BCH 312

Experiment (6)

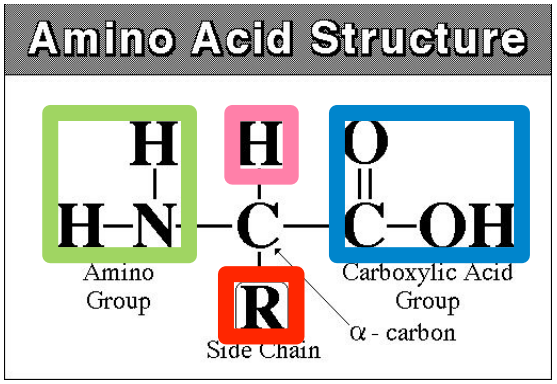
AMINO ACID TITRATION

Objectives

- To study the titration curves of amino acid.
- To determine the pKa values.
- To determine isoelectric point (pI).
- To determine buffering regions.

Amino acid general formula and classification

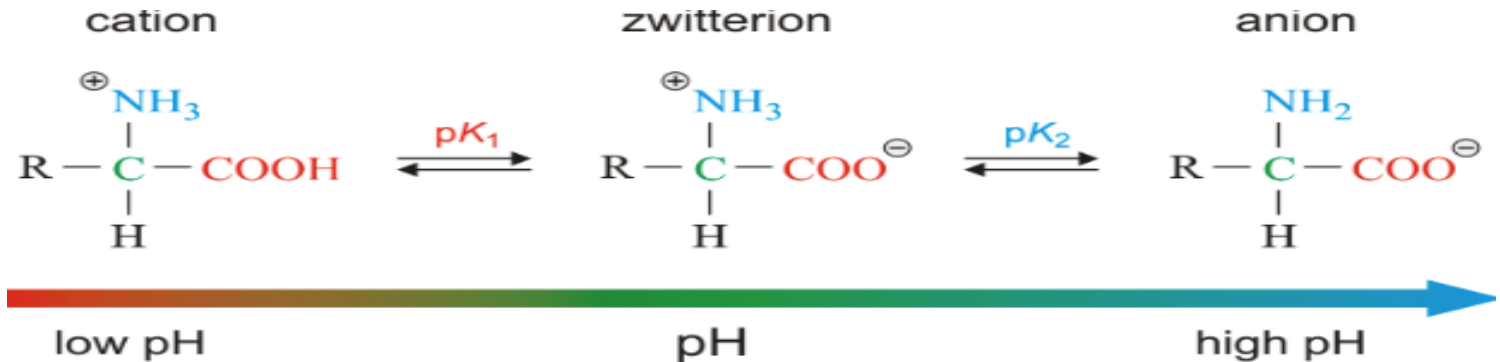
- **Amino acids consist of:**
  - **a basic amino group (—NH_2)**
  - **an acidic carboxyl group (—COOH)**
  - **a hydrogen atom (—H)**
  - **a distinctive side chain (—R).**



- **Classification:**
- Amino acids can be assorted on the basis of the general chemical characteristics of their R groups.
  - **Non-polar side chain:** Non-polar side chain that does not bind or give off or participate in hydrogen or ionic bonds, **e.g. Alanine**
  - **Polar uncharged side chain:** The R groups are not ionized in water, but it can participate in hydrogen bond formation, **e.g. Serine**
  - **Acidic side chain (negatively charge):** Contains $–COO^-$ **e.g. Glutamic acid and Aspartic acid**
  - **Basic side chain (positive charge):** Contains $-NH_3^+$ **e.g. Arginine, Histidine and lysine**

Properties of amino acids

- Amino acids are Amphoteric which means it can react as an acid (donate a proton) as well as a base (accept a proton)
- **Amphoteric properties of amino acids are due to** the presence of their ionizable α-amino and α-carboxylic group can act sometimes as acids and sometimes as bases depending on the pH of their media.
- Each amino acid have a different **isoelectric point (pI)**
- **pI:** It is the pH value at which the positive charge equals the negative charge **(i.e. the net charge of this molecule equals zero) (zwitter ion)**

