Amino Acids as Buffers

Acids

- According to the **Bronsted-Lowry** theory of acids and bases, an acid (HA) can donate a proton (H+) while a base (B) can accept a proton.
- An acid after losing a proton forms a conjugate base (A–), and the protonated base exists as conjugate acid (BH+).
- The dissociation of acid is expressed in terms of the equilibrium equation as:

$$HA \leftrightarrow H^+ + A^-$$

- This relationship can be described in terms of the equilibrium constant as:

pH = -log [H$^+$]

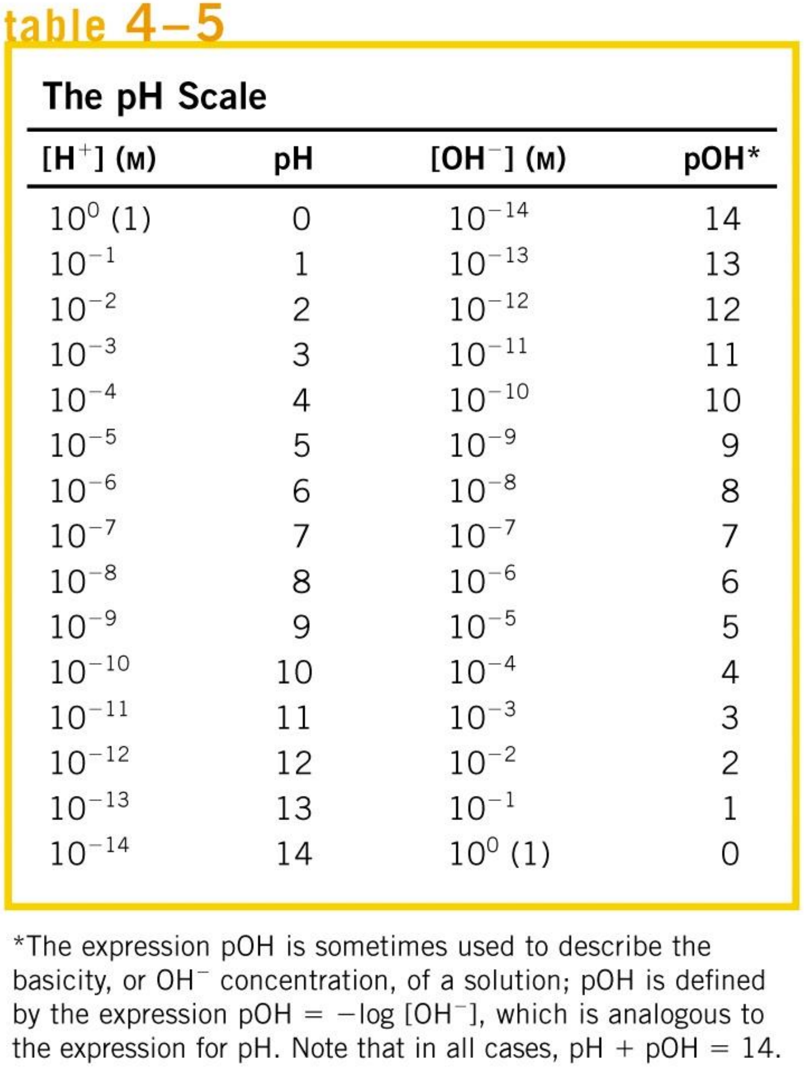
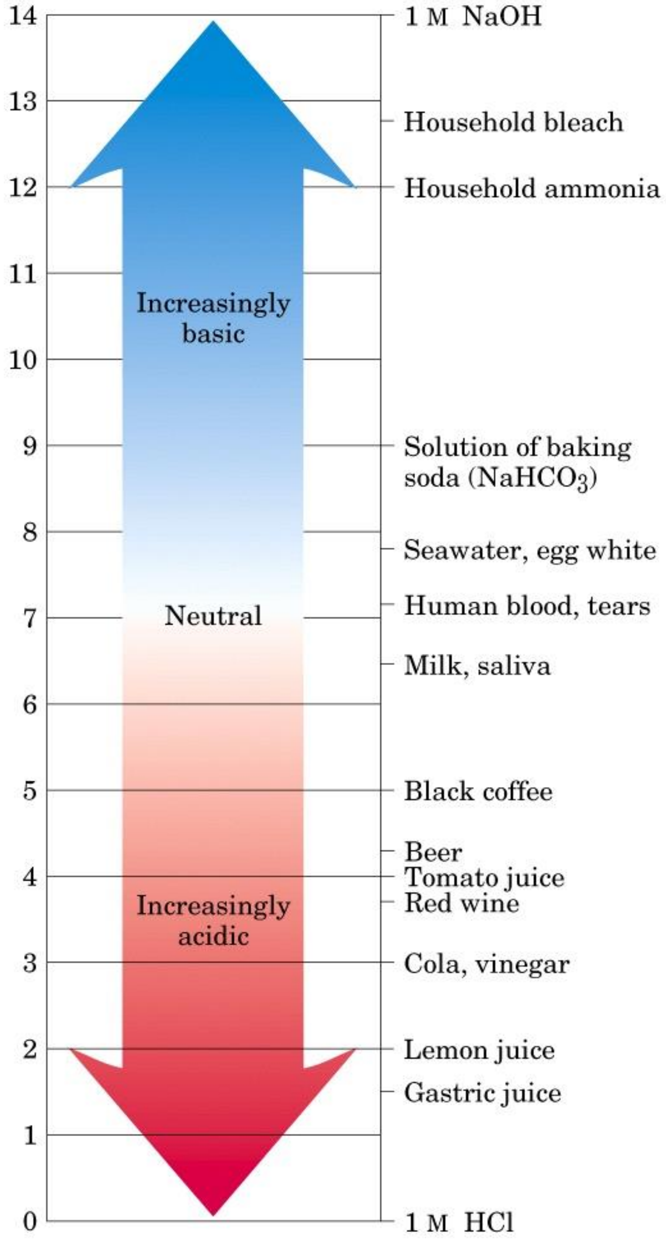


table 4–5

The pH Scale

[H$^+$] (M)	pH	[OH$^-$] (M)	pOH*
10^0 (1)	0	10^{-14}	14
10^{-1}	1	10^{-13}	13
10^{-2}	2	10^{-12}	12
10^{-3}	3	10^{-11}	11
10^{-4}	4	10^{-10}	10
10^{-5}	5	10^{-9}	9
10^{-6}	6	10^{-8}	8
10^{-7}	7	10^{-7}	7
10^{-8}	8	10^{-6}	6
10^{-9}	9	10^{-5}	5
10^{-10}	10	10^{-4}	4
10^{-11}	11	10^{-3}	3
10^{-12}	12	10^{-2}	2
10^{-13}	13	10^{-1}	1
10^{-14}	14	10^0 (1)	0

*The expression pOH is sometimes used to describe the basicity, or OH$^-$ concentration, of a solution; pOH is defined by the expression pOH = $-\log$ [OH$^-$], which is analogous to the expression for pH. Note that in all cases, pH + pOH = 14.



