

biochemistry



Reginald H. Garrett | Charles M. Grisham

SIXTH EDITION

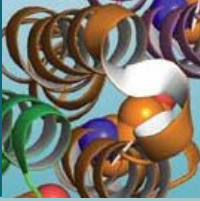
Reginald H. Garrett  
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# Chapter 2

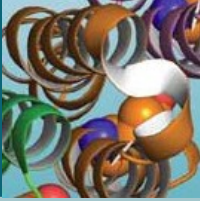
## Water: the Medium of Life

# Outline



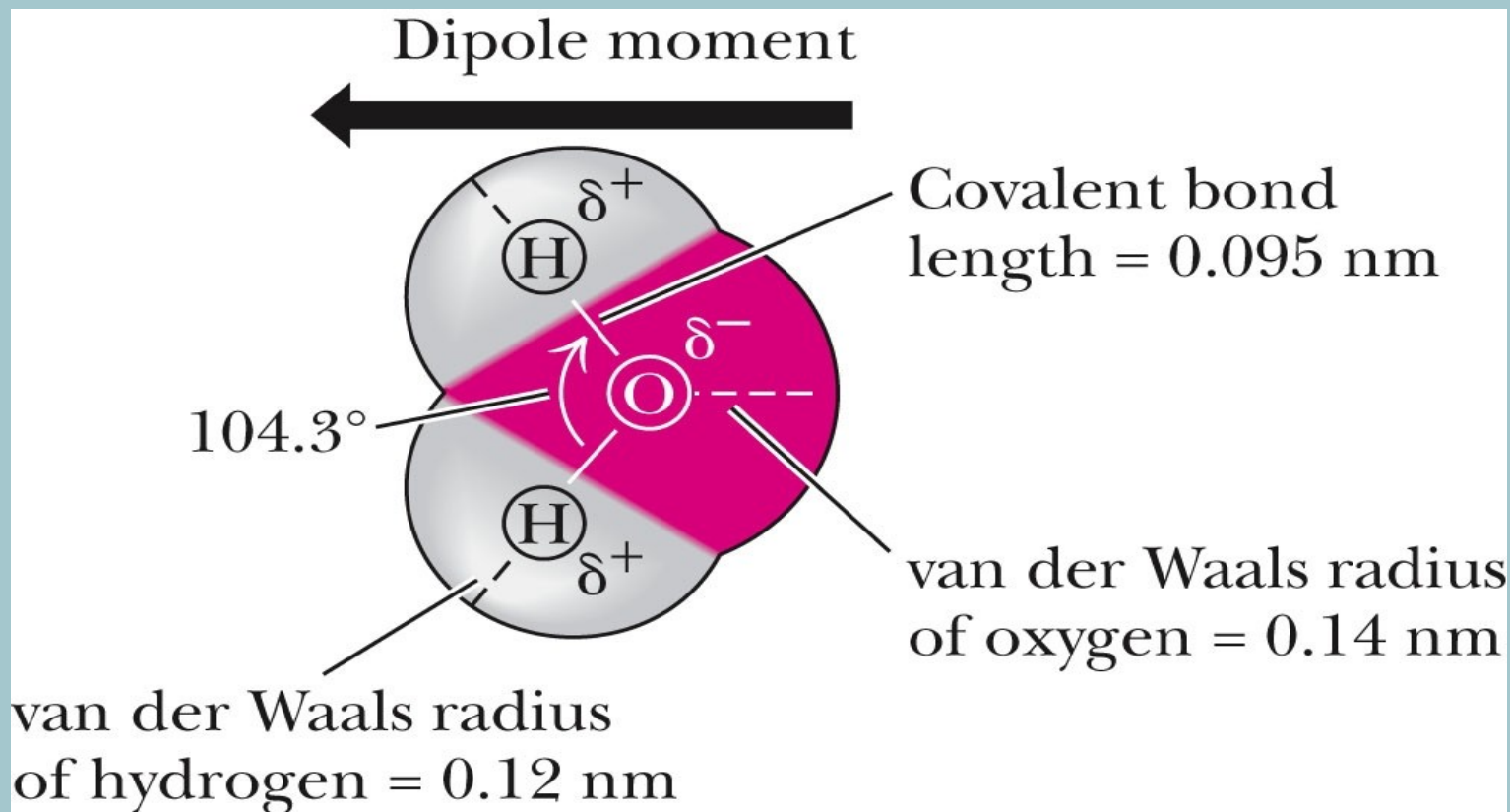
- Properties of water
- Osmotic Pressure
- pH
- Buffers, and how they work

## 2.1 What Are the Properties of Water?



- Water has unusual properties:
  - High b.p., m.p., heat of vaporization, heat capacity, surface tension
  - Polar due to separation of centers of + and – charge
  - High dielectric constant
  - H-bond donor and acceptor
  - Potential to form four H-bonds per water molecule
  - Can ionize into  $\text{H}^+$  and  $\text{OH}^-$
  - Can accept and donate  $\text{H}^+$

## Fig. 2.1 The Structure of Water



Two lobes of negative charge formed by the lone-pair electrons of the oxygen atom lie above and below the plane of the diagram. This electron density contributes substantially to the large dipole moment. Note that the H—O—H angle is 104.3°, *not* 109°, the angular value found in molecules with tetrahedral symmetry, such as CH<sub>4</sub>. (The dipole moment in this figure points in the direction from negative to positive, the convention used by physicists and physical chemists; organic chemists draw it pointing in the opposite direction.)