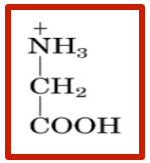


Amino acid titration

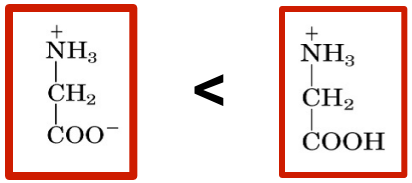
- From the amino acid titration curve, we can get important information about amino acid, for example pKa and also the pI.
  - Amino acids have more than one pka, because it is polyprotic (contain more than one ionizable groups).
- Also it provides information about the buffering range of the amino acid that is studied.
- Based on the number of plateaus on a titration curve, one can determine the number of dissociable protons.

Titration curve of amino acid (glycine)

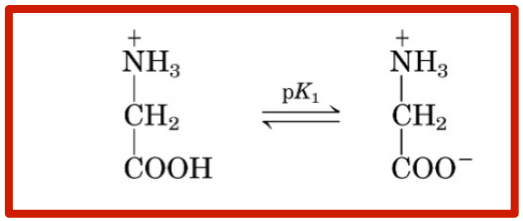
1. At a very low pH (acidic) both groups are fully protonated where the solution predominantly contains:

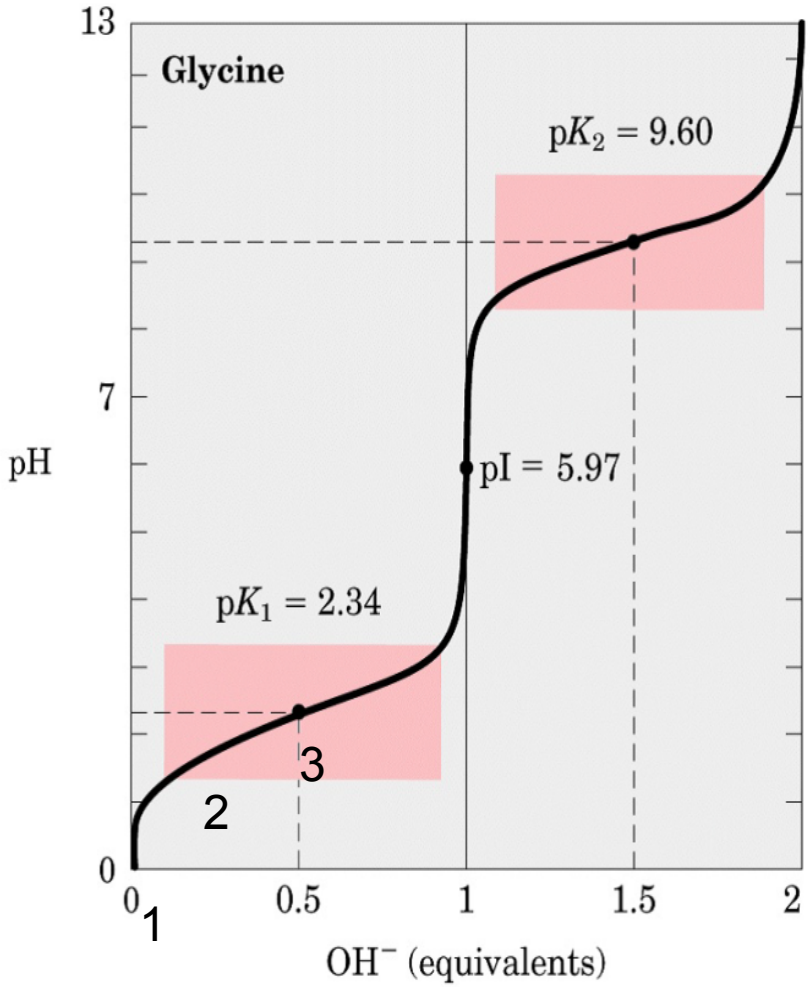


2. When the pH is raised, the –COOH group start to be deprotonated and the proportion will be:



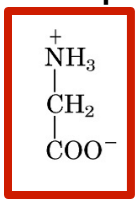
3. pH= pKa_1, where it will act as a buffer and the solution will contain an equal amount of :