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

Now, taking negative log on both sides of the equation gives:

$$-\log K_a = -\log\frac{[\text{H}+][A-]}{[HA]}$$

OR

$$-\log K_a = -\log[\text{H}^+] + (-\log\frac{[A-]}{[HA]})$$

By definition,

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

Thus,

$$pK_a = \text{pH} - \log\frac{[A-]}{[HA]}$$

This equation is then arranged to form the Henderson Hasselbalch equation as:

$$\text{pH} = pK_a + \log\frac{[A-]}{[HA]}$$

Henderson-Hasselbalch Equation

- The **Henderson-Hasselbalch** equation is the equation commonly used in chemistry and biology to determine the pH of a solution.
- This equation shows a relationship between the pH or pOH of the solution, the pK_a or $pK_{b,}$ and the concentration of the chemical species involved.
- This equation was developed by L. J. **Henderson** and K. A. **Hasselbalch** to determine the pH of buffer systems.
- This equation can be considered as the backbone of acid-base physiology.
- This equation is commonly used to determine the amount of acid and conjugate base required to prepare a buffer of the desired pH.

Henderson-Hasselbalch Equation

$$\mathbf{pH = pKa + \log_{10} [conj\text{-}Base] / [conj\text{-}Acid]}$$

For acetic acid, if we know the pH and the pKa, we can calculate the proportion of

$COO^-/COOH$ (salt over acid). E.g.:

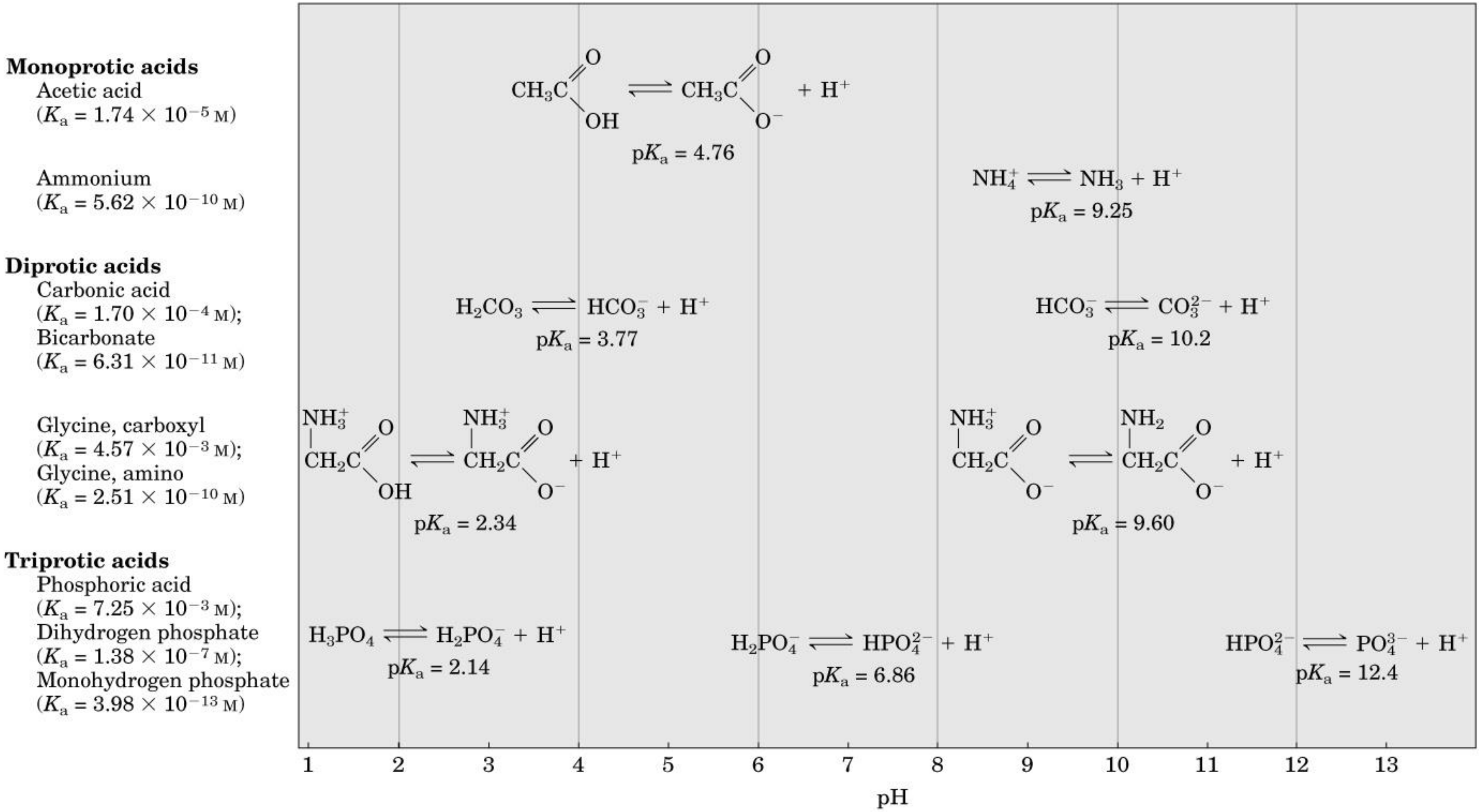
$$pH\ 5.5 = pKa\ 4.76 + \log_{10} [COO^-]/[COOH]$$

$$\text{Log}_{10} [COO^-]/[COOH] = 0.74$$

Taking antilogs (raising 10 to the 0.74 power) we find the ratio:

$$[COO^-]/[COOH) = 5.50/1$$

- (in other words there are 5.5 parts COO^- for 1 part COOH)

