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# Titrimetric Analysis of Amino Acids

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**Abstract- The titrimetric method is used to identify an unknown amino acid or determine an unknown concentration of a known amino acid. This process is carried out by adding H+ or OH- ions dropwise. To perform the titration, it is necessary to know the titration curves, pKa values, buffer regions, protonation states, charges, and isoelectric points. In this work, we will focus on the theoretical principles of amino acid titration and the solution of problems in biochemistry.**

**Keywords— titration curve, amino acids, pH, biochemistry, pKa, isoelectric point.**

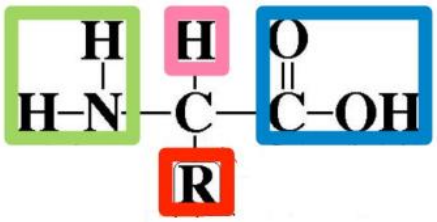
## I. INTRODUCTION

The chemistry of amino acids, including their pKa values, pI values, and protonation states, has been elucidated, and the titration curve for a weak acid has been studied [1,2,3,4].

General Formula and Classification of Amino Acids. Amino acids are bifunctional compounds containing -NH2 and –C (O) OH groups, the first representative of which is aminoacetic acid (glycine, glycocoll, H2NCH2COOH).

Amino acids consist of the following:

- An amino group (-$NH_2$)
- A carboxyl group (-COOH)
- A hydrogen atom (-H)
- A side chain (-R)




Amino acids can be classified as follows based on the general chemical characteristics of their -R group.

Neutral side chain: amino acids that have a nonpolar side chain, do not form ionic or hydrogen bonds, and are non-reactive (do not participate) in chemical processes. For example: alanine, glycine, etc.

Polar uncharged side chain: The R groups are not ionized in water, but they can participate in hydrogen bonding. For example: serine, cysteine, etc.

Acidic side chain (negatively charged): Amino acids whose R group contains a -COO- group. For example: glutamate and aspartic acid.

Basic side chain (positively charged): Amino acids whose R group contains an -NH3+ group. For example: arginine, histidine, and lysine.
Properties of amino acids.

Amino acids are amphoteric, meaning they can react as an acid (donating a proton) or as a base (accepting a proton).

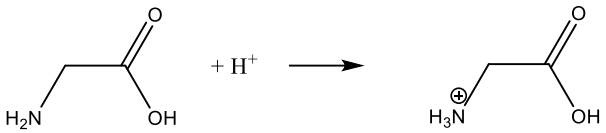
The amphoteric properties of amino acids are due to the presence of their ionizable α-amino and α-carboxyl groups, which, depending on the pH of the medium, can exhibit the properties of either acids or bases.

Each amino acid has a different isoelectric point (pI)

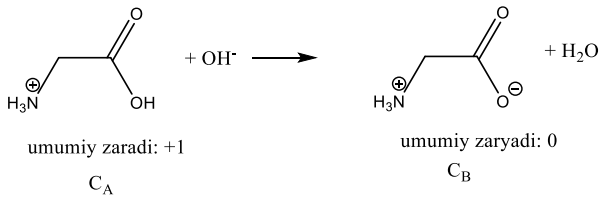
- pI: This is the pH value at which the positive charge equals the negative charge (i.e., the net charge of the molecule is zero), forming a zwitterion.

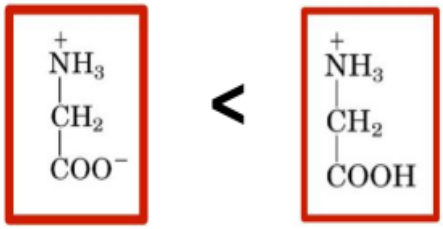
**Alkaline titration of the neutral amino acid glycine.**
For the alkaline titration of an amino acid, an acidic medium is prepared; when acid is added, the pH

approaches ≈0. As a result, the amino group becomes protonated.