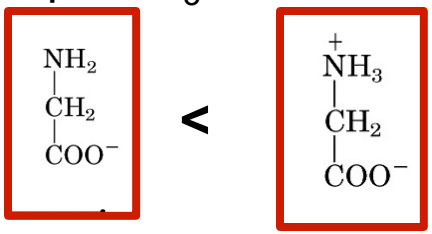


Titration curve of amino acid (glycine)

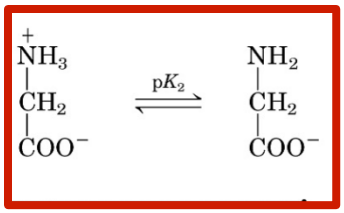
4- Further increase in pH, the solution will predominantly contains zwitter ion and the pH at this point is equal to pI.

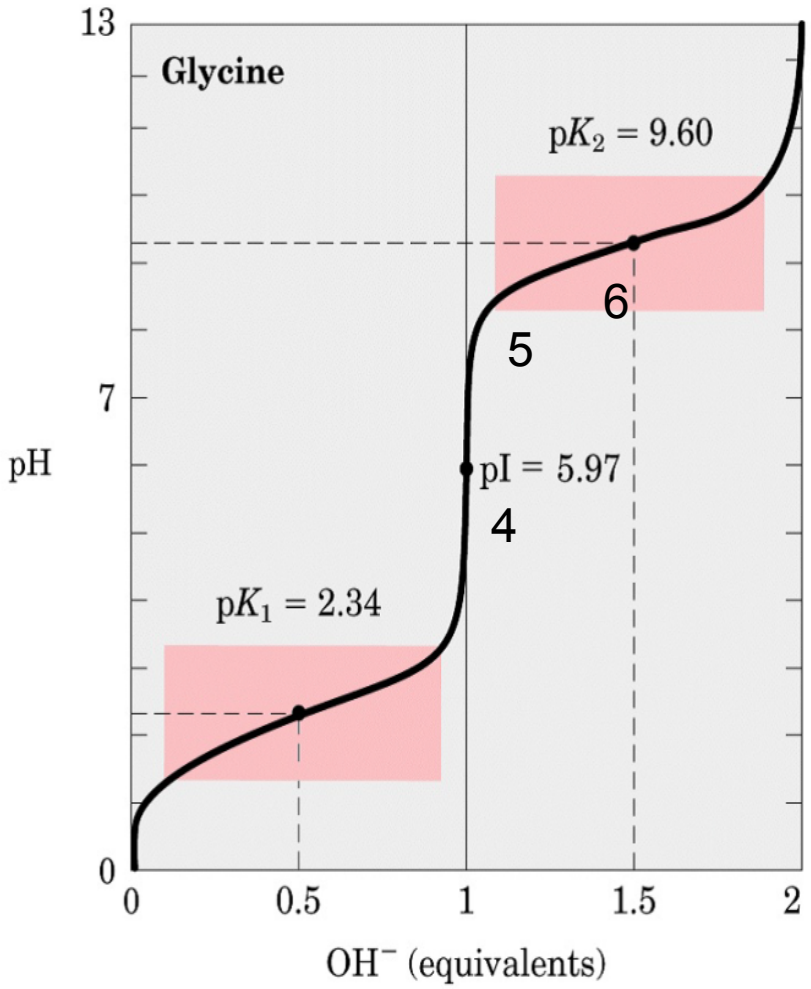


5- As the pH increases, the second group $-NH_3^+$ will be deprotonated