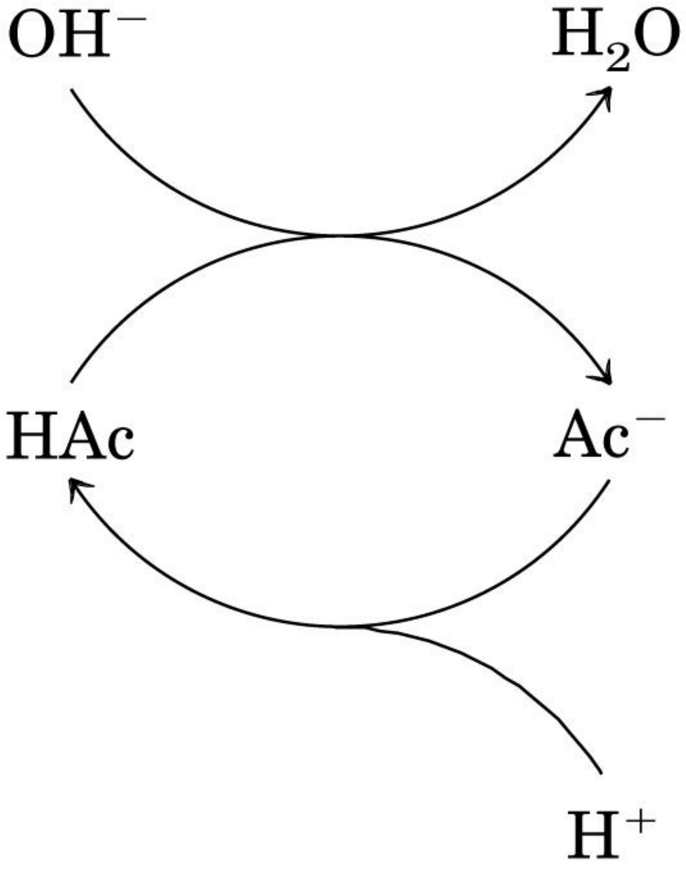


Monoprotic acids
Acetic acid
(K_a = 1.74 × 10^{-5} M)
$CH_3COOH \rightleftharpoons CH_3COO^- + H^+$
pK_a = 4.76
Ammonium
(K_a = 5.62 × 10^{-10} M)
$NH_4^+ \rightleftharpoons NH_3 + H^+$
pK_a = 9.25
Diprotic acids
Carbonic acid
(K_a = 1.70 × 10^{-4} M);
Bicarbonate
(K_a = 6.31 × 10^{-11} M)
$H_2CO_3 \rightleftharpoons HCO_3^- + H^+$
pK_a = 3.77
$HCO_3^- \rightleftharpoons CO_3^{2-} + H^+$
pK_a = 10.2
Glycine, carboxyl
(K_a = 4.57 × 10^{-3} M);
Glycine, amino
(K_a = 2.51 × 10^{-10} M)
$NH_3^+CH_2COOH \rightleftharpoons NH_3^+CH_2COO^- + H^+$
pK_a = 2.34
$NH_3^+CH_2COO^- \rightleftharpoons NH_2CH_2COO^- + H^+$
pK_a = 9.60
Triprotic acids
Phosphoric acid
(K_a = 7.25 × 10^{-3} M);
Dihydrogen phosphate
(K_a = 1.38 × 10^{-7} M);
Monohydrogen phosphate
(K_a = 3.98 × 10^{-13} M)
$H_3PO_4 \rightleftharpoons H_2PO_4^- + H^+$
pK_a = 2.14
$H_2PO_4^- \rightleftharpoons HPO_4^{2-} + H^+$
pK_a = 6.86
$HPO_4^{2-} \rightleftharpoons PO_4^{3-} + H^+$
pK_a = 12.4
1
2
3
4
5
6
7
8
9
10
11
12
13
pH

Buffers

- Ability of an acid-base mixture to resist sudden changes in pH is called its buffer action.
- Buffers work against sudden and large changes in the pH by:
  1. Releasing hydrogen ions (acting as acids) when the pH increases, and
  2. Binding hydrogen ions (acting as bases) when the pH decreases.

$$K_w = [H^+][OH^-]$$



OH^- H_2O

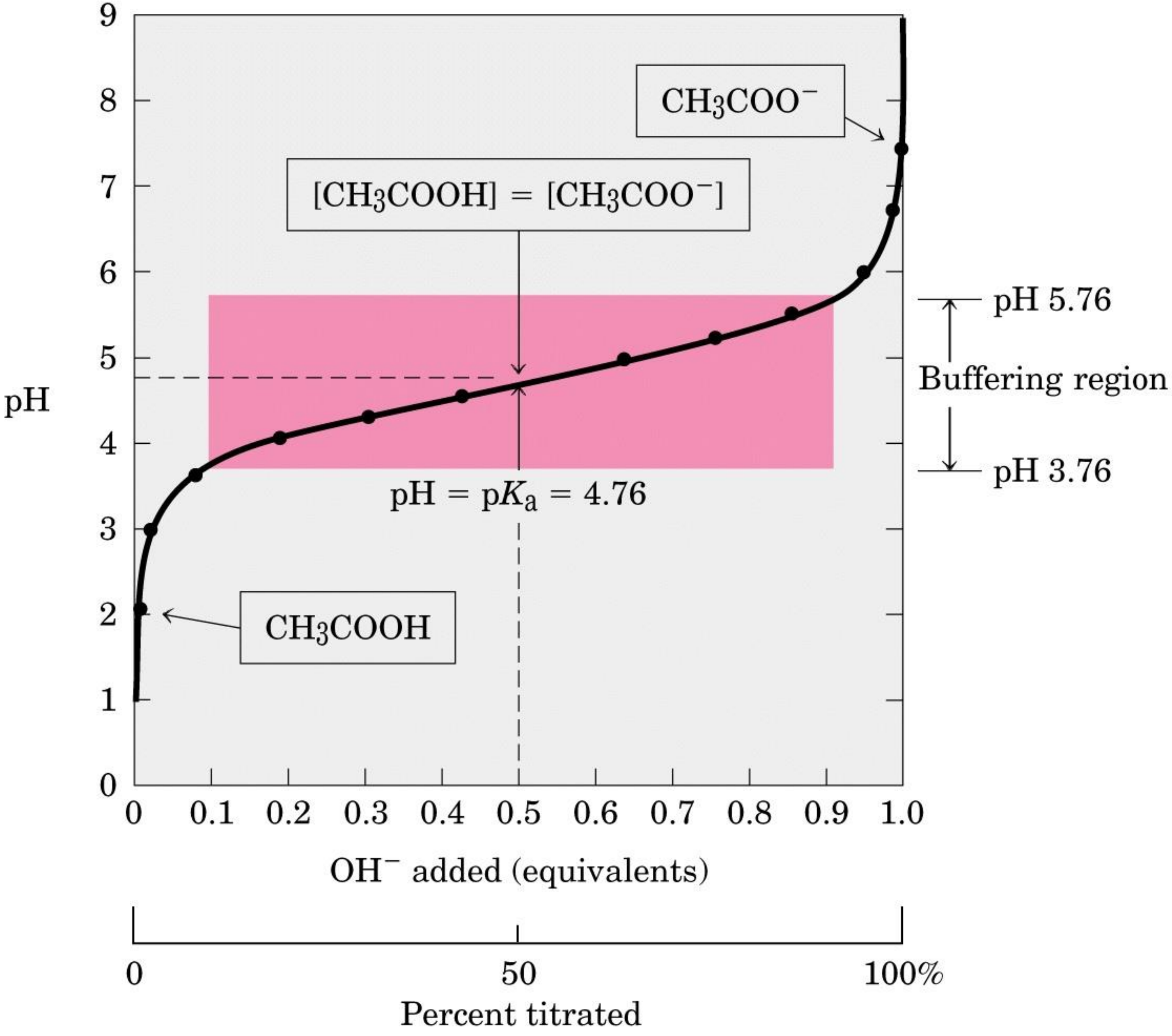
Acetic acid
(CH_3COOH)