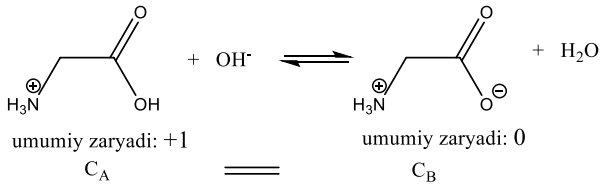


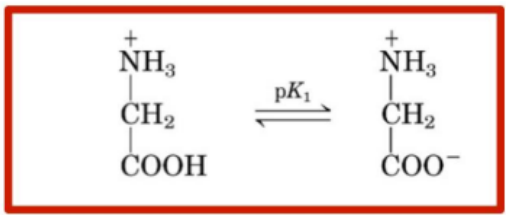
When the pH increases, i.e., when an alkali is added, the -COOH group also begins to deprotonate, and the ratio is as follows:



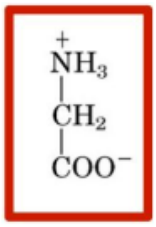
As the addition of alkali continues, a point is reached where the concentrations of CA (cation) and CB (zwitterion) become equal. At this point, pH = pKa1, and the system acts as a buffer.



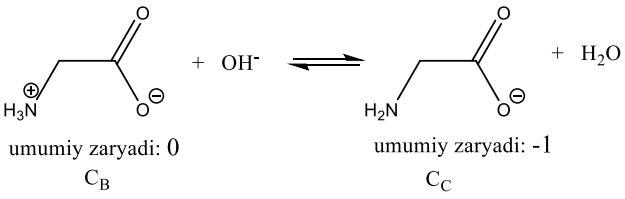



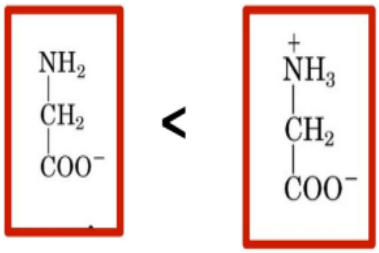
As the pH increases further, the solution will mainly contain the zwitterion, and at this point, the pH is equal to pI (the isoelectric point). A zwitterion is an ion with a net charge of 0, so it is not attracted to the cathode or the anode. The state where the zwitterion concentration is highest is called the isoelectric point. The isoelectric point is found as follows.

$$pI = \frac{pKa1 + pKa2}{2}$$

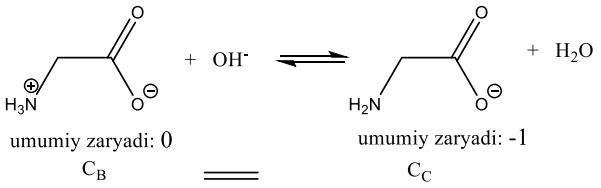


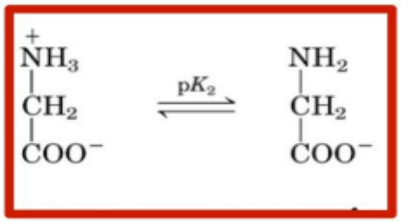
As the pH increases, the second group, -NH3+, also begins to deprotonate.



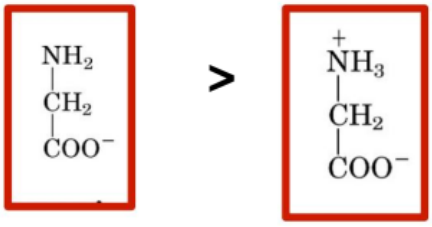



After this, the concentrations of CB and CC become equal. At this point, pH = pKa2, and the system acts as a buffer.

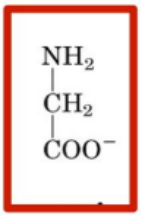


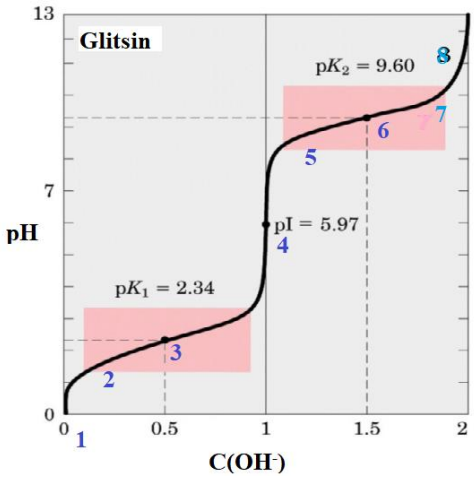

CB < CC, a buffer system also forms in this zone.



The -NH3+ group dissociates, and at the final point, the glycine is completely deprotonated.

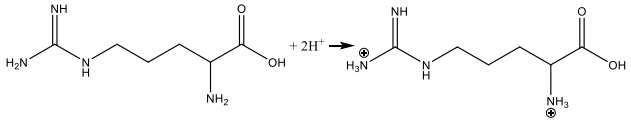


The alkaline titration curve of glycine is as follows (the steps above are shown in full).

