Al al-Bayt University
Faculty of Science

Department of Biological Sciences

Practical Biochemistry-404352

Biochemistry Laboratory-3: Amino acid titration

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Amino acid titration

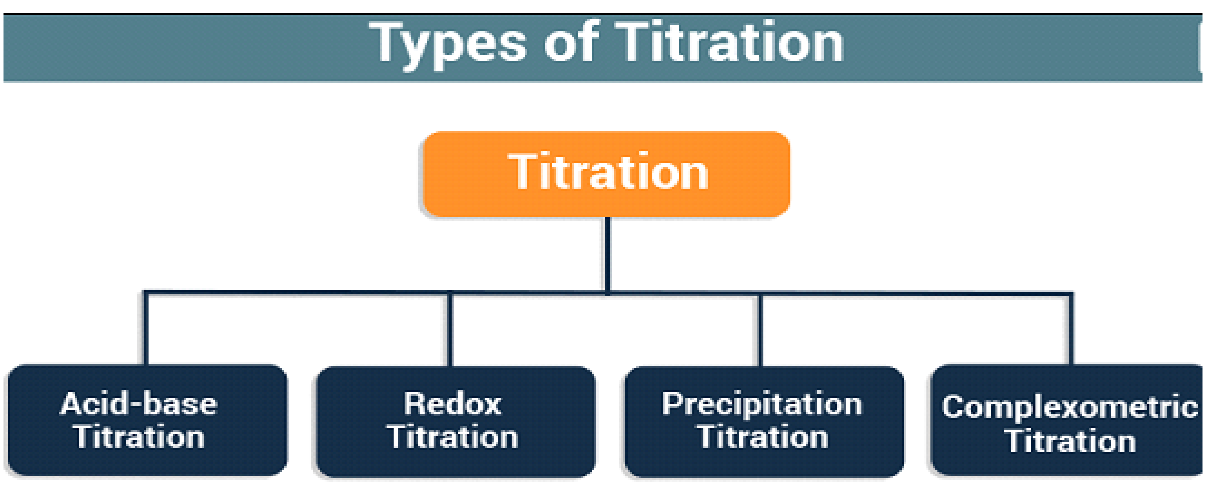
General Concept:

1. **Amino acid titration curve**
2. **Determination of pka values**
3. **Calculation of isoelectric point (PI)**
4. **Identification of amino acid based on PI**

A titration is defined as the process of determining the quantity of a substance A by adding measured increments of substance B, the titrant, with which it reacts until exact chemical equivalence is achieved (the equivalence point).

Titration which is also known as titrimetry is a chemical qualitative analysis technique that is used to calculate the concentration of a given analyte in a mixture.