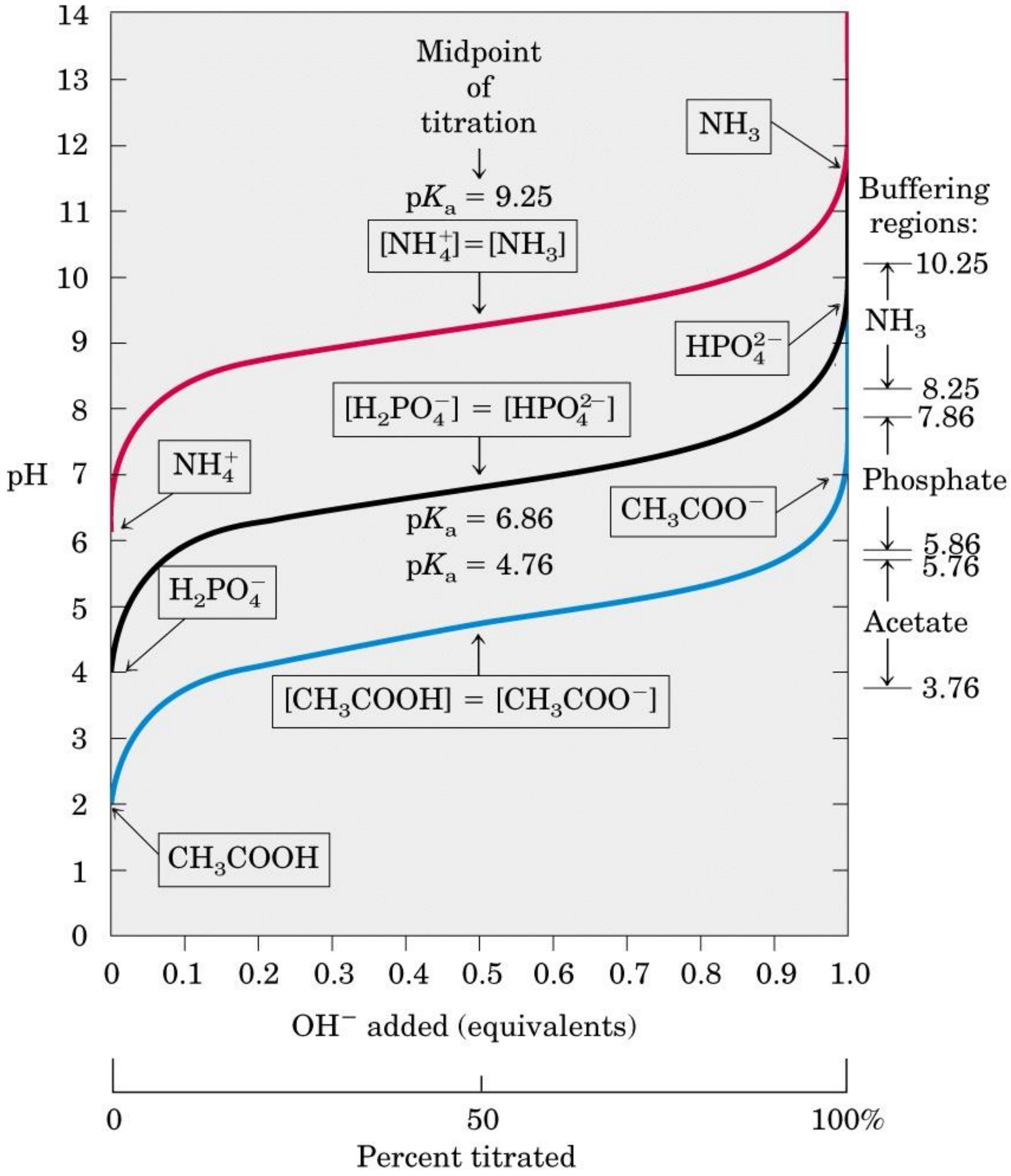
HAc Ac^-

Acetate
(CH_3COO^-)

H^+

$$K_a = \frac{[H^+][Ac^-]}{[HAc]}$$


9
8
7
6
5
4
3
2
1
0
pH
CH_3COO^-
$[CH_3COOH] = [CH_3COO^-]$
pH 5.76
Buffering region
pH 3.76
pH = pK_a = 4.76
CH_3COOH
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
OH^- added (equivalents)
0
50
100%
Percent titrated


Midpoint of titration
pK_a = 9.25
[NH_4^+] = [NH_3]
NH_3
NH_4^+
[$H_2PO_4^-$] = [HPO_4^{2-}]
HPO_4^{2-}
$H_2PO_4^-$
pK_a = 6.86
pK_a = 4.76
CH_3COO^-
[CH_3COOH] = [CH_3COO^-]
CH_3COOH
Buffering regions:
10.25
NH_3
8.25
7.86
Phosphate
5.86
5.76
Acetate
3.76
pH
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
OH^- added (equivalents)
0
50
100%
Percent titrated

$$\mathrm{pH} = \mathrm{p}K_\mathrm{a} + \log \frac{[\mathrm{A}^-]}{[\mathrm{HA}]}$$

The Power of Buffer Solutions

- A buffer solution is made of a mixture of 0.042 M NaH2PO4 and 0.058 M Na2HPO4 and has a pH of 7.0 (pKa is 6.86)

- If 1.0 mL of 10.0 N NaOH is added to a liter of this buffer, how much will the pH change?

- If 1.0 mL of 10.0 N NaOH is added to a liter of pure water at pH 7.0, what is the final pH?

The Power of Buffer Solutions

- Solution:

A liter of the buffer contains 0.042 mol of NaH2PO4. Adding 1.0 mL of 10.0N NaOH (0.010 mol) would titrate an equivalent amount (0.010 mol) of NaH2PO4 to Na2HPO4, resulting in 0.032 mol of NaH2PO4 and 0.068 mol of Na2HPO4.

$$H2PO4^{-} + OH^{-} \rightarrow HPO4^{-2} + H_2O$$

The new pH is

$$\begin{aligned} \mathrm{pH} &= \mathrm{p}K_a + \log \frac{[\mathrm{HPO_4^{2-}}]}{[\mathrm{H_2PO_4^{-}}]} \\ &= 6.86 + \log \frac{0.068}{0.032} = 6.86 + 0.33 = 7.2 \end{aligned}$$

The Power of Buffer Solutions

- If 1.0 mL of 10.0 N NaOH is added to a liter of pure water at pH 7.0, what is the final pH?
- Solution: The NaOH dissociates completely into Na and OH-, giving

[OH-] = 0.010 mol/L = 1.0×10^{-2} M

The pOH is the negative logarithm of [OH-], so pOH = 2.0

Given that in all solutions, pH + pOH = 14, the pH of the solution is 12.

