

Understanding the Nature of Amino Acids

Ionization of Water

Water dissociates into hydronium (H_3O^+) or (H^+) and hydroxide (OH^-). We write the equilibrium as



The equilibrium constant K_{eq} of this dissociation is given by

$$K_{\text{eq}} = \frac{[\text{H}^+][\text{OH}^-]}{[\text{H}_2\text{O}]}$$

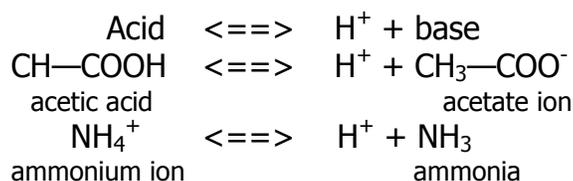
in which the terms in brackets denote molar concentrations. Because the concentration of water is changed little by ionization, expression can be simplified to give

$$K_w = [\text{H}^+][\text{OH}^-] = 1 \times 10^{-14}$$

in which K_w is the ion product of water at 25°C . Note that the concentrations of $[\text{H}^+]$ and $[\text{OH}^-]$ are reciprocally related. If the concentration of H^+ is high and the concentration of OH^- must be low, and vice versa. For example if $[\text{H}^+] = 10^{-4} \text{M}$, then $[\text{OH}^-] = 10^{-10} \text{M}$.

Definition of Acid and Base

An acid is a proton donor. A base is a proton acceptor.



The species formed by the ionization of an acid is its conjugate base. Conversely, protonation (addition of a hydrogen ion) of a base yields its conjugate acid. Acetic acid and acetate ion are a conjugate acid-base pair.

Definition of pH and pK

The pH of a solution is a measure of its concentration of H^+ . The pH is defined as

$$\text{pH} = -\log [\text{H}^+]$$

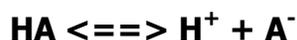
The pOH of a solution is a measure of its concentration of OH^- . The pOH is defined as

$$\text{pOH} = -\log [\text{OH}^-]$$

$$\text{pH} + \text{pOH} = 14$$

Note that $\text{pH} + \text{pOH}$ always equals 14.

A strong acid and strong base always disassociate. The ionization equilibrium of a weak acid is given by



The apparent equilibrium constant K for this ionization is

$$K = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]}$$

Both $[\text{H}^+]$ $[\text{A}^-]$ are equal values since when a weak acid ionizes, half will be of each species.

Inspection of this equation shows that the pK of an acid is the pH at which it is half dissociated. The relationship between pH and the ratio of acid to base is shown by deriving the above equation...

Henderson-Hasselbalch Equation

$$\text{pH} = \text{p}K + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

The pH of a solution can be calculated from the above equation if the molar proportion of A^- (or H^+) to HA and the pK of HA are known. Consider a solution of 0.1 M acetic acid and 0.2 M acetate ion. The pK of acetic acid is 4.8. Determine the pH of the solution below.

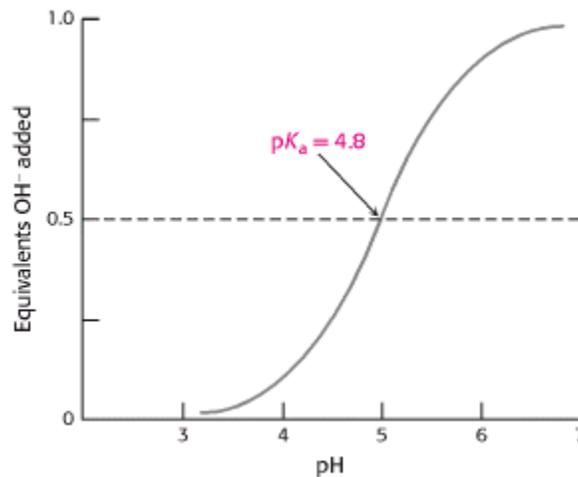
Conversely, the pK of an acid can be calculated if the molar proportion of A^- (or H^+) to HA and the pH of the solution are known.