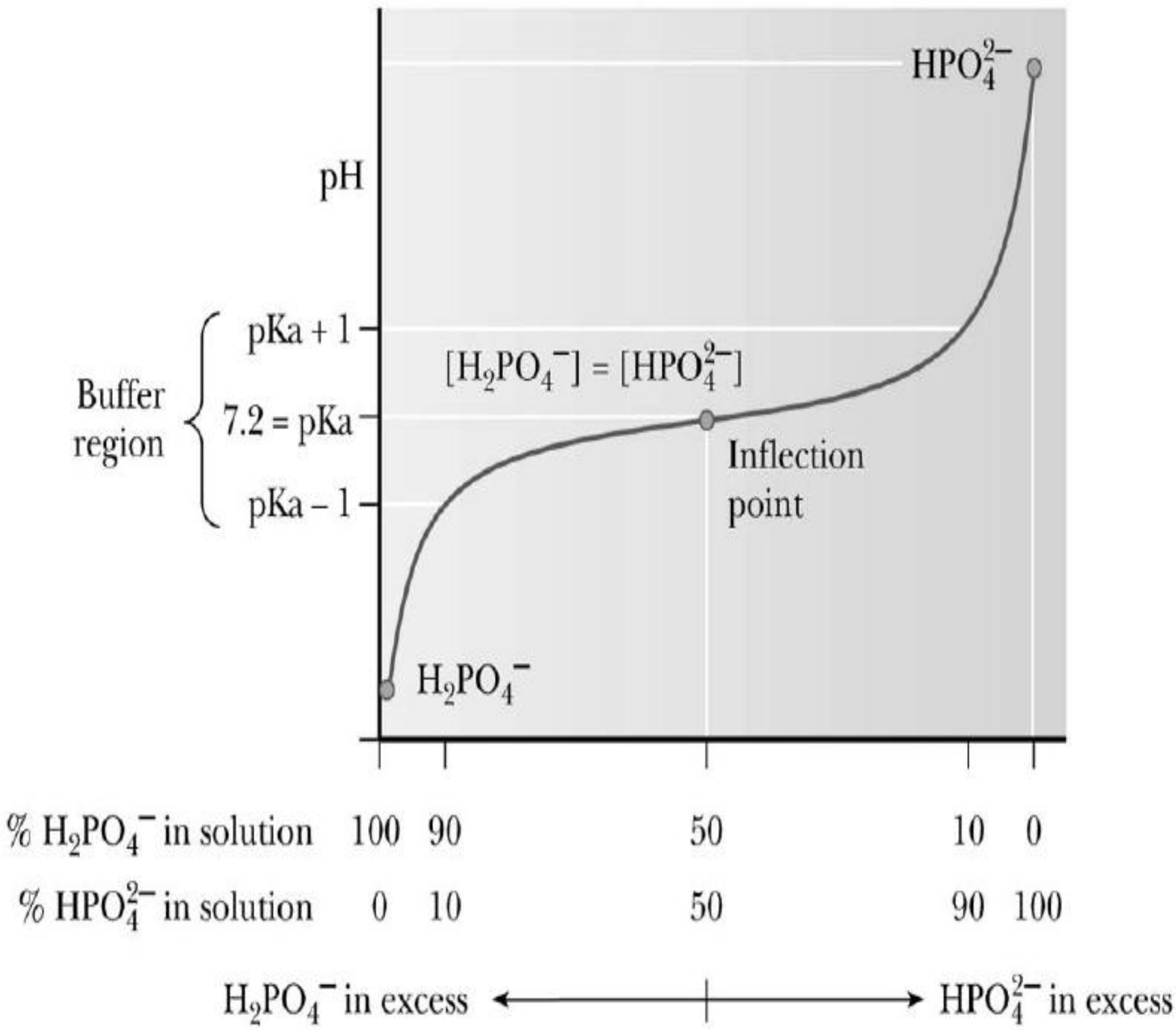
Titration is a way to limit potential side effects by taking time to see how your body will react to a drug. In titration, the medication is started at a low dose. Every couple of weeks, the dose is raised ("up-titrated") until the maximum effective dose ("target dose") has been achieved or side effects occur.



Types of Titrations

- **Acid-base titrations**, in which an acidic or basic titrant reacts with an analyte that is a base or an acid.
- **Complexometric titrations** involving a metal-ligand complexation reaction.
- **Precipitation titrations**, in which the analyte and titrant react to form a precipitate.
- **Redox titrations**, where the titrant is an oxidizing or reducing agent.

The titration curve is flat near the pKa of the buffer



At the midpoint, 1/2 of $H_2PO_4^-$ has been ionized and this means [$H_2PO_4^-$] = [HPO_4^{2-}]. Thus, the pKa of the solution equal the pH.

<u>Inflection point -</u> the point at which enough base has been added to neutralize the 50 % of the acid (or vice versa), [Acid] = [Base].

<u>End or Equivalence point:</u> The point at which enough base has been added to exactly neutralize the acid (or vice versa).

Isoelectric points and zwitterions

Each amino acid has a particular pH called the **isoelectric point** at which the overall charge on an amino acid molecule is zero.