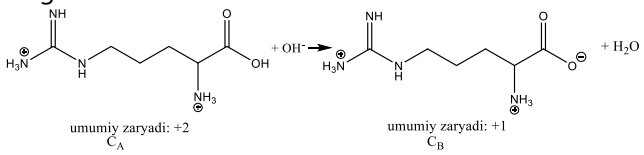



Alkaline titration of the basic amino acid arginine.

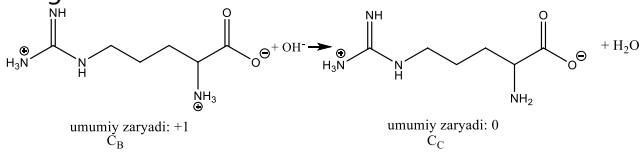
For alkaline titration, the medium is made acidic, where the pH approaches ≈0 upon addition of acid. As a result, the amino group is protonated.




Stage I.

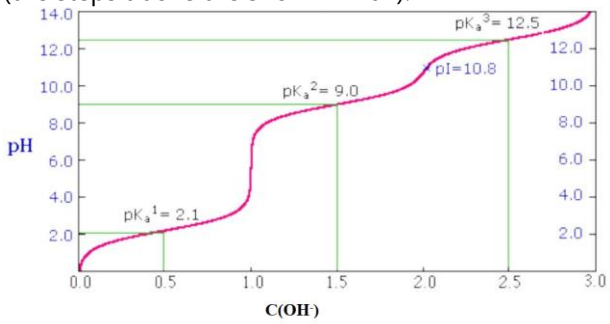



Stage II.



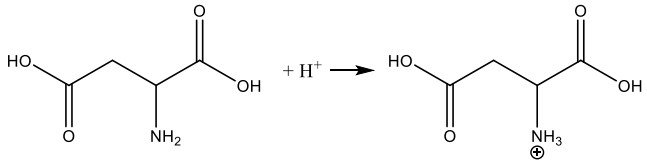

Stage III.

The alkaline titration curve for arginine is as follows (the steps above are shown in full).

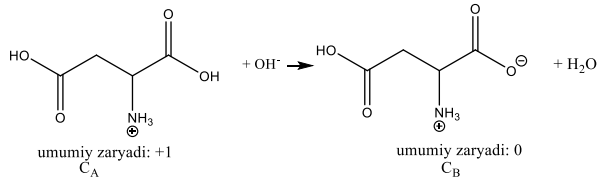



**Alkaline titration of the acidic amino acid aspartic acid.**

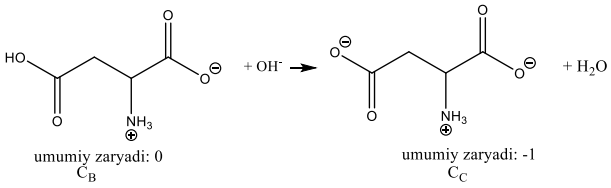
For alkaline titration, an acidic medium is prepared, where the pH approaches ≈0 upon acid addition. As a result, the amino group becomes protonated.



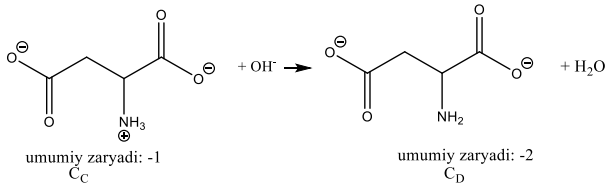
Stage I.



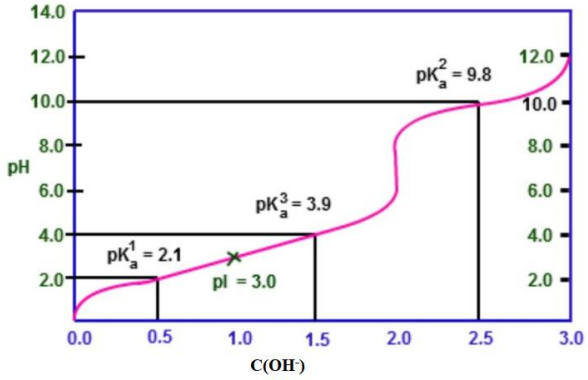

Stage II.