- So, an amount of NaOH that increases the pH of water from 7 to 12 increases the pH of a buffered solution, from 7.0 to just 7.2.

Such is the power of buffering!

Amino Acids as Acids and Bases

- Since free amino acids, as well as peptides and proteins, incorporate **both acidic and basic functional groups**, the predominant molecular species present in an aqueous solution will depend on the pH of the solution.

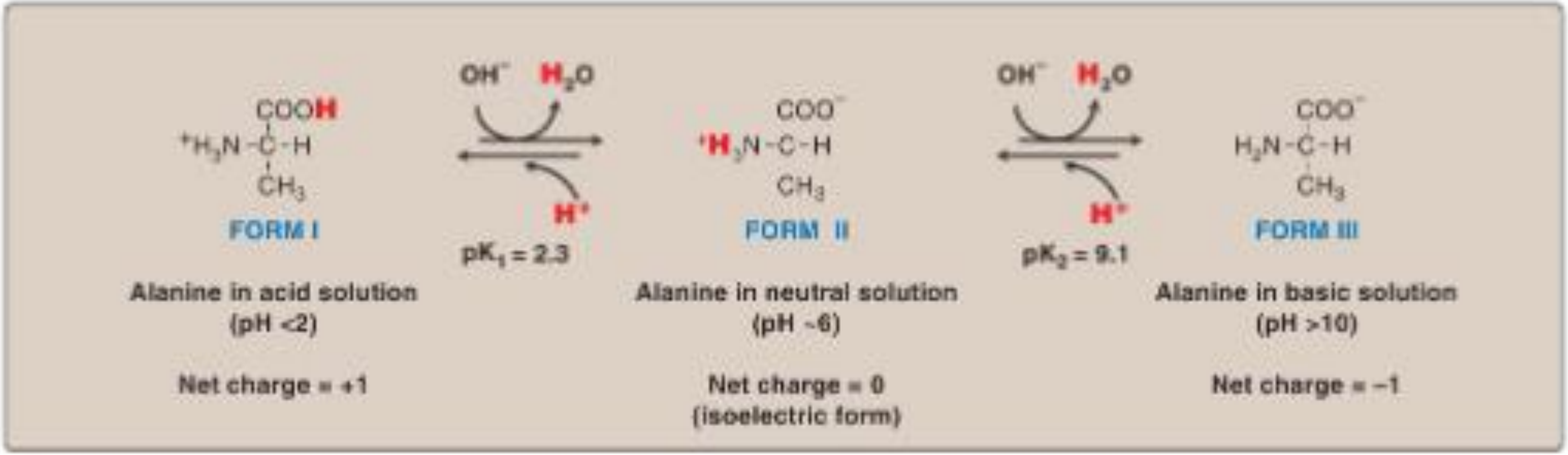
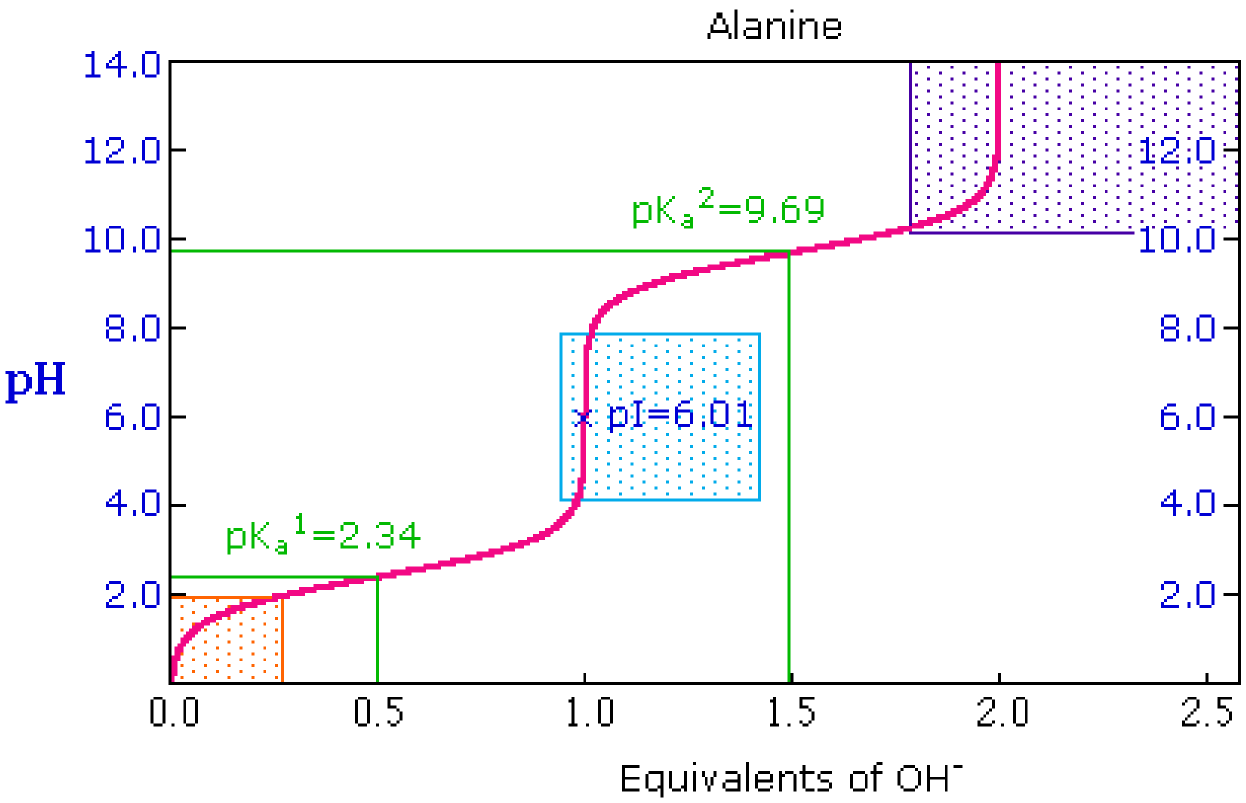


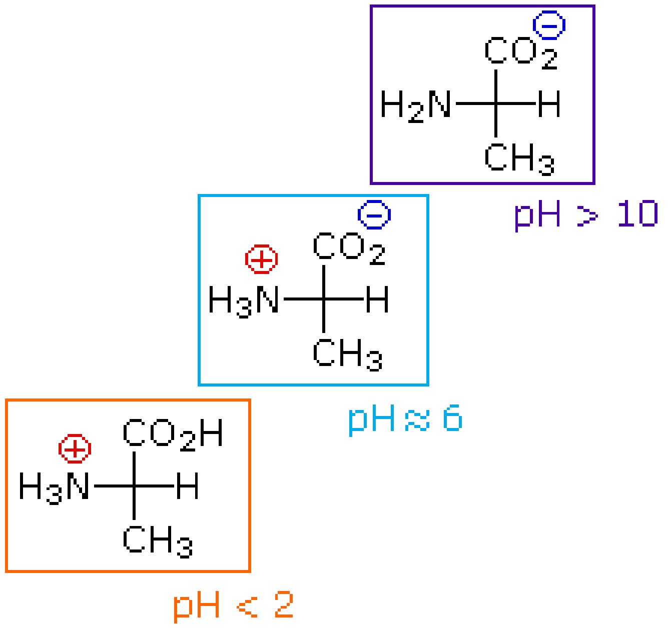
Figure 1.10

Ionic forms of alanine in acidic, neutral, and basic solutions.