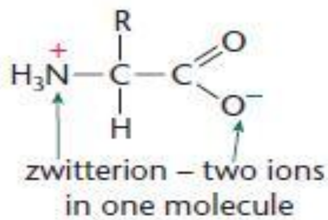
Examples of isoelectric points

amino acid	aspartic acid	glycine	histidine	arginine
isoelectric point	3.0	6.1	7.6	10.8

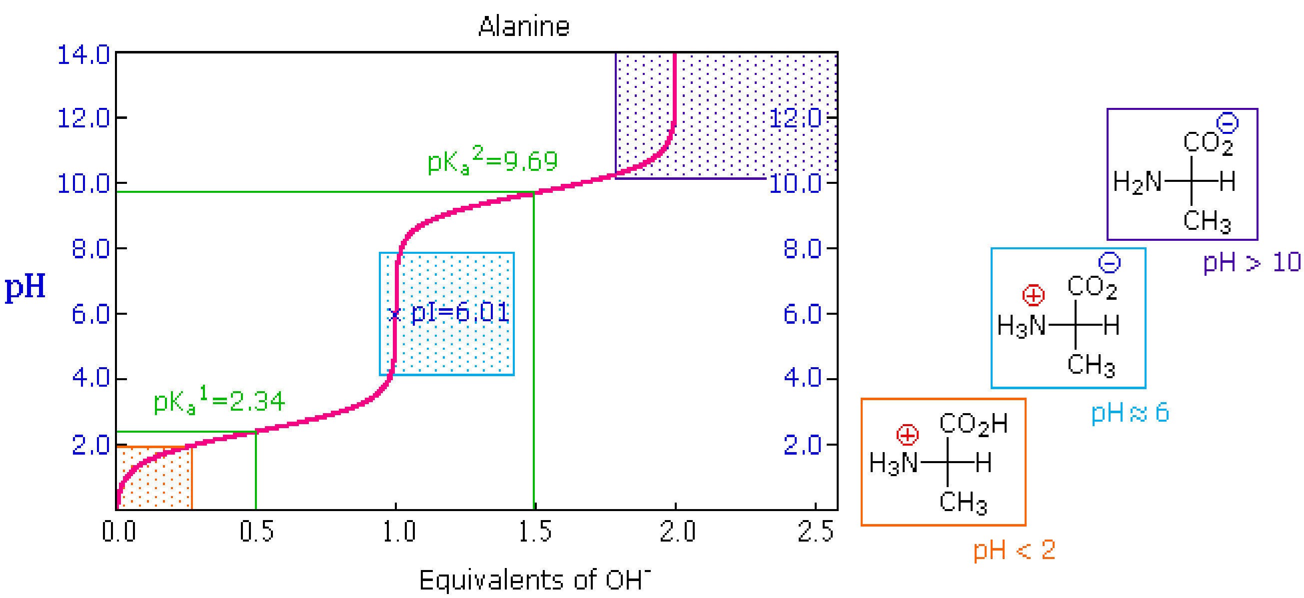
At the isoelectric point, an amino acid exists as a zwitterion:

- the **carboxyl** group **has donated** a proton to the **amino** group which form a positive NH_3^+ ion.

A **zwitterion** is a dipolar ion with both positive and negative charges in different parts of the molecule.