Buffering Power

An acid-base conjugate pair (such as acetic acid and acetate ion) has an important property: it resists changes in the pH of a solution. In other words, it acts as a buffer. Consider the addition of OH⁻ to a solution of acetic acid (HA):



A plot of the dependence of the pH of this solution on the amount of OH⁻ added is called a *titration curve*. Note that there is an inflection point in the curve at pH 4.8, which is the p*K* of acetic acid. In the vicinity of this pH, a relatively large amount of OH⁻ produces little change in pH. **In general, an acid is most effective in buffering against pH change in the vicinity of its p*K* value.**



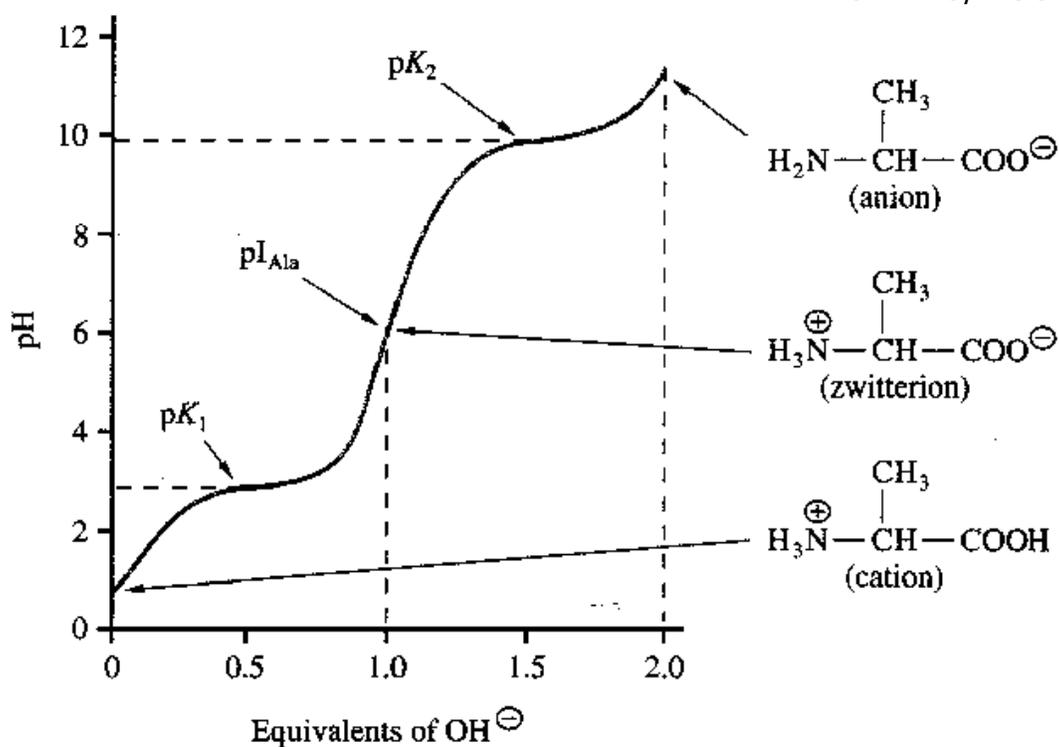
p*K* Values of Amino Acids

An amino acid such as alanine contains two ionizable groups: an α -carboxyl group and a protonated amino group. As base is added (as the pH increases), these two groups are titrated. The p*K* of the α -COOH group is 2.3, whereas that of the α -NH₃⁺ group is 9.9. The values of these groups in other amino acids are similar. Some amino acids, such as aspartic acid, also contain ionizable side chains (R). These contribute highly to the overall net charge and final shape of the protein.

In a pH below the p*K* of the ionizable groups, these groups are protonated. In a pH above the p*K* of these groups, they lose their H⁺. At a pH at the p*K* values of these groups, **half of the groups have a hydrogen associated with them (protonated) and half do not.** *At different pH values, the charge on the amino acid species varies—which in a protein, can affect its shape both locally and globally!*

The **isoelectric point** is the pH at which a molecule carries no charge. To calculate this for a diprotonated amino acids, for an amino acid with only one amine and one carboxyl group, the pI can be calculated from the p*K*s of this molecule in the formula below. See the titration curve for alanine on the next page for a nice look at the ions.

