Physical Biochemistry Lab

Experiment no.:

Titration curve of amino acids

Objective:

To study the acidic and basic properties of amino acids through its titration curve and determine pKa values to recognize the unknown amino acid.
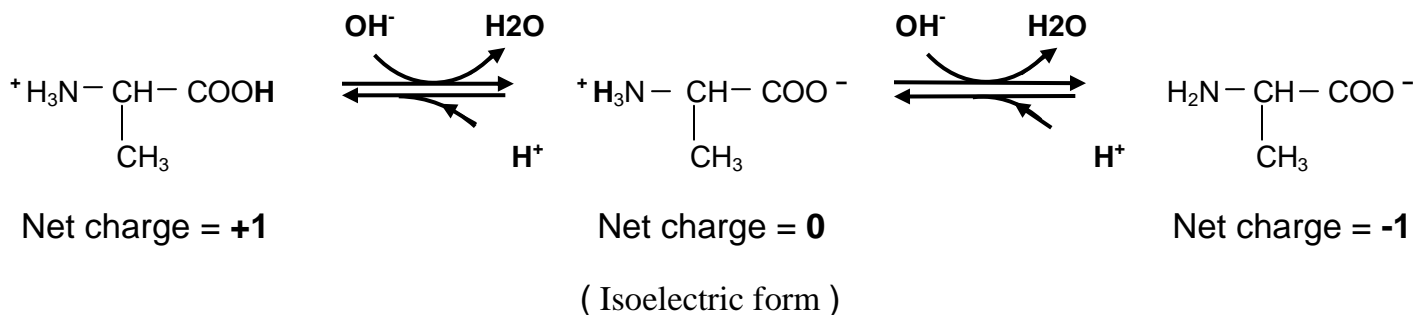
Introduction and principle:

Amino acids are organic compounds containing both an amino group (- NH_3) and a carboxyl group (- COOH).

$$H_2N-CH-COOH$$
$$|$$
$$R$$
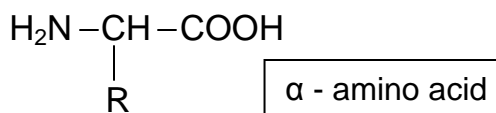
α - amino acid

Each of these amino acids has a side chain with distinctive chemical properties. Amino acids are grouping into five main classes based on the properties of their R group. These classes are non-polar aliphatic R groups, aromatic R groups, polar uncharged R groups, positively charged (basic) R group, and negatively charged (acidic) R groups. When amino acid is dissolved in water, it exists in solution as the dipolar ion, or "zwitterion".

$$^+H_3N-CH-COO^-$$
$$|$$
$$R$$
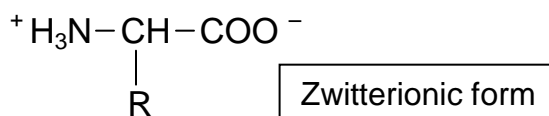
Zwitterionic form

A zwitterion can act as either an acid (proton donor) or a base (proton acceptor).

$^+H_3N-CH-COOH$, CH_3 ⇌ (OH⁻, H2O / H⁺) $^+H_3N-CH-COO^-$, CH_3 ⇌ (OH⁻, H2O / H⁺) $H_2N-CH-COO^-$, CH_3

Net charge = **+1**   Net charge = **0**   Net charge = **-1**

(Isoelectric form)

Substances having this dual nature are amphoteric. So, amino acids in aqueous solution are exist predominantly in isoelectric form. The characteristic pH at which the net electric charge is zero is called the isoelectric point (pI). So, an amino acid has a net negative charge at any pH above its pI, and has a net positive charge at any pH below its pI. Each amino acid has its own pI value. The Ionizable groups of amino acids act as weak acids or bases, giving off or taking on protons when the pH is altered. Simply, common amino acids are weak polyprotic acids.

Titration curves are produced by monitoring the pH of given volume of a sample solution after successive addition of acid or alkali. Titration curves are usually plots of pH against the volume of titrant added or more correctly against the number of equivalents added per mole of the sample.

Upon titration of amino acid with acid, it acts as a base, and upon titration with base, it acts as an acid.