# The Solvent Properties of Water Derive from Its Polar Nature

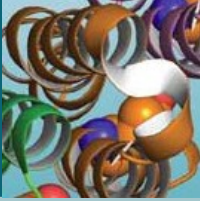
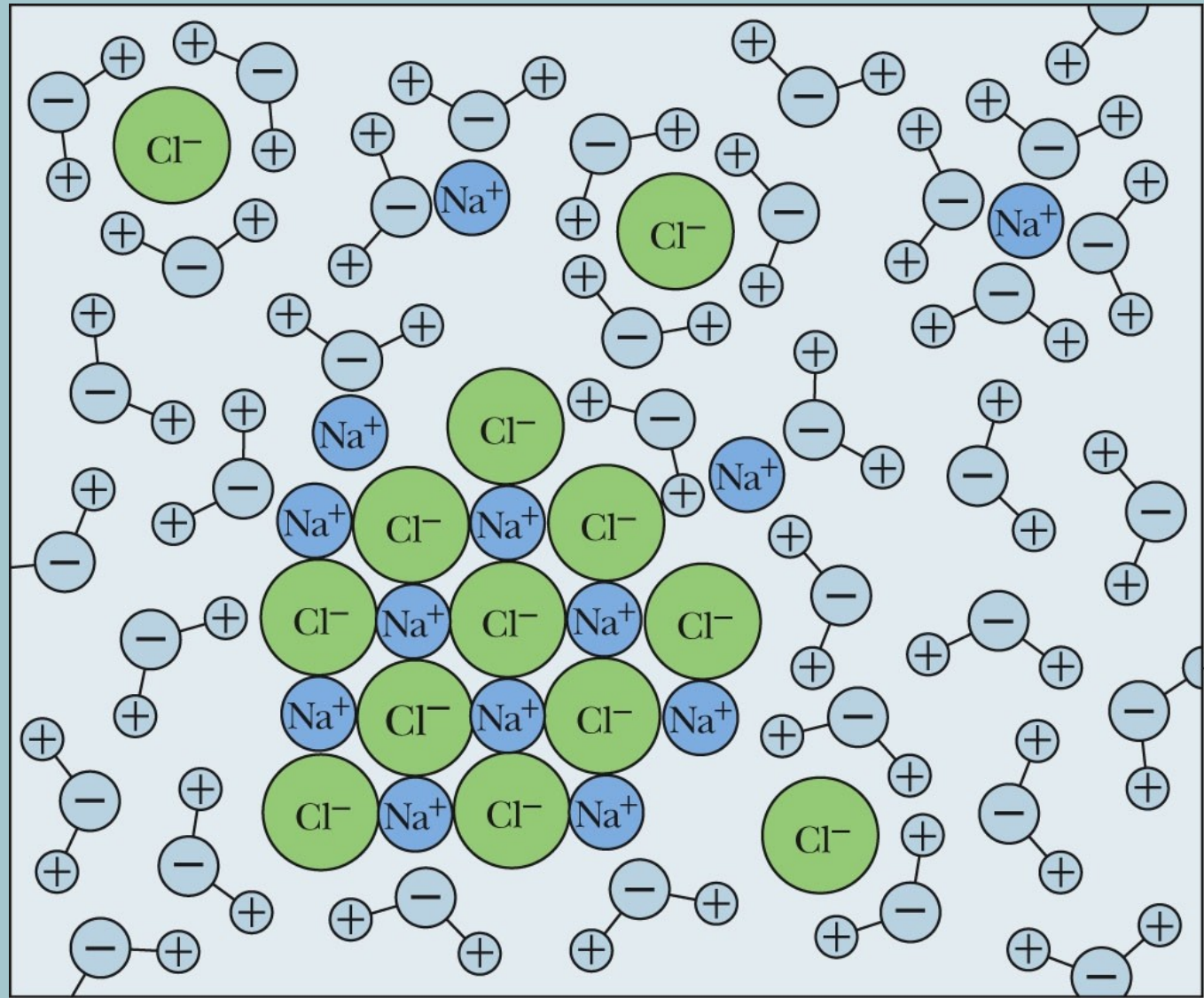


Figure 2.4  
Hydration shells  
surrounding ions  
in solution.



# The Solvent Properties of Water Derive from Its Polar Nature



**TABLE 2.1**

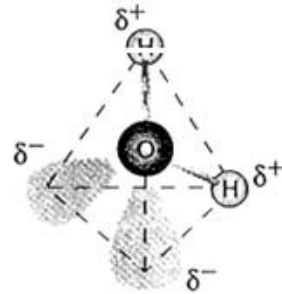
Dielectric Constants\* of Some Common Solvents at 25°C

Solvent	Dielectric Constant ( $D$ )
Formamide	109
Water	78.5
Methyl alcohol	32.6
Ethyl alcohol	24.3
Acetone	20.7
Acetic acid	6.2
Chloroform	5.0
Benzene	2.3
Hexane	1.9

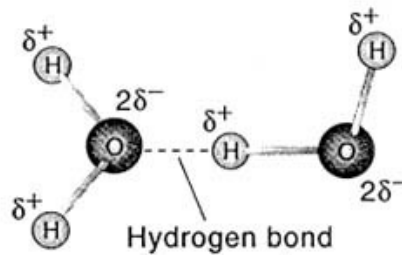
\*The dielectric constant is also referred to as *relative permittivity* by physical chemists.



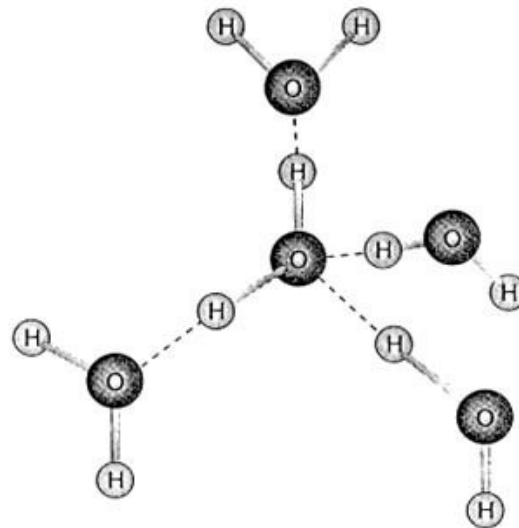
# H<sub>2</sub>O Structure and H-bonding



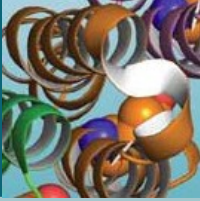
(a) Single water molecule



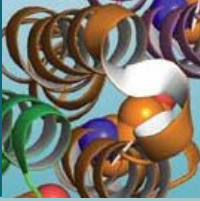
(b) Two interacting water molecules



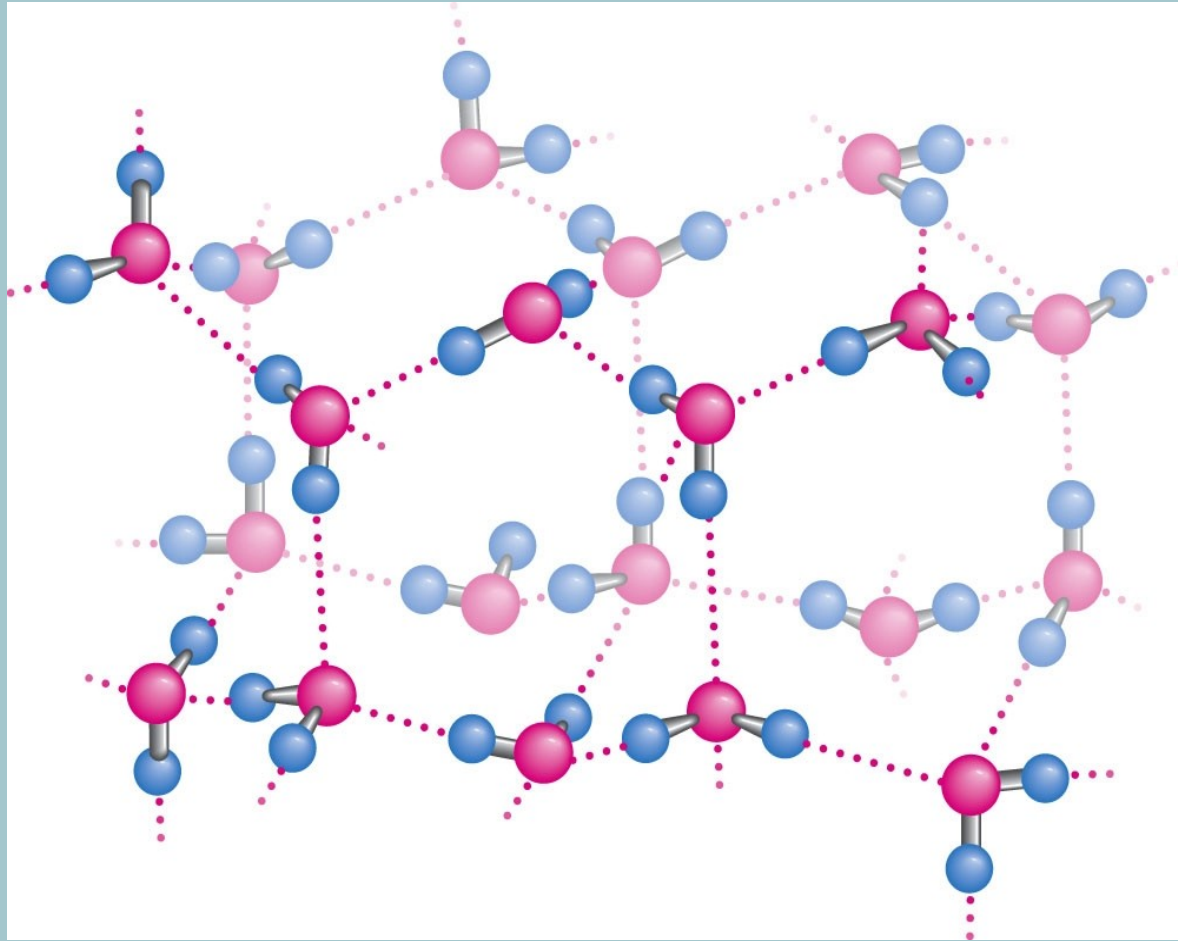
(c) Cluster of interacting water molecules