$$\text{pI} = \frac{\text{p}K_1 + \text{p}K_2}{2}$$



pK and pI Values of Amino Acids

Name	pK			pI at 25°C
	pK α-COOH	pK α-NH ₃	pK R-group	
Alanine	2.35	9.87		6.11
Arginine	2.18	9.09	13.2	10.76
Asparagine	2.18	9.09	13.2	10.76
Aspartic Acid	1.88	9.60	3.65	2.98
Cysteine	1.71	10.78	8.33	5.02
Glutamic Acid	2.19	9.67	4.25	3.08
Glutamine	2.17	9.13		5.65
Glycine	2.34	9.60		6.06
Histidine	1.78	8.97	5.97	7.64
Isoleucine	2.32	9.76		6.04
Leucine	2.36	9.60		6.04
Lysine	2.20	8.90	10.28	9.47
Methionine	2.28	9.21		5.74
Phenylalanine	2.58	9.24		5.91
Proline	1.99	10.60		6.30
Serine	2.21	9.15		5.68
Threonine	2.15	9.12		5.60
Tryptophan	2.38	9.39		5.88
Tyrosine	2.20	9.11	10.07	5.63
Valine	2.29	9.74		6.02

MOLE BIO/BIOCHEMISTRY

$\begin{array}{c} \text{H} \\ \\ \text{H}_3\text{N}^+ - \text{C} - \text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{O} \end{array} \\ \\ (\text{CH}_2)_3 \\ \\ \text{NH} \\ \\ \text{C}=\text{NH}_2 \\ \\ \text{NH}_2 \end{array}$ <p>Arginine (Arg / R)</p>	$\begin{array}{c} \text{H} \\ \\ \text{H}_3\text{N}^+ - \text{C} - \text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{O} \end{array} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{C}=\text{O} \\ \\ \text{NH}_2 \end{array}$ <p>Glutamine (Gln / Q)</p>	$\begin{array}{c} \text{H} \\ \\ \text{H}_3\text{N}^+ - \text{C} - \text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{O} \end{array} \\ \\ \text{CH}_2 \\ \\ \text{C}_6\text{H}_5 \end{array}$ <p>Phenylalanine (Phe / F)</p>	$\begin{array}{c} \text{H} \\ \\ \text{H}_3\text{N}^+ - \text{C} - \text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{O} \end{array} \\ \\ \text{CH}_2 \\ \\ \text{C}_6\text{H}_4 \\ \\ \text{OH} \end{array}$ <p>Tyrosine (Tyr / Y)</p>	$\begin{array}{c} \text{H} \\ \\ \text{H}_3\text{N}^+ - \text{C} - \text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{O} \end{array} \\ \\ \text{CH}_2 \\ \\ \text{C}_8\text{H}_6\text{N} \\ \\ \text{H} \end{array}$ <p>Tryptophan (Trp, W)</p>
$\begin{array}{c} \text{H} \\ \\ \text{H}_3\text{N}^+ - \text{C} - \text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{O} \end{array} \\ \\ (\text{CH}_2)_4 \\ \\ \text{NH}_2 \end{array}$ <p>Lysine (Lys / K)</p>	$\begin{array}{c} \text{H} \\ \\ \text{H}_3\text{N}^+ - \text{C} - \text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{O} \end{array} \\ \\ \text{H} \end{array}$ <p>Glycine (Gly / G)</p>	$\begin{array}{c} \text{H} \\ \\ \text{H}_3\text{N}^+ - \text{C} - \text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{O} \end{array} \\ \\ \text{CH}_3 \end{array}$ <p>Alanine (Ala / A)</p>	$\begin{array}{c} \text{H} \\ \\ \text{H}_3\text{N}^+ - \text{C} - \text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{O} \end{array} \\ \\ \text{CH}_2 \\ \\ \text{C}_4\text{H}_3\text{N}_2 \end{array}$ <p>Histidine (His / H)</p>	$\begin{array}{c} \text{H} \\ \\ \text{H}_3\text{N}^+ - \text{C} - \text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{O} \end{array} \\ \\ \text{CH}_2 \\ \\ \text{OH} \end{array}$ <p>Serine (Ser / S)</p>
$\begin{array}{c} \text{H}_2 \\ \\ \text{C} \\ / \quad \backslash \\ \text{H}_2\text{C} \quad \text{CH}_2 \\ \backslash \quad / \\ \text{H}_2\text{N}^+ - \text{C} - \text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{O} \end{array} \end{array}$ <p>Proline (Pro / P)</p>	$\begin{array}{c} \text{H} \\ \\ \text{H}_3\text{N}^+ - \text{C} - \text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{O} \end{array} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{COOH} \end{array}$ <p>Glutamic Acid (Glu / E)</p>	$\begin{array}{c} \text{H} \\ \\ \text{H}_3\text{N}^+ - \text{C} - \text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{O} \end{array} \\ \\ \text{CH}_2 \\ \\ \text{COOH} \end{array}$ <p>Aspartic Acid (Asp / D)</p>	$\begin{array}{c} \text{H} \\ \\ \text{H}_3\text{N}^+ - \text{C} - \text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{O} \end{array} \\ \\ \text{H} - \text{C} - \text{OH} \\ \\ \text{CH}_3 \end{array}$ <p>Threonine (Thr / T)</p>	$\begin{array}{c} \text{H} \\ \\ \text{H}_3\text{N}^+ - \text{C} - \text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{O} \end{array} \\ \\ \text{CH}_2 \\ \\ \text{SH} \end{array}$ <p>Cysteine (Cys / C)</p>
$\begin{array}{c} \text{H} \\ \\ \text{H}_3\text{N}^+ - \text{C} - \text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{O} \end{array} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{S} \\ \\ \text{CH}_3 \end{array}$ <p>Methionine (Met / M)</p>	$\begin{array}{c} \text{H} \\ \\ \text{H}_3\text{N}^+ - \text{C} - \text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{O} \end{array} \\ \\ \text{CH}_2 \\ \\ \text{CH} \\ / \quad \backslash \\ \text{CH}_3 \quad \text{CH}_3 \end{array}$ <p>Leucine (Leu / L)</p>	$\begin{array}{c} \text{H} \\ \\ \text{H}_3\text{N}^+ - \text{C} - \text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{O} \end{array} \\ \\ \text{CH}_2 \\ \\ \text{C}=\text{O} \\ \\ \text{NH}_2 \end{array}$ <p>Asparagine (Asn / N)</p>	$\begin{array}{c} \text{H} \\ \\ \text{H}_3\text{N}^+ - \text{C} - \text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{O} \end{array} \\ \\ \text{HC} - \text{CH}_3 \\ \\ \text{CH}_2 \\ \\ \text{CH}_3 \end{array}$ <p>Isoleucine (Ile / I)</p>	$\begin{array}{c} \text{H} \\ \\ \text{H}_3\text{N}^+ - \text{C} - \text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{O} \end{array} \\ \\ \text{CH} \\ / \quad \backslash \\ \text{CH}_3 \quad \text{CH}_3 \end{array}$ <p>Valine (Val / V)</p>

