Water: The Solvent for Biochemical Reactions

2.1 Water and Polarity

Water is the principal component of most cells. The geometry of the water molecule and its properties as a solvent play major roles in determining the properties of living systems.

The tendency of an atom to attract electrons to itself in a chemical bond (i.e., to become negative) is called **electronegativity.** Atoms of the same element, of course, share electrons equally in a bond—that is, they have equal electronegativity—but different elements do not necessarily have the same electronegativity. Oxygen and nitrogen are both highly electronegative, much more so than carbon and hydrogen (Table 2.1).

What is polarity?

When two atoms with the same electronegativity form a bond, the electrons are shared equally between the two atoms. However, if atoms with differing electronegativity form a bond, the electrons are not shared equally and more of the negative charge is found closer to one of the atoms. In the $O-H$ bonds in water, oxygen is more electronegative than hydrogen, so there is a higher probability that the bonding electrons are closer to the oxygen. The difference in electronegativity between oxygen and hydrogen gives rise to a *partial* positive and negative charge, usually pictured as δ^+ and δ^-, respectively (Figure 2.1). Bonds such as this are called **polar bonds.** In situations in which the electronegativity difference is quite small, such as in the C—H bond in methane (CH_4), the sharing of electrons in the bond is very nearly equal, and the bond is essentially **nonpolar.**

Table 2.1

Electronegativities of Selected Elements

Element	Electronegativity*
Oxygen	3.5
Nitrogen	3.0
Sulfur	2.6
Carbon	2.5
Phosphorus	2.2
Hydrogen	2.1

*Electronegativity values are relative, and are chosen to be positive numbers ranging from less than 1 for some metals to 4 for fluorine.

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Life processes depend on the properties of water.

Chapter Outline

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Dipole moment

Covalent bond length = 0.095 nm

104.3°

Van der Waals radius of oxygen = 0.14 nm

Van der Waals radius of hydrogen = 0.12 nm
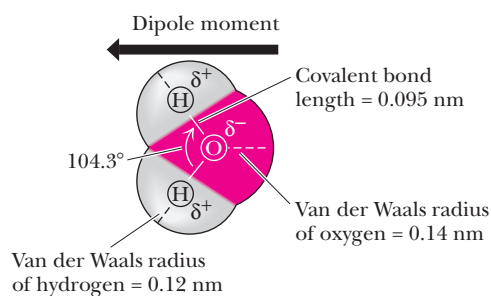
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■ **ACTIVE FIGURE 2.1** **The structure of water.** Oxygen has a partial negative charge, and the hydrogens have a partial positive charge. The uneven distribution of charge gives rise to the large dipole moment of water. The dipole moment in this 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. **Sign in at www.thomsonedu.com/login to explore an interactive version of this figure.**

The bonds in a molecule may be polar, but the molecule itself can still be nonpolar because of its geometry. Carbon dioxide is an example. The two C=O bonds are polar, but because the CO_2 molecule is linear, the attraction of the oxygen for the electrons in one bond is cancelled out by the equal and opposite attraction for the electrons by the oxygen on the other side of the molecule.

$$\overset{\delta^-}{O}=\overset{2\delta^+}{C}=\overset{\delta^-}{O}$$
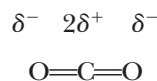
Water is a bent molecule with a bond angle of 104.3° (Figure 2.1), and the uneven sharing of electrons in the two bonds is not cancelled out as it is in CO_2. The result is that the bonding electrons are more likely to be found at the oxygen end of the molecule than at the hydrogen end. Bonds with positive and negative ends are called **dipoles.**

Solvent Properties of Water

Why do some chemicals dissolve in water while others do not?