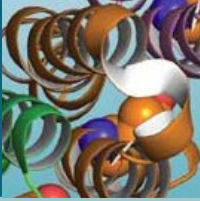
## Figure 2.2 The structure of normal ice.



The hydrogen bonds in ice form a three-dimensional network. The smallest number of  $\text{H}_2\text{O}$  molecules in any closed circuit of H-bonded molecules is six, so this structure bears the name hexagonal ice. Covalent bonds are represented as solid lines, whereas hydrogen bonds are shown as dashed lines. The directional preference of H bonds leads to a rather open lattice structure for crystalline water and, consequently, a low density for the solid state. The distance between neighboring oxygen atoms linked by a hydrogen bond is 0.274nm. Since the covalent H-O bond is 0.095nm, the H-O hydrogen bond length in ice is 0.18 nm.







# Comparison of Ice and Water

## *Issues: H-bonds and Motion*

- Ice: 4 H-bonds per water molecule
- Water: 2.3 H-bonds per water molecule at 10°C
- Ice: H-bond lifetime - about 10 microsec
- Water: H-bond lifetime - about 10 psec

# Important Colligative Property of Solutes: Osmotic Pressure

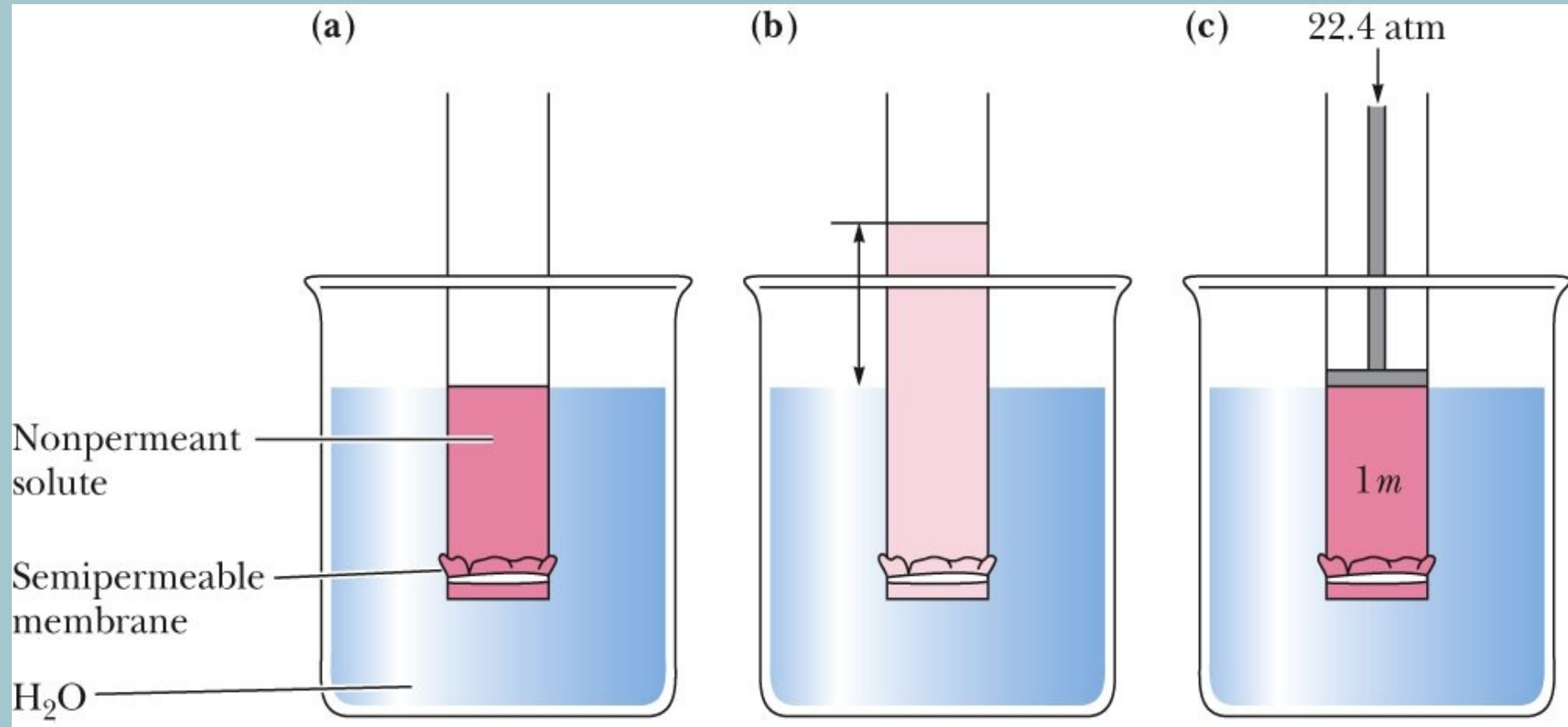
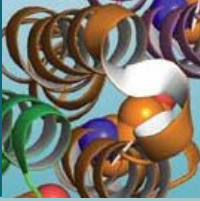


Figure 2.8 The osmotic pressure of a 1 molal ( $m$ ) solution is equal to 22.4 atmospheres. Osmotic pressure is directly proportional to the concentration of the nonpermeant solute.