Practice Problems

1. Calculate the pH of the following solutions: (HINT—NaOH and HCl are a **strong** base and **strong** acid)

a) 0.001 M HCl

b) 0.01 M NaOH

c) 1×10^{-5} M HCl

d) 0.03 M HCl

e) 3.4×10^{-3} M NaOH

2) What is the actual hydrogen ion concentration in the following fluids:

a) Lemon juice (pH 2.3)

b) Coca-cola (pH 2.8)

c) Saliva (pH 6.5)

d) gastric juice (pH 1.8)

e) milk (pH 6.7)

3) At what hydrogen ion concentration will an amino acid having a pK_{COOH} of 2.48 and a pK_{NH_3} of 9.42 remain stationary in an electric field?

- 4) It is desired to prepare a solution which can serve as a buffer at pH 4.0. Which of the following would be best for this purpose? Why?
- a) propionic acid (pK 4.67)
 - b) lactic acid (pK 3.86)
 - c) phosphoric acid (pK_{COOH} 2.14; pK_{NH_3} 7.20)
- 5) A mixture of alanine, valine, aspartic acid, threonine, and lysine at pH 6.0 was subjected to paper electrophoresis.
- a) Which amino acid(s) moved furthest toward the anode?
 - b) Which amino acid(s) moved furthest toward the cathode?
 - c) Which amino acid(s) remained at or near the origin?
- 6) Write the structure of the following peptide chain at a pH of 6.0. What is the net charge of this tripeptide?

Alanine—Leucine—Glycine

- 7) What is the net charge of glutamic acid at pH 1, pH 4, pH 7, and pH 11. Draw all species and label where the charges (or lack thereof) are for all groups that can be ionized. (HINT: There are three!)