The Titration Curve of Alanine

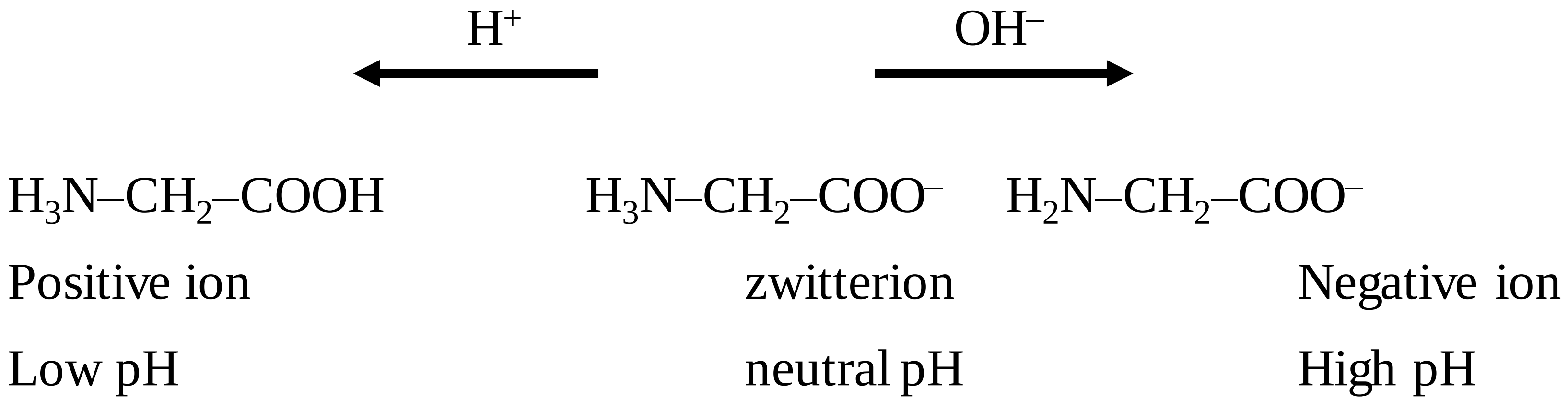
- The titration curve for alanine demonstrates this relationship. **Titration curves show the neutralization of these acids by added base, and the change in pH during the titration.**
- At a **pH lower than 2**, both the carboxylate and amine functions are protonated, so the alanine molecule has a net 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.
- Starting from a fully protonated state, the pKa's of the acidic functions 2.34 for -CO2H, and 9.96 for -NH3(+). The isoelectric points range from 5.5 to 6.15.


Alanine
pH
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0.5
1.0
1.5
2.0
2.5
pKa2=9.69
pKa1=2.34
pI=6.01
Equivalents of OH⁻