In this experiment, the amino acid represents either the conjugate base A- or the conjugate acid HA from in the Henderson-Hasselbalch equation, depending on the titration.

For particular Ionizable group
$$pH = pKa + log\ \frac{unprotonated\ \ form\,(conjugate\ \ base)}{protonated\ \ form\,(conjugate\ \ acid)}$$

pKa for particular Ionizable group is equal the pH at which the Ionizable group is at its best buffering capacity. Considering applying the Henderson-Hasselbalch equation to the titration of glycine with acid and base, glycine has two Ionizable groups: a carboxyl group and an amino group, with pKa values of 2.34 and 9.6 respectively. In water at pH 6, glycine exists as a dipolar ion, or zwitterion, in which the carboxyl group is unprotonated ($-COO^-$) and the amino group is protonated to give the substituted ammonium ion ($-NH_3^+$). Addition of the acid to the solution lowers the pH rapidly at first and then more slowly as the buffering action of the carboxyl is exerted. At pH 2.34 the pKa is reached, one-half the acid has been consumed, and the carboxyl group is half ionized and the most effective as a buffer. Titration of the amino group with base follows a similar curve into the alkali region. The intersection between the titration of the carboxyl group and the titration of the amino group describes in this case the point at which glycine has no net charge, and is called the isoelectric point (pI). For simple amino acids such as glycine, the pI is an average of the pKa's of the carboxyl (2.34) and ammonium (9.6) groups. Thus the pI for glycine is calculated to be: (2.34 + 9.6) / 2 = 5.97 . If additional acidic or basic groups are present as side-chain functions, the pI is the average of the pKa's of the two most similar acids or bases.
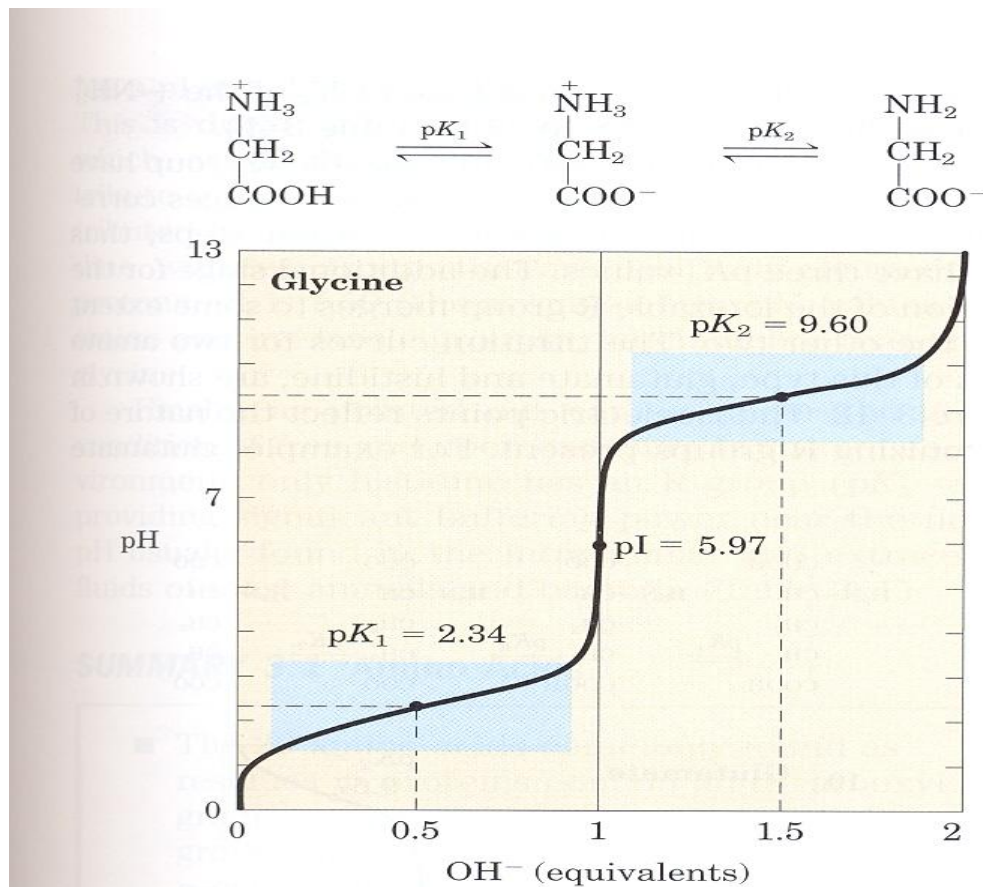
FIGURE 3–10 **Titration of an amino acid.** Shown here is the titration curve of 0.1 M glycine at 25 °C. The ionic species predominating at key points in the titration are shown above the graph. The shaded boxes, centered at about $pK_1 = 2.34$ and $pK_2 = 9.60$, indicate the regions of greatest buffering power.