# Water Can Ionize to Form $\text{H}^+$ and $\text{OH}^-$

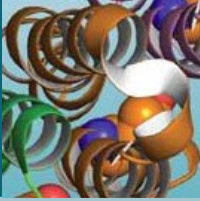
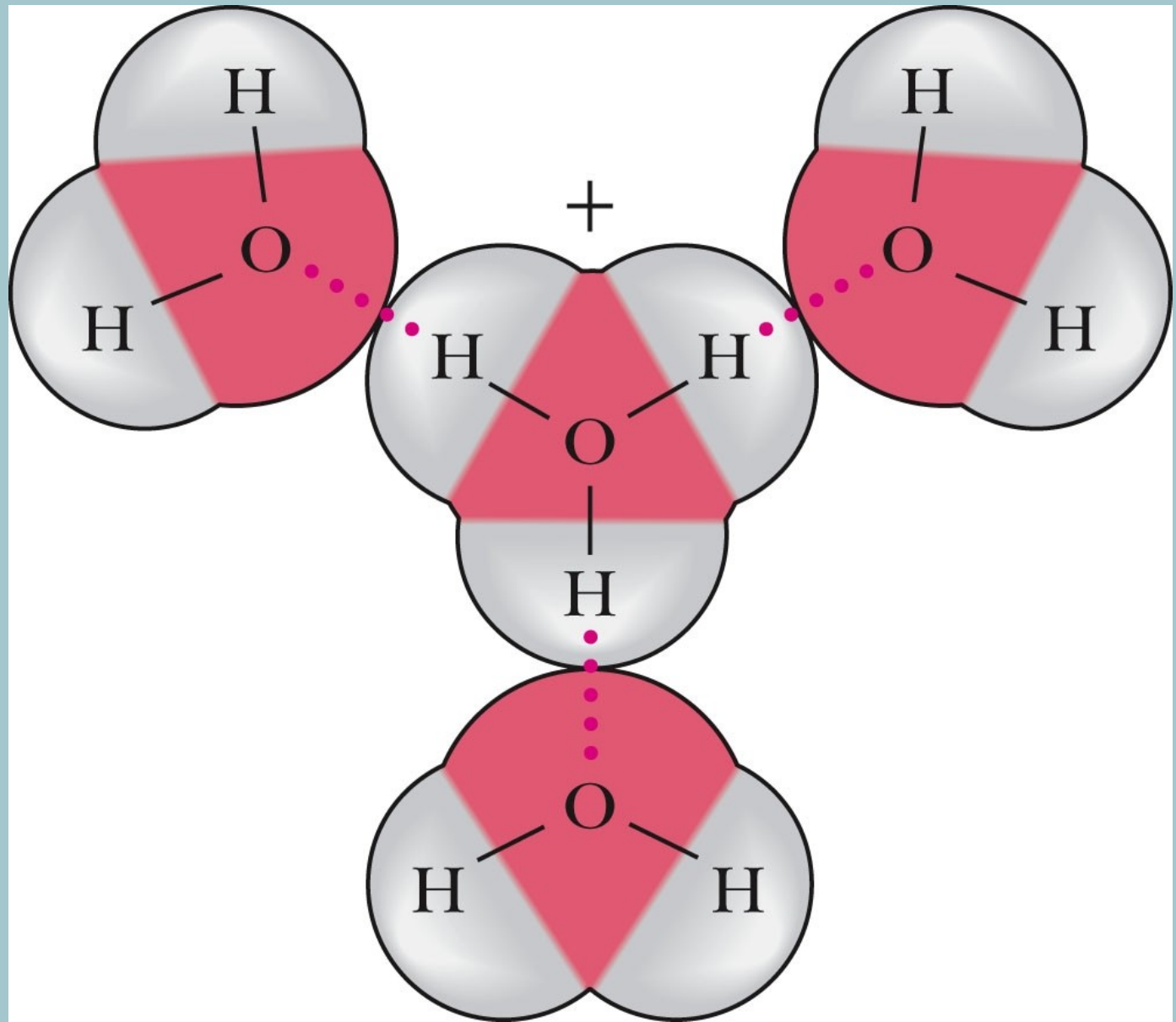
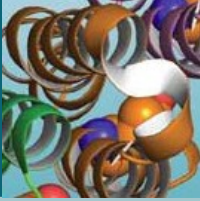
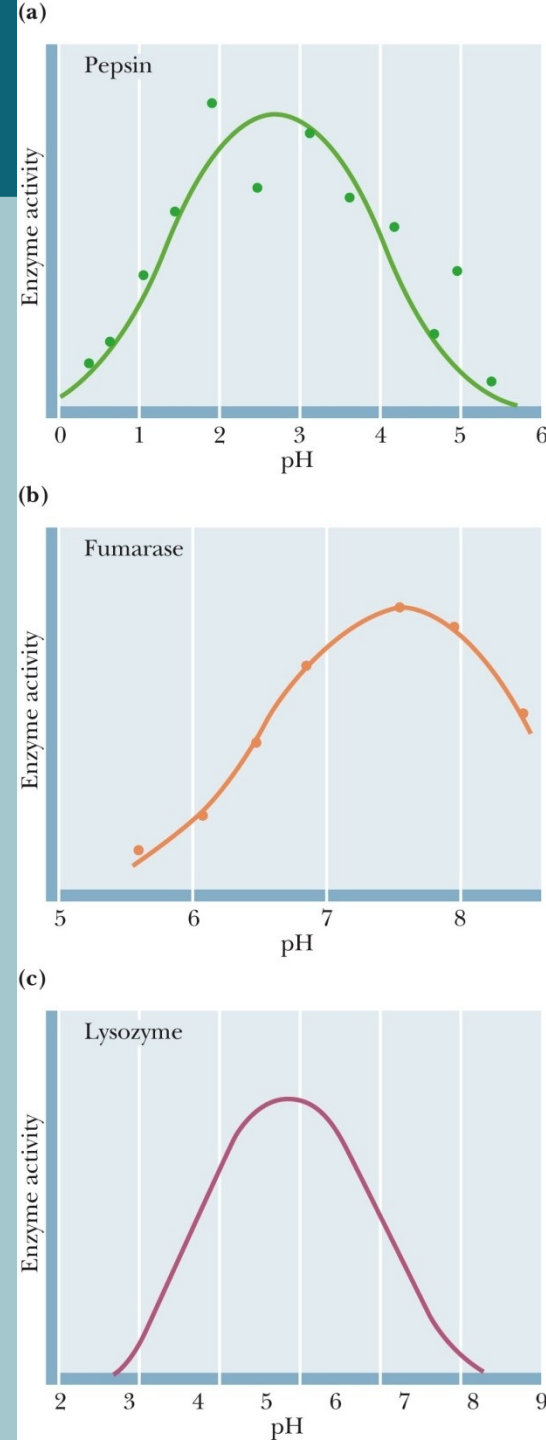


Figure 2.10  
The hydration  
of  $\text{H}_3\text{O}^+$ .