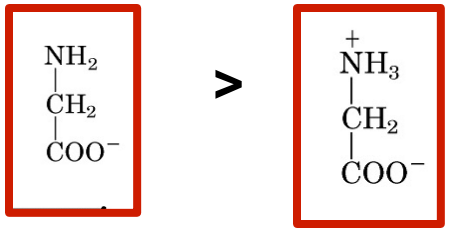
6- After that, pH=pKa_2 where it will as a buffer and the solution will contain an equal amount of:



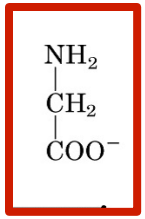


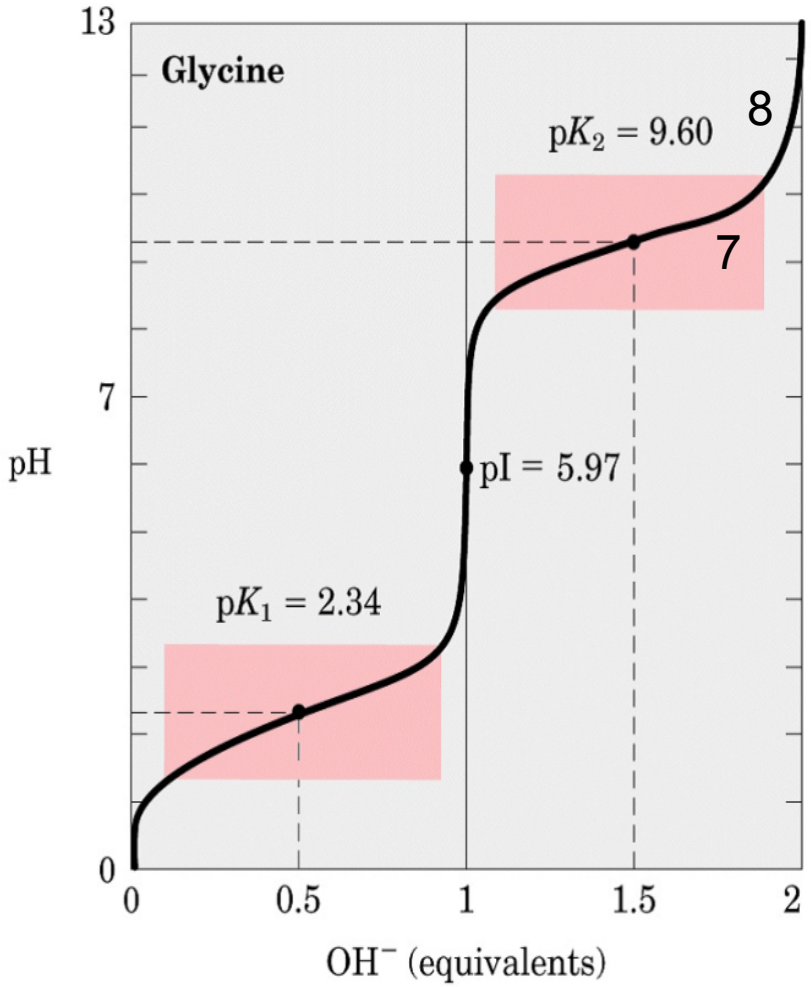
Titration curve of amino acid (glycine)

- **7-**



- **8-** the NH_3^+ group will dissociate and at the same time the glycine full dissociate in end point