Most amino acids contain carboxyl and amino groups having pKa values similar to those of glycine. In addition to these groups, some amino acids contain other Ionizable groups, which introduce other "steps" or pKa values into their titration curves.
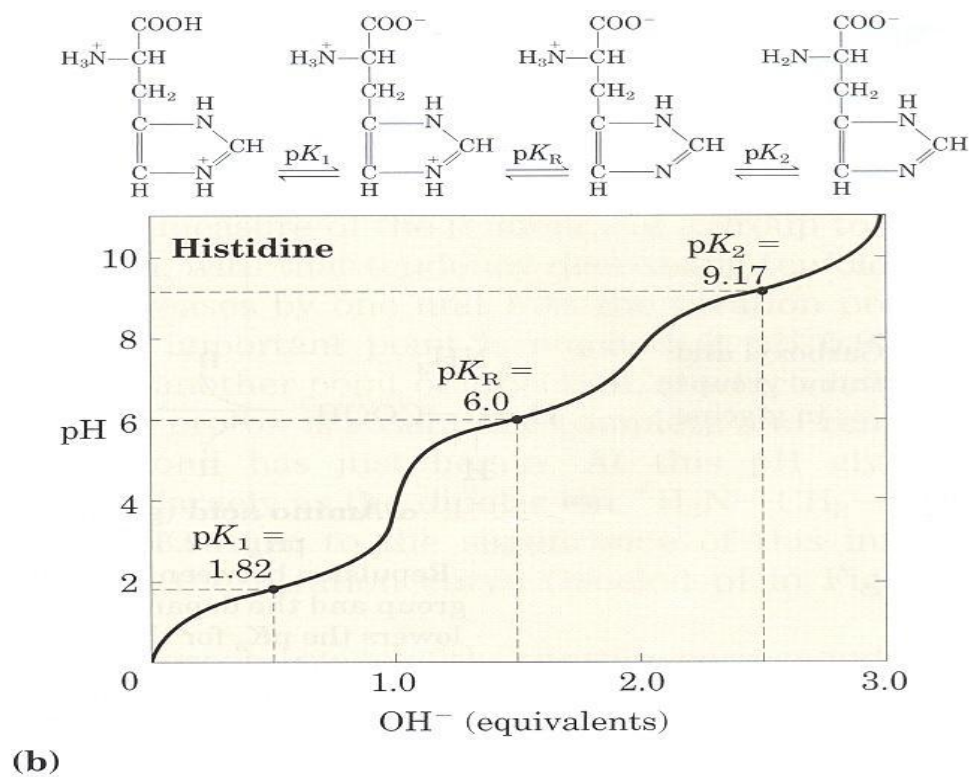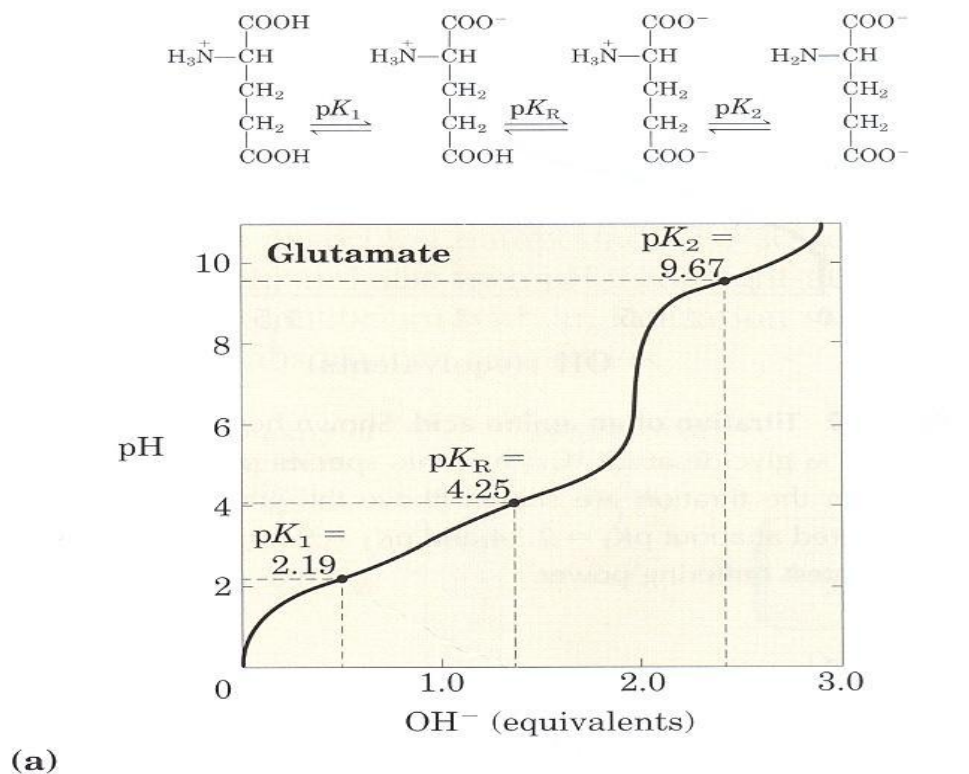
FIGURE 3–12 Titration curves for **(a)** glutamate and **(b)** histidine. The pK$_a$ of the R group is designated here as pK$_R$.