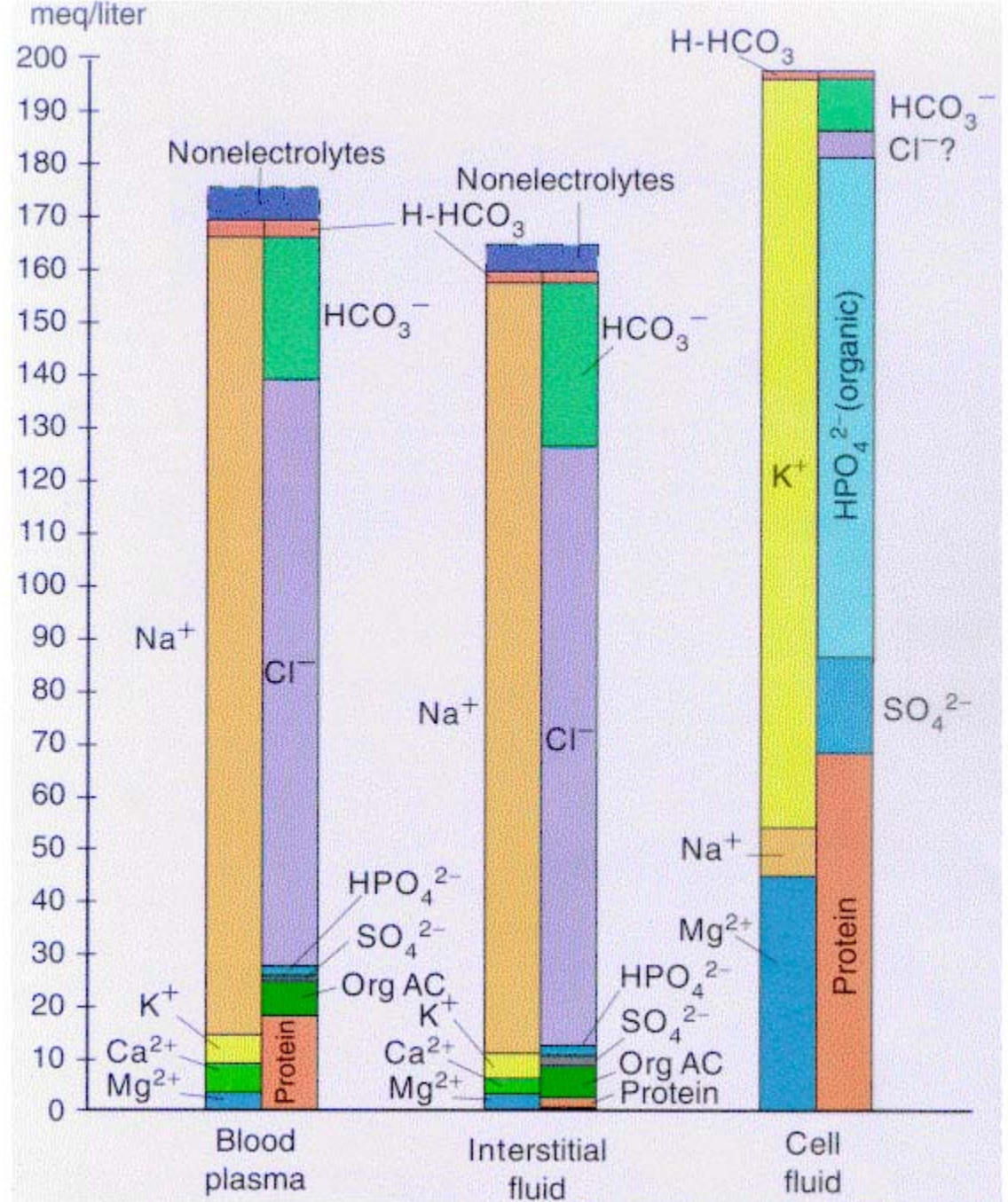


## 2.2 Enzyme Activity is Influenced by pH

Figure 2.15 pH versus enzymatic activity.  
Pepsin is a protein-digesting enzyme active in gastric fluid.  
Fumarase is a metabolic enzyme found in mitochondria.  
Lysozyme digests the cell walls of bacteria. It is found in tears.



# What are the pH Buffers of Life?



Electrolyte Composition of Body Fluids

Source: Modified from Ganong, From M. I. Gossens, Medical Physiology, 11th ed., by P. Band, Mosby, St. Louis, 1961, p. 307.  
Geoffrey L. Zubay, Biochemistry, 4th, Copyright © 1989 The McGraw-Hill Companies, Dubuque, Iowa. All Rights Reserved.



# pH Equations

pH vs. [acid]

For strong acid



$$[\text{H}^+] \equiv [\text{H}_3\text{O}^+] = [\text{HA}]_0$$

$$\text{pH} = -\log[\text{H}^+] = -\log[\text{HA}]_0$$

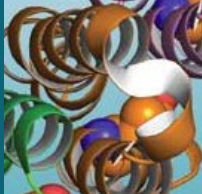
For weak acid



i.e.






$$K_{eq} = \frac{[A^-][H^+]}{[HA^+][H_2O]}$$

$$K_a = K_{eq}[H_2O] = \frac{[A^-][H^+]}{[HA^+]}$$

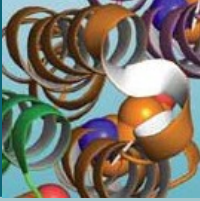
Since  $[A^-] = [H^+]$

and  $[HA^+] = [HA^+]_0 - [H^+]$

$$K_a = \frac{[H^+]^2}{[HA^+]_0 - [H^+]}$$

$$[H^+]^2 + K_a[H^+] - K_a[HA^+]_0 = 0$$

Solve quadratic eq. for  $[H^+]$   
and convert to pH



$$ax^2 + bx + c = 0$$

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

where

$$x = [H^+]$$

$$a = 1$$

$$b = K_a$$

$$c = -K_a [HA^{0+}]_0$$

**TABLE 2.4** Acid Dissociation Constants and  $pK_a$  Values for Some Weak Electrolytes (at 25°C)

Acid	$K_a$ (M)	$pK_a$
HCOOH (formic acid)	$1.78 \times 10^{-4}$	3.75
CH <sub>3</sub> COOH (acetic acid)	$1.74 \times 10^{-5}$	4.76
CH <sub>3</sub> CH <sub>2</sub> COOH (propionic acid)	$1.35 \times 10^{-5}$	4.87
CH <sub>3</sub> CHOHCOOH (lactic acid)	$1.38 \times 10^{-4}$	3.86
HOOCCH <sub>2</sub> CH <sub>2</sub> COOH (succinic acid) $pK_1^*$	$6.16 \times 10^{-5}$	4.21
HOOCCH <sub>2</sub> CH <sub>2</sub> COO <sup>-</sup> (succinic acid) $pK_2$	$2.34 \times 10^{-6}$	5.63
H <sub>3</sub> PO <sub>4</sub> (phosphoric acid) $pK_1$	$7.08 \times 10^{-3}$	2.15
H <sub>2</sub> PO <sub>4</sub> <sup>-</sup> (phosphoric acid) $pK_2$	$6.31 \times 10^{-8}$	7.20
HPO <sub>4</sub> <sup>2-</sup> (phosphoric acid) $pK_3$	$3.98 \times 10^{-13}$	12.40
C <sub>3</sub> N <sub>2</sub> H <sub>5</sub> <sup>+</sup> (imidazole)	$1.02 \times 10^{-7}$	6.99
C <sub>6</sub> O <sub>2</sub> N <sub>3</sub> H <sub>11</sub> <sup>+</sup> (histidine–imidazole group) $pK_R^\dagger$	$9.12 \times 10^{-7}$	6.04
H <sub>2</sub> CO <sub>3</sub> (carbonic acid) $pK_1$	$1.70 \times 10^{-4}$	3.77
HCO <sub>3</sub> <sup>-</sup> (bicarbonate) $pK_2$	$5.75 \times 10^{-11}$	10.24
(HOCH <sub>2</sub> ) <sub>3</sub> CNH <sub>3</sub> <sup>+</sup> ( <i>tris</i> -hydroxymethyl aminomethane)	$8.32 \times 10^{-9}$	8.07
NH <sub>4</sub> <sup>+</sup> (ammonium)	$5.62 \times 10^{-10}$	9.25
CH <sub>3</sub> NH <sub>3</sub> <sup>+</sup> (methylammonium)	$2.46 \times 10^{-11}$	10.62