The titration curve for alanine

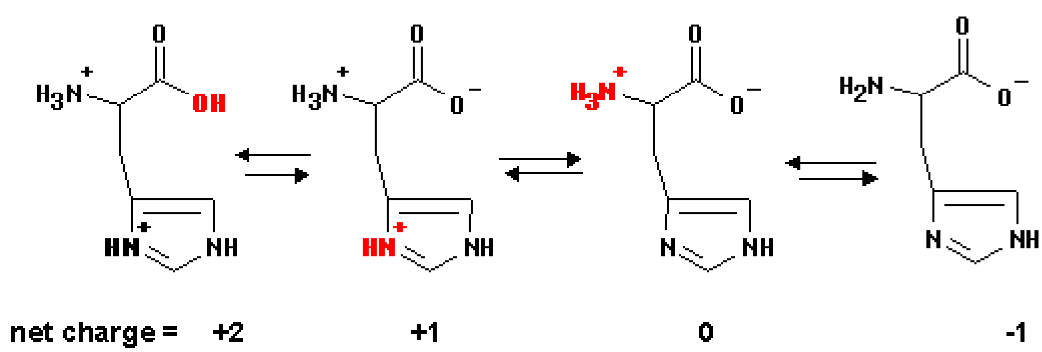


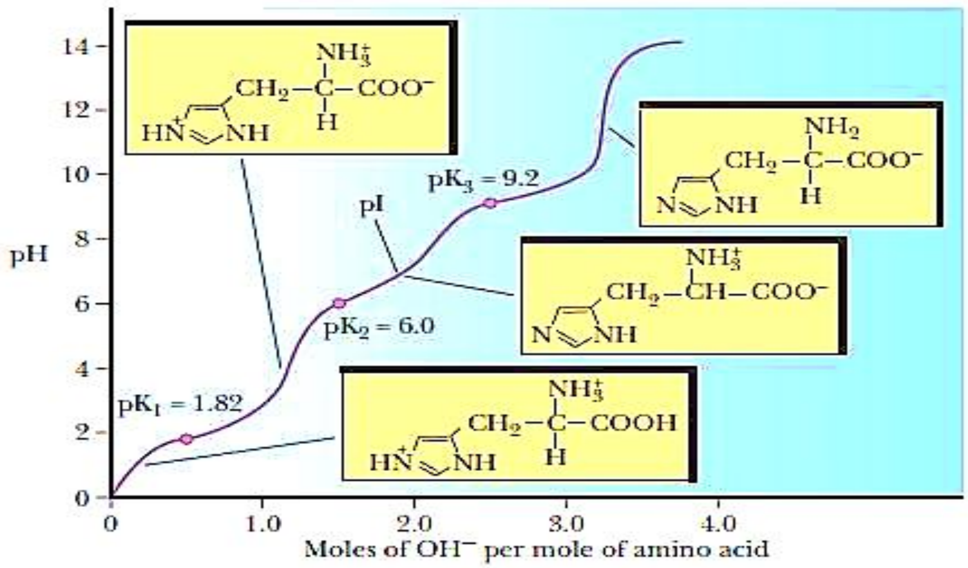
The isoelectric point (pI) of an amino acid is the pH of an aqueous solution of an amino acid (or peptide) at which the molecules on average have no net charge or have an average net charge of zero. Therefore, does not migrate in an electric field.

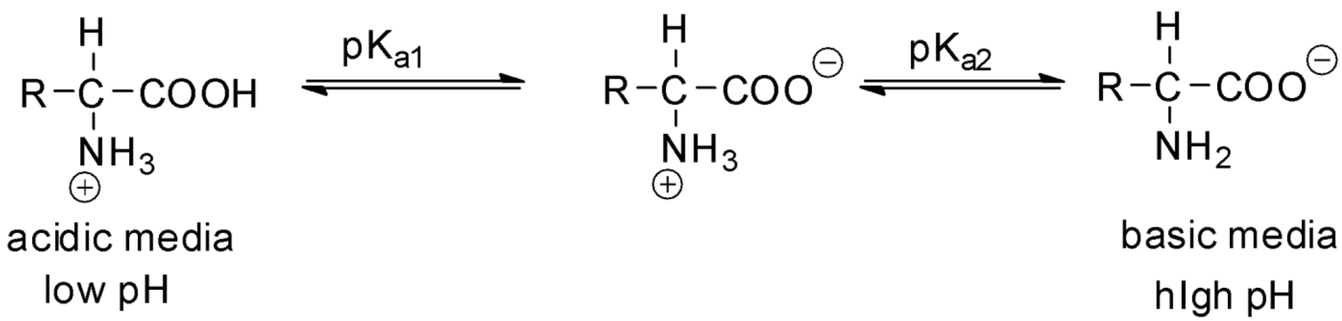
At a pH lower than 2, both the carboxylate and amine functions are protonated, so the alanine molecule has a net positive charge. At a pH greater than 10, the amine exists as a neutral base and the carboxyl as its conjugate base, so the alanine molecule has a net negative charge. At intermediate pH's the zwitterion concentration increases, and at a characteristic pH, called the **isoelectric point** (**pI**), the negatively and positively charged molecular species are present in equal concentration.

For simple amino acids such as alanine, the pI is an average of the pK_a's of the carboxyl (2.34) and ammonium (9.69) groups. Thus, the pI for alanine is calculated to be: (2.34 + 9.69)/2 = 6.02.

Histidine is a basic amino acid as it contains basic site at its side chain (R). General structure for amino acids is shown below.