TABLE 3–1   Properties and Conventions Associated with the Common Amino Acids Found in Proteins

Amino acid	Abbreviation/ symbol	M_r	pK_1 (—COOH)	pK_2 (—NH$_3^+$)	pK_R (R group)	pI	Hydropathy index*	Occurrence in proteins (%)†
Nonpolar, aliphatic R groups								
Glycine	Gly G	75	2.34	9.60		5.97	−0.4	7.2
Alanine	Ala A	89	2.34	9.69		6.01	1.8	7.8
Proline	Pro P	115	1.99	10.96		6.48	1.6	5.2
Valine	Val V	117	2.32	9.62		5.97	4.2	6.6
Leucine	Leu L	131	2.36	9.60		5.98	3.8	9.1
Isoleucine	Ile I	131	2.36	9.68		6.02	4.5	5.3
Methionine	Met M	149	2.28	9.21		5.74	1.9	2.3
Aromatic R groups								
Phenylalanine	Phe F	165	1.83	9.13		5.48	2.8	3.9
Tyrosine	Tyr Y	181	2.20	9.11	10.07	5.66	−1.3	3.2
Tryptophan	Trp W	204	2.38	9.39		5.89	−0.9	1.4
Polar, uncharged R groups								
Serine	Ser S	105	2.21	9.15		5.68	−0.8	6.8
Threonine	Thr T	119	2.11	9.62		5.87	−0.7	5.9
Cysteine	Cys C	121	1.96	10.28	8.18	5.07	2.5	1.9
Asparagine	Asn N	132	2.02	8.80		5.41	−3.5	4.3
Glutamine	Gln Q	146	2.17	9.13		5.65	−3.5	4.2
Positively charged R groups								
Lysine	Lys K	146	2.18	8.95	10.53	9.74	−3.9	5.9
Histidine	His H	155	1.82	9.17	6.00	7.59	−3.2	2.3
Arginine	Arg R	174	2.17	9.04	12.48	10.76	−4.5	5.1
Negatively charged R groups								
Aspartate	Asp D	133	1.88	9.60	3.65	2.77	−3.5	5.3
Glutamate	Glu E	147	2.19	9.67	4.25	3.22	−3.5	6.3

*A scale combining hydrophobicity and hydrophilicity of R...