\*The  $pK$  values listed as  $pK_1$ ,  $pK_2$ , or  $pK_3$  are in actuality  $pK_a$  values for the respective dissociations. This simplification in notation is used throughout this book.

<sup>†</sup> $pK_R$  refers to the imidazole ionization of histidine.




$$\text{pH vs. } \frac{[A^-]}{[HA^+]}$$

For most applications in biochemistry this is the relationship used and is easily seen in the Henderson-Hasselbalch rearrangement of the  $K_a$  equality:

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

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

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

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

Figure 2.11 The titration curve for acetic acid. Note that the titration curve is relatively flat at pH values near the  $pK_a$ . In other words, the pH changes relatively little as  $OH^-$  is added in this region of the titration curve.

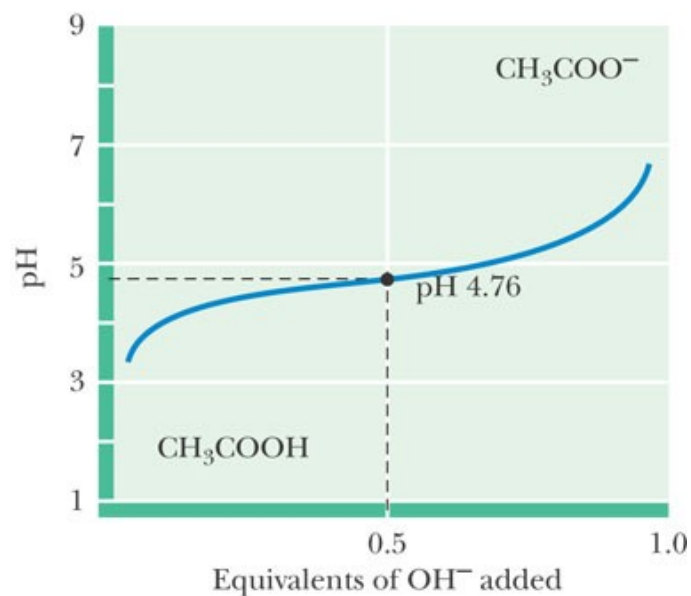
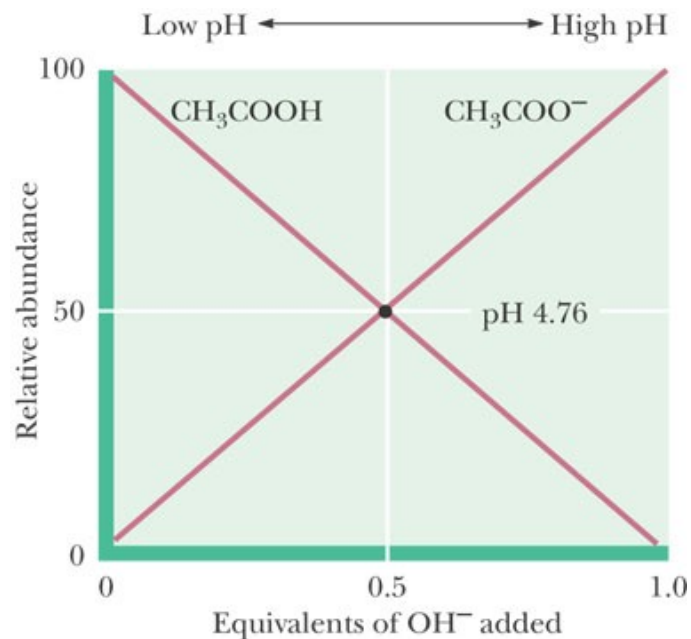
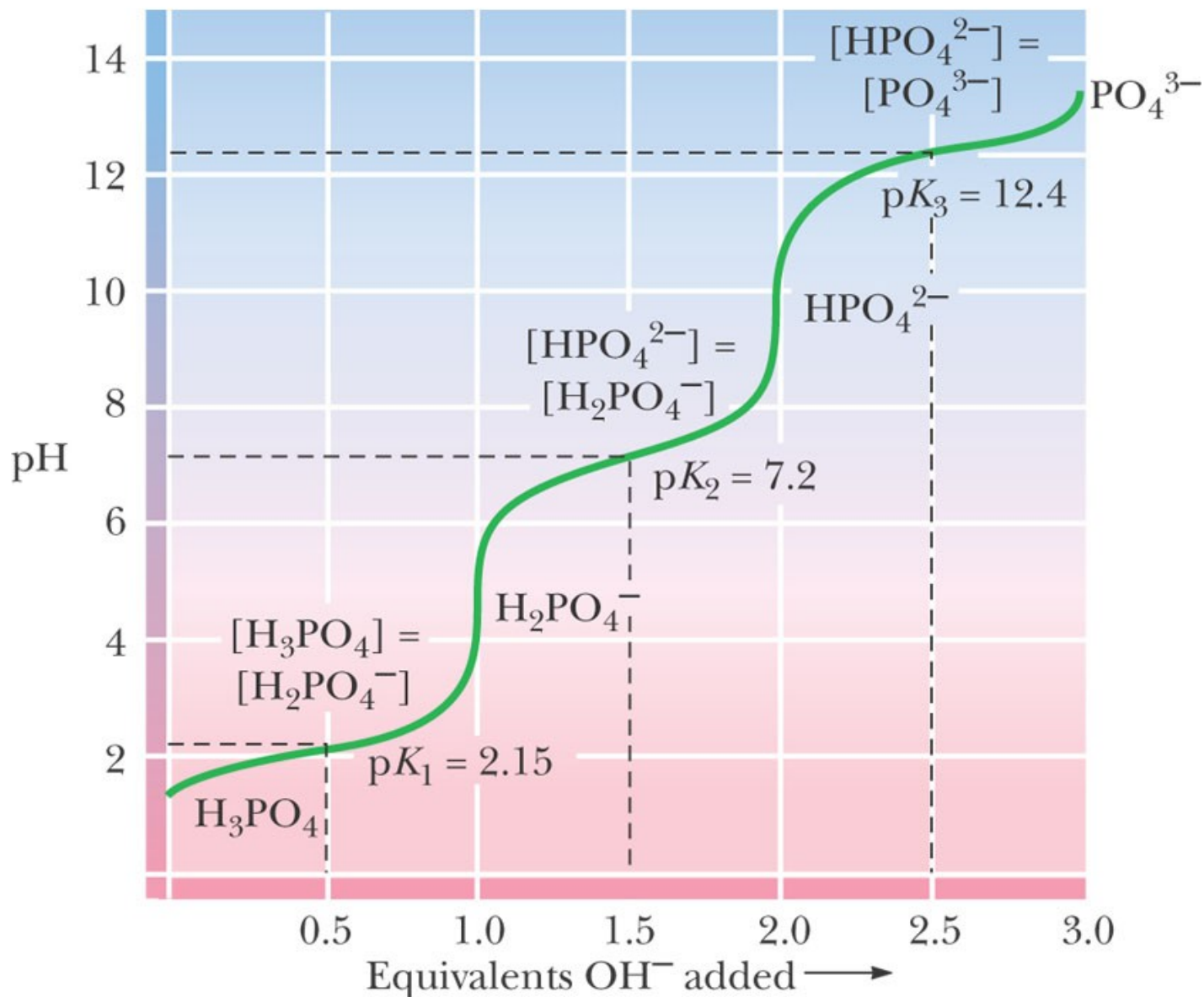