CO2⁻
H2N
H
CH3
pH > 10
+
H3N
CO2⁻
H
CH3
pH ≈ 6
+
H3N
CO2H
H
CH3
pH < 2

Amino Acids as Acids and Bases

pH and ionization of amino acids

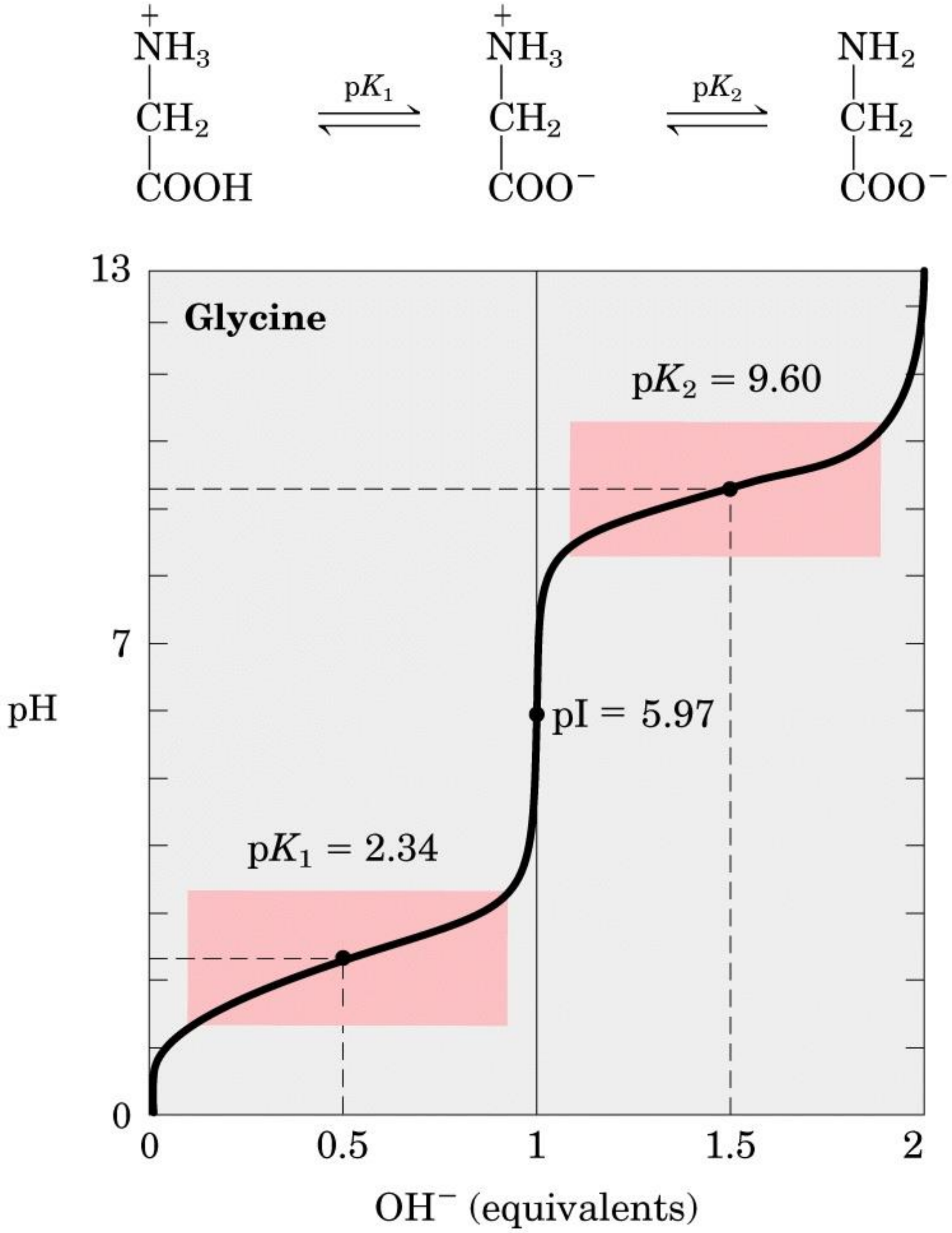


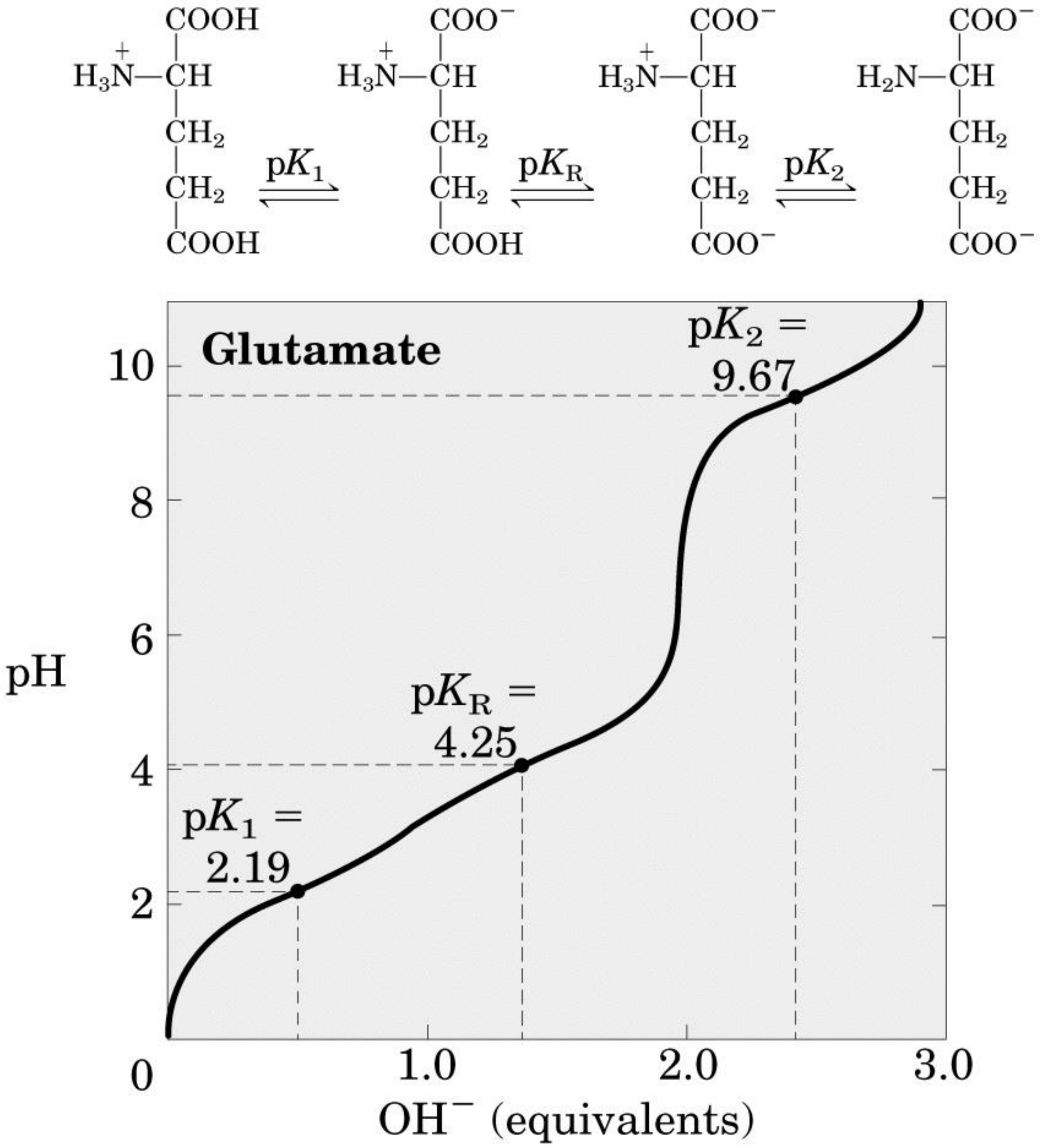
$$H_3N–CH_2–COOH \xleftarrow{H^+} H_3N–CH_2–COO^- \xrightarrow{OH^-} H_2N–CH_2–COO^-$$

Positive ion	zwitterion	Negative ion
Low pH	neutral pH	High pH

- Since amino acids, as well as peptides and proteins, incorporate both acidic and basic functional groups, the predominant molecular species present in an aqueous solution will depend on the pH of the solution.