Table 1-3   Aspartic Acid

	Predominant Ionic Form at Different Positions along the Titration Curve						
Ionizable Group	Start	pK_{a_1}	First Equivalence	pK_{a_2}	Second Equivalence	pK_{a_3}	Third Equivalence
α-carboxyl	COOH	COOH($\frac{1}{2}$) COO$^-$($\frac{1}{2}$)	COO$^-$	COO$^-$	COO$^-$	COO$^-$	COO$^-$
β-carboxyl	COOH	COOH	COOH	COOH($\frac{1}{2}$) COO$^-$($\frac{1}{2}$)	COO$^-$	COO$^-$	COO$^-$
α-amino	NH$_3^+$	NH$_3^+$	NH$_3^+$	NH$_3^+$	NH$_3^+$	NH$_3^+$($\frac{1}{2}$) NH$_2$($\frac{1}{2}$)	NH$_2$
Net charge	+1	+$\frac{1}{2}$	0	−$\frac{1}{2}$	−1	−1$\frac{1}{2}$	−2

Table 1-4   Lysine

	Predominant Ionic Form at Different Positions along the Titration Curve						
Ionizable Group	Start	pK_{a_1}	First Equivalence	pK_{a_2}	Second Equivalence	pK_{a_3}	Third Equivalence
α-carboxyl	COOH	COOH($\frac{1}{2}$) COO$^-$($\frac{1}{2}$)	COO$^-$	COO$^-$	COO$^-$	COO$^-$	COO$^-$
α-amino	NH$_3^+$	NH$_3^+$	NH$_3^+$	NH$_3^+$($\frac{1}{2}$) NH$_2$($\frac{1}{2}$)	NH$_2$	NH$_2$	NH$_2$
ε-amino	NH$_3^+$	NH$_3^+$	NH$_3^+$	NH$_3^+$	NH$_3^+$	NH$_3^+$($\frac{1}{2}$) NH$_2$($\frac{1}{2}$)	NH$_2$
Net charge	+2	+1$\frac{1}{2}$	+1	+$\frac{1}{2}$	0	−$\frac{1}{2}$	−1

Figures 3-10, 2-12, and table 3-1 are taken from Lehninger principles of biochemistry by Cox & Nelson, fourth edition. Table 1-3, and 1-4 are taken from Biochemical calculations by Segel, second edition.

Materials and apparatus:

1. 0.1 M NaOH solution
2. 0.1 M HCl solution
3. 0.1 M unknown amino acid A in form of zwitterion
4. 0.1 M unknown amino acid B in form of full protonated
5. Distilled water
6. pH meter
7. burette
8. conical flask
9. pipette 10 ml
10. pipette bulb
11. funnel

Methods:

1) Pipette 10 ml of amino acid solution (A) into a 200 ml conical flask. Add to it 10 ml of distilled water. Mix well and determine the pH of the solution. Titrate this mixture with 0.1 M HCl solution by adding aliquot volume of 1 ml each time and read the pH value. Run on the titration until the pH reach to 1.6 . Plot the titration curve (pH verses titrant in ml).

2) Repeat the titration for amino acid solution (A) using 0.1 M NaOH solution as a titrant. Run on the titration until the pH reach to 12.5 .

3) Pipette 10 ml of amino acid solution (B) into a 200 ml conical flask. Add to it 10 ml distilled water. Mix well and determine the pH of the solution. Titrate this mixture with 0.1 M NaOH solution by adding aliquot volume of 1 ml each time and reading the pH value. Run on the titration until the pH reach to 10.8 . Plot the titration curve (pH versus titrant in ml).

Results: Amino acid (A)

Volume of 0.1 M HCl added (ml)	pH	Volume of 0.1 M NaOH added (ml)	pH

Amino acid (B)

Volume of 0.1 M NaOH added (ml)	pH

Note: Mix well after each addition of titrant, don't remain the pH meter electrode inside the flask during addition of titrant, and never use the electrode for mixing.

Question:
1) In the titration of amino acids you have made, mark the points on the curve and diagram the chemical structure of amino acid composition of the flask in case of:
    a) In form of fully protonated
    b) In the solution of pKa_1 point
    c) In the solution of pI point
    d) In the solution of pKa_2 point
    e) In the solution of pKa_3 point (if applicable)
    f) In form of unprotonated.

➢ Amino acid A is arginine, and amino acid B is glycine.