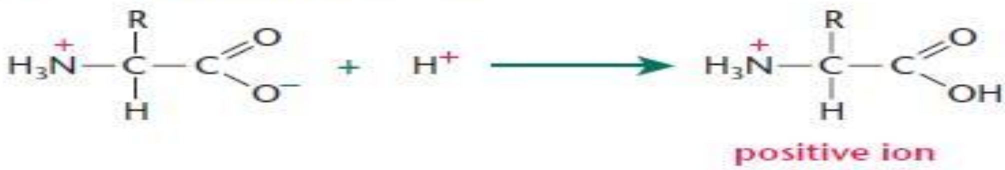

pH
Moles of OH⁻ per mole of amino acid
pK1 = 1.82
pK2 = 6.0
pK3 = 9.2
pI
0
2
4
6
8
10
12
14
0
1.0
2.0
3.0
4.0


pKa1
pKa2
acidic media
low pH
basic media
hIgh pH

Amino acids as bases

In strongly acidic conditions a positive ion forms:

- an amino acid behaves as a base
- the COO^- ion gains a proton.



Amino acids as acids

In strongly alkaline conditions a negative ion forms:

- an amino acid behaves as an acid
- the NH_3^+ ion loses a proton.

➢ To calculate the isoelectric point of amino acids having other ionizable functional groups, we must also take into account the pKa of the additional functional group in the side chain. a. Indicate which pKa values must be used to calculate the pI of each of the following amino acids. Be sure to answer all parts.
- Aspartic acid:
- Lysine:
- Arginine:
- Histidine:
- Glutamic acid:
- Isoleucine:

For an acidic amino acid (one with an additional acidic OH group):

$$pI = \frac{pK_a(\alpha\text{-COOH}) + pK_a(\text{side COOH})}{2}$$

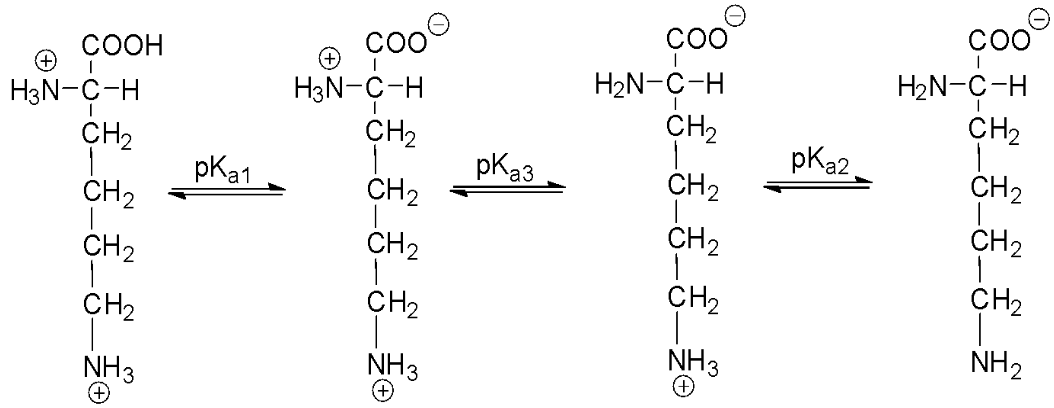
For a basic amino acid (one with an additional basic NH group):

$$pI = \frac{pK_a(\alpha\text{-NH}_3^+) + pK_a(\text{side NH})}{2}$$

b. In general, how does the pI of an acidic amino acid compare to that of a neutral amino acid?

c. In general, how does the pI of a basic amino acid compare to that of a neutral amino acid?

PI for lysine, we can write:



Net charge: **+2** **+1** **0** **-1**

Since the isoelectric point is given by the average of the pKa values that involve the zwitterion, so we can write the formula for lysine as: Now, for lysine, the pKa1 is equal to 2.18, pKa2 is equal to 8.95 and pKa3 is equal to 10.53.

Therefore, by replacing the equation by these values we will get, the isoelectric point for lysine is:

$$\mathbf{pI = \frac{pKa_2 + pKa_3}{2} = \frac{8.95 + 10.53}{2} = 9.74}$$