Stage III.




The alkaline titration curve for aspartate is as follows (the stages above are shown in full).




## II. CALCULATION PROBLEM (35TH IBO OLYMPIAD, STAGE II SELECTION QUESTION).

**Task 1: Biochemistry (12 points) TITRATION OF AMINO ACIDS.**

Amino acids are organic molecules that contain carboxyl and amino groups. It is known that natural proteins can contain 20 different types of amino acids. The table below shows the formulas for these 20 amino acids. Most amino acids exhibit amphoteric properties in a solution environment; this is due to the properties of the amino (-NH2) and carboxyl -(COOH) groups within the amino acid molecule. The radical (R) group of neutral amino acids has no charge.

Amino group (-NH2): exhibits basic properties. At a low pH (in an acidic medium), the amino group is protonated (H+) and transitions to a positively charged state (NH3+) .

Carboxyl group (-COOH) : exhibits acidic properties. At a high pH (in an alkaline medium), the carboxyl group is deprotonated (H+) and transitions to a negatively charged state (COO-) .

Zwitterion: At an intermediate pH, amino acids can exist in a zwitterion form. In a zwitterion, the amino group is protonated (NH3+) , and the carboxyl group is deprotonated (COO-) .

The overall charge of an amino acid in its zwitterionic form is neutral. The pH at which an amino acid exists as a zwitterion (with a neutral charge) is called its isoelectric point (pI) . The specific pKa values for the amino and carboxyl groups vary among different amino acids, which causes their isoelectric points to differ. Using a titration curve, the pI (isoelectric point) can be empirically determined as the inflection point between the pKa values of the amino acid's anionic and cationic forms.

The general equilibrium for the dissociation of an acid is: HA ⇌ H+ + A-. The acid dissociation constant, Ka, for this equilibrium is found using the following formula:

$$\mathrm{Ka} = \frac{[\mathrm{H^+}][\mathrm{A^-}]}{[\mathrm{HA}]}$$

Typically, the strength of acids is expressed through their pKa values.

$$pKa = -\log_{10}(Ka)$$

When titrating such diprotic amino acids, the titration occurs in two stages because the more acidic carboxyl group (lower pKa1) and the less acidic amino group (pKa2) lose their protons sequentially.

The apparent pKa values for the two dissociation stages can be calculated based on the midpoints of each stage.

The Henderson-Hasselbalch equation can be used for this purpose.

$$\mathrm{pH} = \mathrm{pKa} + \log\left(\frac{[\mathrm{A}^-]}{[\mathrm{HA}]}\right)$$

pKa1 (the pKa for the carboxyl group) is the point at which half of the acid group has been titrated; that is, the point where the anion (A-) and the acid group (HA) are present in equal amounts in the solution.

Thus, the equation at this point is as follows:

$$\mathrm{pH} = \mathrm{pKa}$$

Similarly, pKa2 (the pKa for the amino group) can be determined.

The data below were obtained from an experiment titrating an unknown amino acid, X, with 0.3024 M NaOH (a 25 mL burette was used in the experiment).

| Volume of NaOH added (mL) | pH |
|---|---|
| 0.00 | 1.4 |
| 1.00 | 1.4 |
| 2.00 | 1.5 |
| 3.00 | 1.6 |
| 4.00 | 1.7 |
| 5.00 | 1.8 |
| 6.00 | 1.9 |
| 7.00 | 2.1 |
| 8.00 | 2.2 |
| 9.00 | 2.4 |
| 10.00 | 2.7 |
| 11.00 | 3.2 |
| 12.00 | 9.4 |
| 13.00 | 9.9 |
| 14.00 | 10.2 |
| 15.00 | 10.4 |
| 16.00 | 10.6 |
| 17.00 | 10.8 |
| 18.00 | 11.0 |
| 19.00 | 11.3 |
| 20.00 | 11.6 |
| 21.00 | 11.8 |
| 22.00 | 12.0 |
| 23.00 | 12.1 |
| 24.00 | 12.2 |
| 25.00 | 12.3 |

Q1.1. Using the data in the table above, draw a graph of the titration process (Volume of NaOH (mL) vs. pH) on the graph paper provided.

Q1.2. From your titration curve, find the pI point, mark it on the graph, and write the value of the pI point on the answer sheet.

Q1.3. From your titration curve, find pKa1 and pKa2, mark them on the graph, and write the values of the pKa1 and pKa2 points on the answer sheet.