Figure 2.13

The titration curve for phosphoric acid. The chemical formulas show the prevailing ionic species present at various pH values. Phosphoric acid ( $\text{H}_3\text{PO}_4$ ) has three titratable hydrogens and therefore three midpoints are seen: at pH 2.15 ( $\text{p}K_1$ ), pH 7.20 ( $\text{p}K_2$ ), and pH 12.4 ( $\text{p}K_3$ ).





What is the pH of a phosphate solution if

$$\frac{[H_2PO_4^-]}{[HPO_4^{2-}]} = \frac{1}{10} \quad ?$$

$$pH = pK_{a2} + \log \frac{[HPO_4^{2-}]}{[H_2PO_4^-]}$$

$$pH = 7.2 + \log \frac{10}{1}$$

$$= 7.2 + 1$$

$$= 8.2$$

What is pH of  $10^{-2} \text{ M H}_3\text{PO}_4$ ?

$$K_a = \frac{[\text{H}^+]^2}{[\text{H}_3\text{PO}_4]_0 - [\text{H}^+]}$$

use  $K_{a1}$

$$7.08 \times 10^{-3} \text{ M} = \frac{[\text{H}^+]^2}{10^{-2} \text{ M} - [\text{H}^+]}$$

$$[\text{H}^+]^2 + 7.08 \times 10^{-3} \text{ M} [\text{H}^+] - 7.08 \times 10^{-5} \text{ M}^2 = 0$$

$$a = 1$$

$$b = 7.08 \times 10^{-3} \text{ M}$$

$$c = -7.08 \times 10^{-5} \text{ M}^2$$

$$[\text{H}^+] =$$

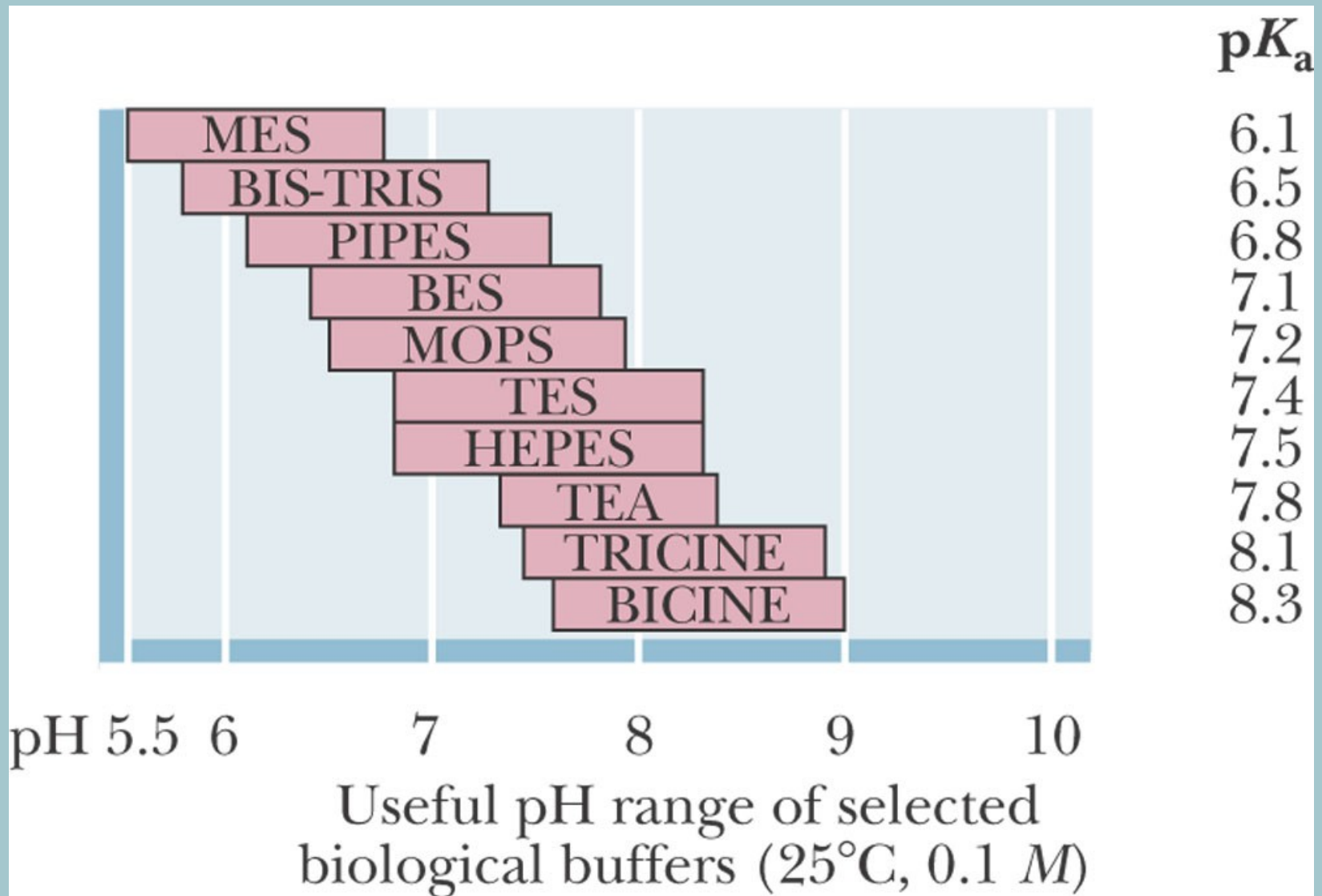
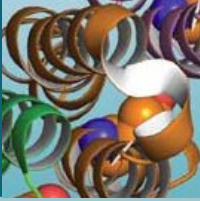
$$\text{pH} = -\log [\text{H}^+] =$$