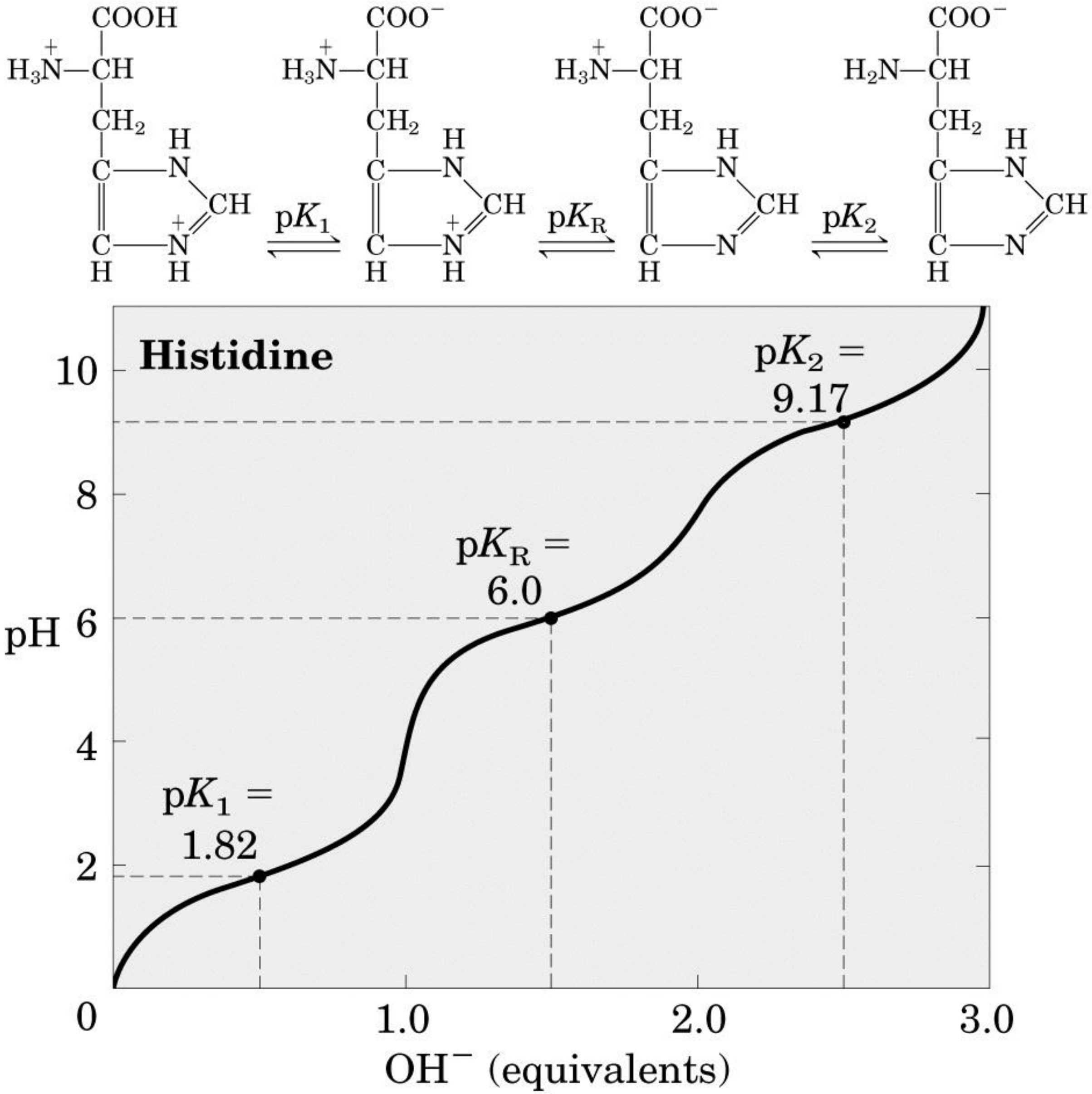
Titration of Glycine



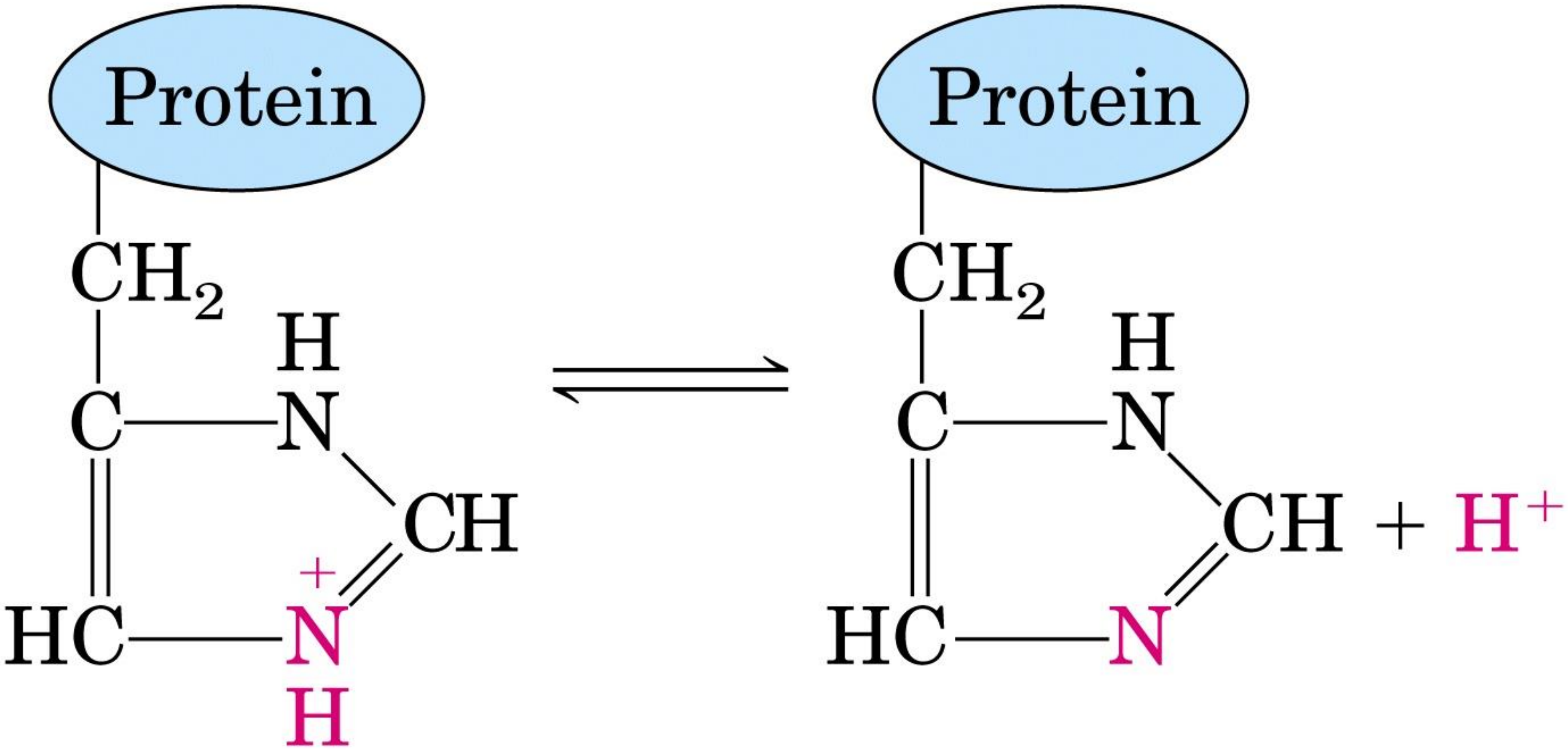


pK1
pKR
pK2
Glutamate
pK2 = 9.67
pKR = 4.25
pK1 = 2.19
pH
0
2
4
6
8
10
1.0
2.0
3.0
OH− (equivalents)

(a)


COOH
$H_3\overset{+}{N}$—CH
CH_2
COO⁻
H_2N—CH
pK_1
pK_R
pK_2
Histidine
pK_2 =
9.17
pK_R =
6.0
pK_1 =
1.82
pH
10
8
6
4
2
0
1.0
2.0
3.0
OH⁻ (equivalents)

(b)


Protein
CH_2
H
C
N
CH
HC
$\overset{+}{N}$
H
Protein
CH_2
H
C
N
CH
HC
N
$+ H^+$