Q1.4. 0.866 g of an unknown amino acid X was dissolved in 75 ml of deionized water. Determine the molecular weight of the unknown amino acid X and, based on the molecular weights of the amino acids provided below, write the name of the titrated amino acid on the answer sheet. Note: To start the titration process from the fully protonated form of the amino acid, 3.2 ml of a 0.3024 M HCl solution was added to the amino acid solution. This is equivalent to 3.2 ml of a 0.3024 M NaOH solution. To determine the actual molar amount of NaOH required to reach the pI point, subtract 3.2 ml from the volume of NaOH used to reach the pI point.

| Aminokislota | Molekulyar og'irlik |
|---|---|
| Glitsin | 75 |
| Alanin | 89 |
| Valin | 117 |
| Leysin | 131 |
| Metionin | 149 |
| Izoleysin | 131 |
| Prolin | 115 |
| Sistein | 121 |
| Serin | 105 |
| Treonin | 119 |

| Aminokislota | Molekulyar og'irlik |
|---|---|
| Asparagin | 132 |
| Glutamin | 146 |
| Lizin | 146 |
| Arginin | 174 |
| Gistidin | 155 |
| Aspartat | 133 |
| Glutamat | 147 |
| Fenilalanin | 165 |
| Tirozin | 181 |
| Triptofan | 204 |

**Solution to the Problem**

Using the data from the table above, the following graph was plotted.

The graph shows that the pH changed sharply when 11 ml and 12 ml of alkali were consumed. This means the isoelectric point corresponds to the point where approximately 11.5 ml of alkali has been added. At this point, the H+ ions of the amino acid are completely neutralized, and if we find the pH corresponding to this volume, it is approximately pH≈6.

The isoelectric point is pH ≈ pI ≈ 6.

After determining the isoelectric point on the graph, we will determine pKa1 and pKa2.

pKa1 is determined by finding the pH at the point where half the amount of alkali required to reach the isoelectric point has been added. At 11.5 ml / 2 = 5.75 ml, the pH is equal to pKa1. This value is pH≈1.8, so pKa1≈1.8.

pKa2 is determined by the pH value when an additional half of the volume of alkali used to reach the isoelectric point is added. At 11.5 ml + 11.5 ml/2 = 17.25 ml, pH = pKa2. This value is pH ≈ 10.8, so pKa2 ≈ 10.8.

1.4. Initially, 3.2 ml of a 0.3024 M HCl solution was added to convert the amino acid to its acidic form. Therefore, since the concentrations of the alkali and the acid (HCl) are equal, 3.2 ml of the alkali was consumed to neutralize the added HCl. The remaining volume, 11.5 ml – 3.2 ml = 8.3 ml, was used to neutralize the amino acid. The amount of substance is:

n (NaOH) = CM·V = 0.0083 L · 0.3024 M = 0.00251 mol.

If 0.866 g of an unknown amino acid X was dissolved in 75 ml of deionized water and a 25 ml aliquot was taken for the experiment (a 25 ml burette was used), then the 25 ml aliquot contains 3 times less amino acid (75 ml / 25 ml = 3): 0.866 g / 3 = 0.28867 g.

Since the graph corresponds to a monobasic amino acid:
X (amino acid) + NaOH → Salt + H2O
0.28867 g corresponds to 0.00251 mol
Mr (X) = 115 g/mol corresponds to 1 mol
If Mr (X) = 115 g/mol, this amino acid is proline.

## III. CONCLUSION

This work helps develop the ability to identify unknown amino acids and to create and analyze titration curves. It provides an understanding of protonation states, buffer regions, and isoelectric points, as well as practical skills in solving related problems.

## REFERENCES

1. Berg, J. M., Tymoczko, J. L., Stryer, L.: Biochemistry (2012) 7th edition, W. H. Freeman and Company, New York; ISBN-13: 9781429229364
2. Nelson, D. L., Cox, M. M.: Principles of Biochemistry (2008) 5th edition, W. H. Freeman and Company, New York; ISBN-13: 9780716771081
3. Voet, D., Voet, J. G., Prat, C. W.: Fundamentals of Biochemistry (2009) 3rd edition, John Wiley & Sons, ISBN: 978-0-470-22842-5
4. Voet, D., Voet, J. G.: Biochemistry (2011) 4th edition, John Wiley & Sons, ISBN-13: 978-0470570951