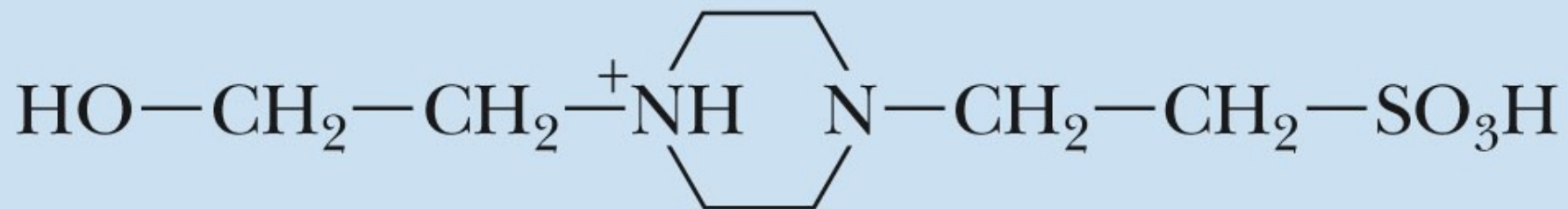
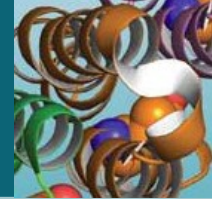
## 2.3 What Are Buffers, and What Do They Do?

- Buffers are solutions that resist changes in pH as acid and base are added.
- Most buffers consist of a weak acid and its conjugate base.
- Buffer range is  $\text{pK}_a \pm 1.0$ . This is the range in which a buffer can be used reliably.

# What are Buffers and What Do They Do?



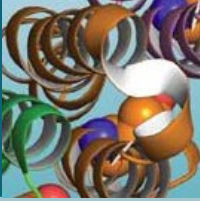
# What are Buffers and What Do They Do?



**HEPES**

Figure 2.17 The structure of HEPES, in its fully protonated form.

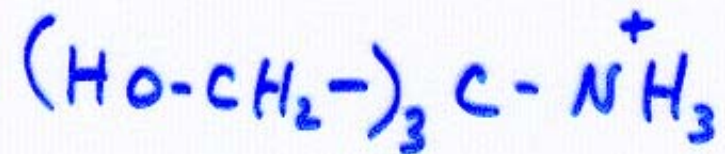
# Most Common Biochemistry Lab Buffer



Most common lab buffer:

Tris

Tris (hydroxymethyl)aminomethane



$\text{pK}_a = 8.1$



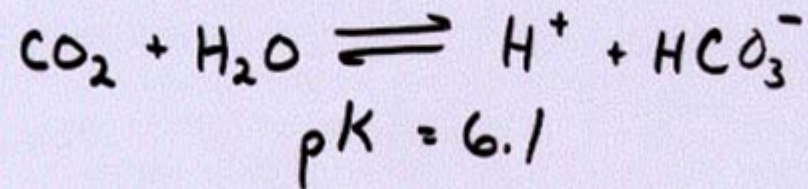
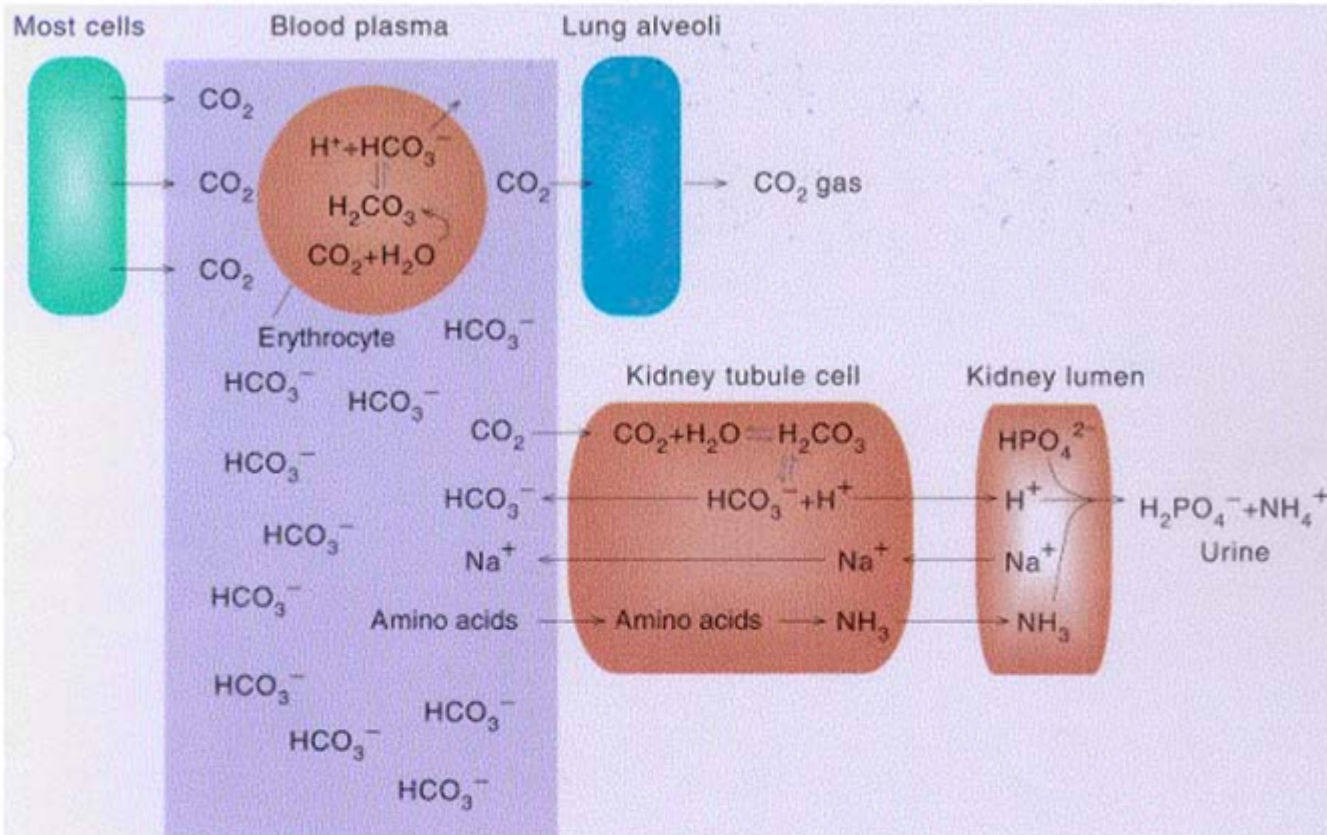
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<sup>†</sup> $pK_R$  refers to the imidazole ionization of histidine.

## Blood Buffering; CO<sub>2</sub> and H<sup>+</sup> Removal



$$pH = 6.1 + \log \frac{[\text{HCO}_3^-]}{[\text{CO}_2]}$$



NB: If you are using the G&G 4<sup>th</sup> ed., in the text box 'The Bicarbonate Buffer System of Blood Plasma' on p.43, middle of right column, there is a typo.

The overall equilibrium for the ionization of  $\text{H}_2\text{CO}_3$  in equilibrium with  $\text{CO}_2(\text{d})$  should be

$$K_a K_h = [\text{H}^+][\text{HCO}_3^-]/[\text{CO}_2(\text{d})]$$

i.e.,  $K_h$  should not be present on the right side of the equation