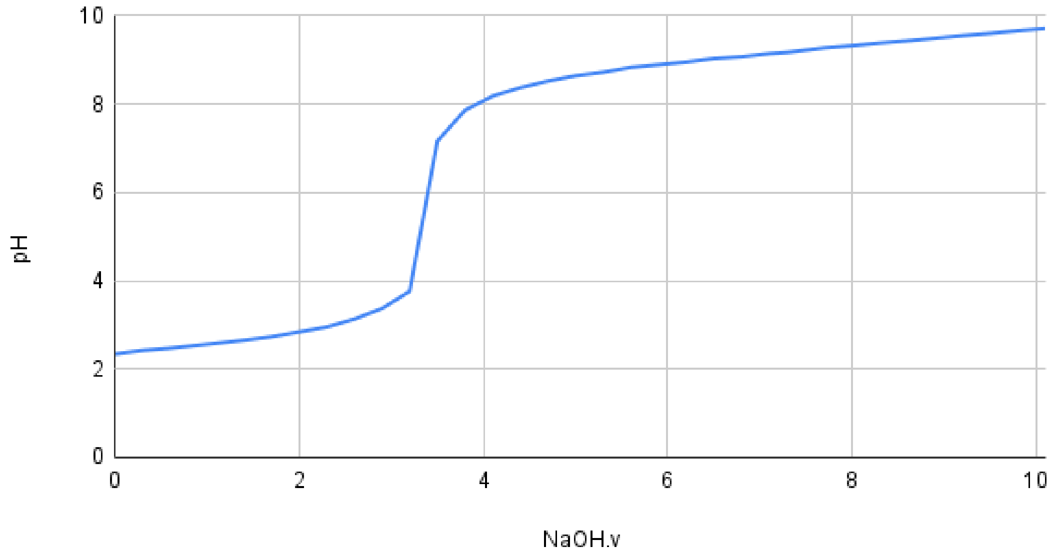
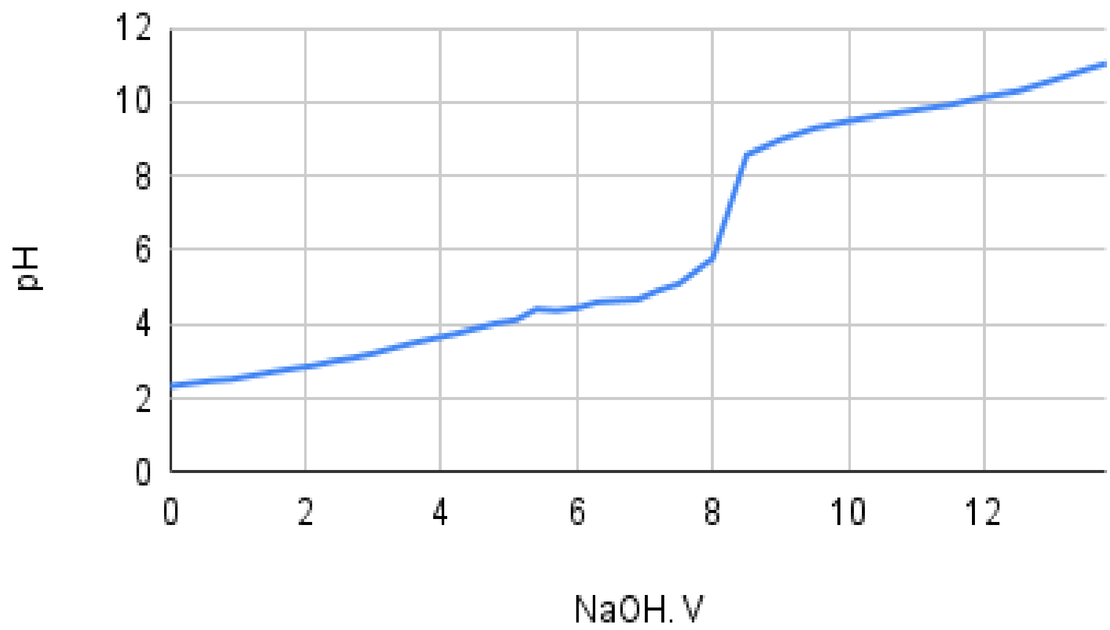


image 5 titration curve (NaOH vs pH of glutamic acid)



Discussion & Comments:

This experiment involves the recognition of different dissociations of different amino acids. some amino acids lose either a proton from their carboxyl group and gain one in their amino group (making them amorphous with two pKAs, and non-ionizing), or even lose an extra R-group (making them ionizing). The formation from acid to neutral is pK1, and from neutral to alkaline is pK2. in ionizing amino acids, however, there is an extra pK3 which makes a strong base.

referencing the images above, both image 1 and image 2 are non-ionizing amino acids. Hence, they only contain pk1 and pk2. image 1 consists of alanine that had a pk1 of about 2.6 and a pk2 of 9.8, which is really close to its literature value. Its pI is

6.2. image 2 consists of glycine, this curve has a pk1 of around 2.7 and a pk2 of 9.7, and a pI of 6.2.

image 3, histidine, one of my favorite amino acids. This amino acid showed 3 clear amphoteric zones in its titration curve. The presence of these zones proves that histidine is ionizing and loses its r-group (imidazole). The pk values obtained from the graph were pk1: 2.2, pk2: 9.4, pk3: 6.0. with a pI of 7.7.

image 4 lysine, doesn't have an obvious transition in pk values, but it is there. lysine has a pk1 of 2.3, pk2 of 9.0, and pk3 of around 10. with a pI of 9.47. lysine is an ionizing amino acid.

image 5, Glutamic acid, is similar to that of lysine in the sense that its pk transitions aren't very obvious. However, glutamic acid is ionizing and has 3 buffer regions, pk1 is 2.3, pk2 is 9.8, and pk3 is 4.3. Its pI is 3.08.

conclusion:

all amino acids are ionized in the solution they're in, but they do not all ionize the same. some amino acids lose their r-groups making them more alkaline than others. The pI of amino acids could help one better understand an amino acid's capability of ionization. Overall, the results graphed in the titration curves were almost precise compared to the literature values in chart 1.

chart 1 literature PKA values of amino acids.

Amino acid	pK_1 $-COOH$	pK_2 $-NH_2$	pK_3 side chain (R)
Glycine	2.4	9.7	---
Alanine	2.3	9.9	---
Glutamic acid	2.2	9.7	4.3-carboxyl
Histidine	1.8	9.2	6.0-imidazole
Lysine	2.2	9.0	10.5-amino

appendix:

- pI= (pK1+pK2)/2
- pR=(pk2+pk3)/2

references:

(1) The titration of amino- and carboxyl-groups in amino-acids, polypeptides, etc. Parts I—III.—Investigations with aqueous solutions. (1923). *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character*, *95*(670), 440–484. https://doi.org/10.1098/rspb.1923.0047

(2) *Zwitterions and Amino Acids*. (2012). Zwitterions. https://www.aqion.de/site/zwitterions