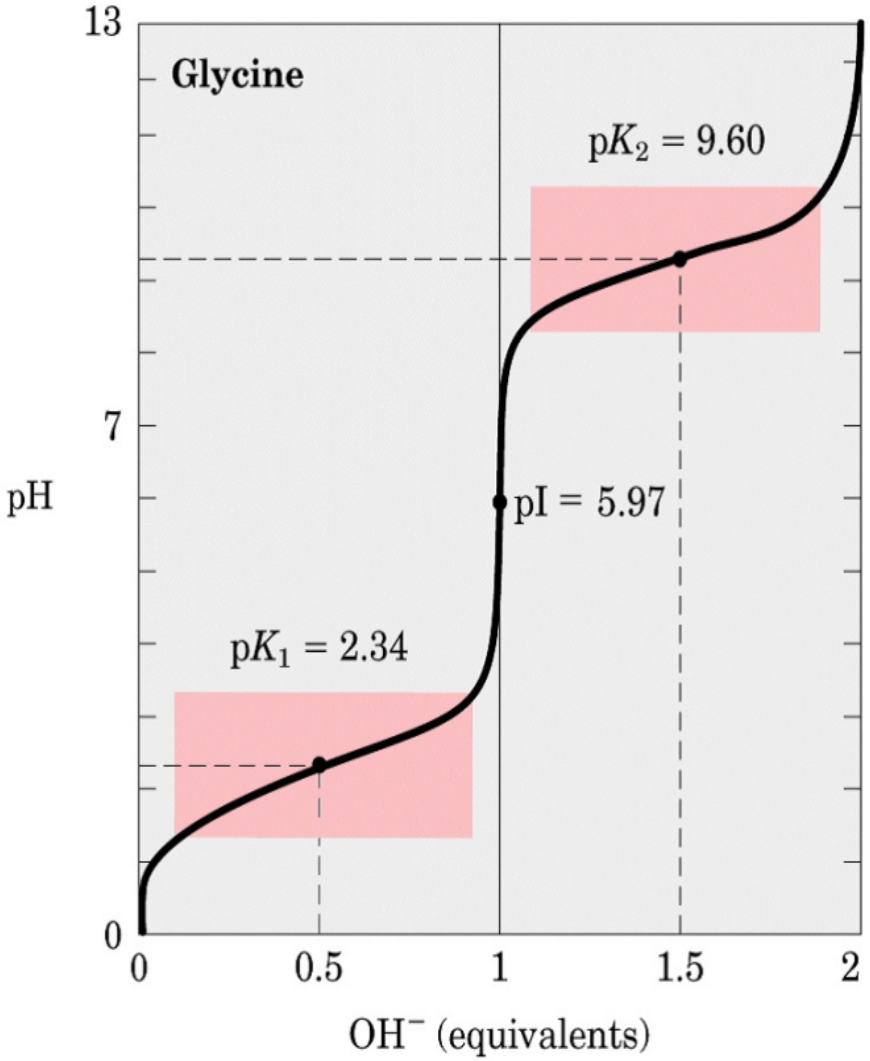




How to determine pI from the curve

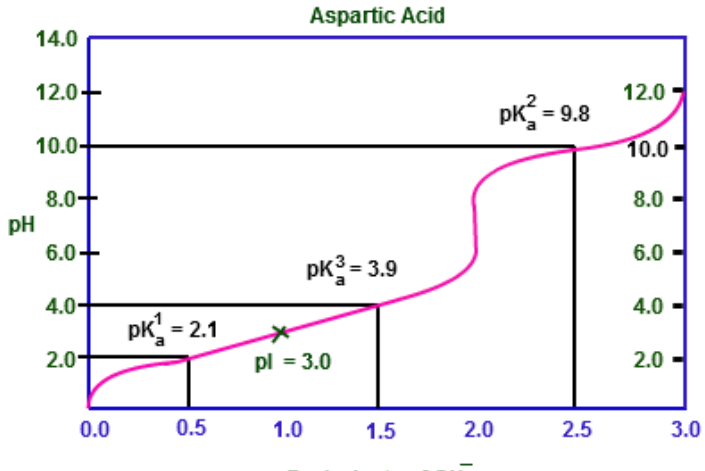
- In diprotic amino acids such as glycine,
  - The pI is an average of the pK_a's of the carboxyl (2.34) and ammonium (9.60) groups. Thus, the pI for alanine is calculated to be:

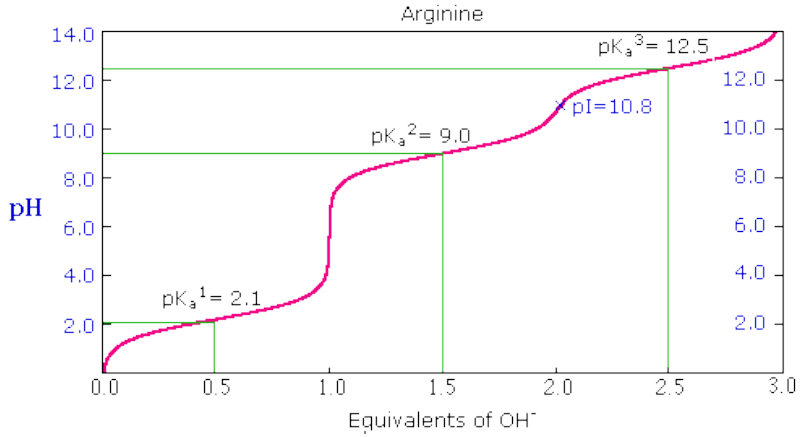
    (2.34 + 9.60)/2 = 5.97.



Triprotic amino acids

- Titration curves of triprotic a.a. are more complex with three stages→ They have 3 pKa values.
- If additional acidic or basic groups are present as side-chain functions, **the pI is the average of the pK_a's of the two most similar acids (value).**
- In the case of aspartic acid, the similar acids are the alpha-carboxyl group (pK_a = 2.1) and the side-chain carboxyl group (pK_a = 3.9), so pI = (2.1 + 3.9)/2 = 3.0.
- For arginine, the similar pKa`s values are the pKa for guanidinium group on the side-chain (pK_a = 12.5) and pKa for alpha-ammonium group (pK_a = 9.0), so the calculated pI = (12.5 + 9.0)/2 = 10.75.





Note:

The pH calculated by different way :

- **[1] At starting point** pH= (pKa+p[HA]) /2
- **[2] At any point within the curve (after , in or after middle titration)**

  pH=pKa+ log[A-]/[HA] ***(Henderson-Hasselbalch equation)***
- **[3] At end point** pOH=(pKb+p[A-]) /2

  pH=pKw – pOH

Method:

- Pipette 10 ml of alanine solution (0.1 M) into a 50 ml breaker.
- Add 0.5 ml of (0.1 M) HCl from the burette and determine the pH of the solution after each addition.
- Continue adding acid in until pH falls to about 1.3 .
- Wash the electrode in distilled water titrate a further 10 ml of alanine solution with 0.1 M NaOH until pH reaches 12.5.
- Plot a titration curve for alanine (pH verses titrant in ml).
- Do the titration of arginine in a similar way and plot their titration curves.

Result:

- Record the titration table and Plot a Curve of pH versus ml of NaOH and HCl added.
- Determine the pka and pI values from your curves and compare them with the standard values.
  - **For alanine:** pK_1= 2.34 , pK_2= 9.69, pI= 6.01
  - **For arginine:** pK_1= 2.17 , pK_2= 9.04, pK_3= 12.48, pI= 10.76

Discussion :

[1] Calculate the pH of the alanine solution after the addition of 0ml, 5ml of 0.1M NaOH and calculate pH after addition of 1ml , 2.5ml of HCl

[2] Calculate the pH of the arginine solution after the addition of 0ml, 5ml of 0.1M NaOH and calculate pH after addition of 0.5 ml , 2 ml of HCl

[3] Compare your calculated pH values with those obtained from Curve.

[4] At what pH-range did the acid show buffering behavior? What are the chemical species at that region, what are their proportions?