The polar nature of water largely determines its solvent properties. *Ionic* compounds with full charges, such as potassium chloride (KCl, K⁺ and Cl⁻ in solution), and *polar* compounds with partial charges (i.e., dipoles), such as ethyl alcohol (C_2H_5OH) or acetone [$(CH_3)_2C$=O], tend to dissolve in water (Figures 2.2 and 2.3). The underlying physical principle is electrostatic attraction
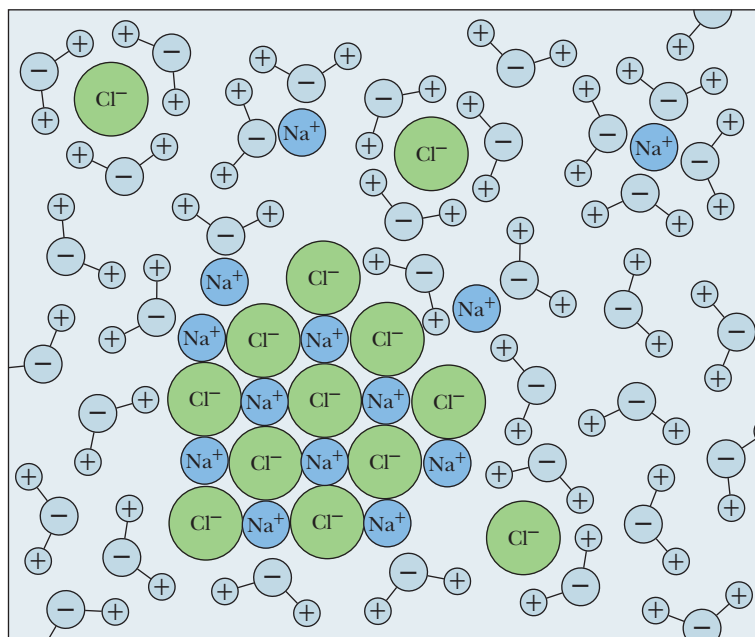
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■ **ANIMATED FIGURE 2.2** **Hydration shells surrounding ions in solution.** Unlike charges attract. The partial negative charge of water is attracted to positively charged ions. Likewise, the partial positive charge on the other end of the water molecule is attracted to negatively charged ions. **Sign in at www.thomsonedu.com/login to see an animated version of this figure.**
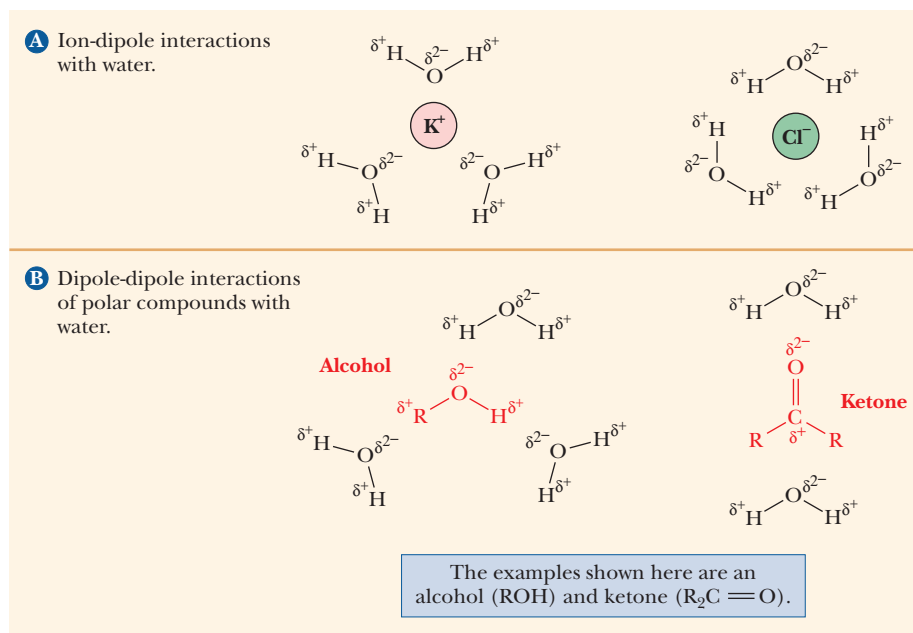
A Ion-dipole interactions with water.

B Dipole-dipole interactions of polar compounds with water.

Alcohol

Ketone

The examples shown here are an alcohol (ROH) and ketone ($R_2C=O$).

■ **FIGURE 2.3** **Ion–dipole and dipole–dipole interactions.** Ion–dipole and dipole–dipole interactions help ionic and polar compounds dissolve in water.

between unlike charges. The negative end of a water dipole attracts a positive ion or the positive end of another dipole. The positive end of a water molecule attracts a negative ion or the negative end of another dipole. The aggregate of unlike charges, held in proximity to one another because of electrostatic attraction, has a lower energy than would be possible if this interaction did not take place. The lowering of energy makes the system more stable and more likely to exist. These *ion–dipole* and *dipole–dipole* interactions are similar to the interactions between water molecules themselves in terms of the quantities of energy involved. Examples of polar compounds that dissolve easily in water are small organic molecules containing one or more electronegative atoms (e.g., oxygen or nitrogen), including alcohols, amines, and carboxylic acids. The attraction between the dipoles of these molecules and the water dipoles makes them tend to dissolve. Ionic and polar substances are referred to as **hydrophilic** ("water-loving," from the Greek) because of this tendency.

Hydrocarbons (compounds that contain only carbon and hydrogen) are nonpolar. The favorable ion–dipole and dipole–dipole interactions responsible for the solubility of ionic and polar compounds do not occur for nonpolar compounds, so these compounds tend not to dissolve in water. The interactions between nonpolar molecules and water molecules are weaker than dipolar interactions. The permanent dipole of the water molecule can induce a temporary dipole in the nonpolar molecule by distorting the spatial arrangements of the electrons in its bonds. Electrostatic attraction is possible between the induced dipole of the nonpolar molecule and the permanent dipole of the water molecule (a *dipole–induced dipole interaction*), but it is not as strong as that between permanent dipoles. Hence, its consequent lowering of energy is less than that produced by the attraction of the water molecules for one another. The association of nonpolar molecules with water is far less likely to occur than the association of water molecules with themselves.

A full discussion of why nonpolar substances are insoluble in water requires the thermodynamic arguments that we shall develop in Chapters 4 and 15. However, the points made here about intermolecular interactions will be useful background information for that discussion. For the moment, it is enough to know that it is less favorable thermodynamically for water molecules to be associated with nonpolar molecules than with other water molecules. As a result, nonpolar molecules do not dissolve in water and are referred to as **hydrophobic** ("water-hating," from the Greek). Hydrocarbons in particular tend to sequester themselves from an aqueous environment. A nonpolar solid leaves undissolved material in water. A nonpolar liquid forms a two-layer system with water; an example is an oil slick. The interactions between nonpolar molecules are called **hydrophobic interactions** or, in some cases, **hydrophobic bonds.**

Table 2.2 gives examples of hydrophobic and hydrophilic substances.

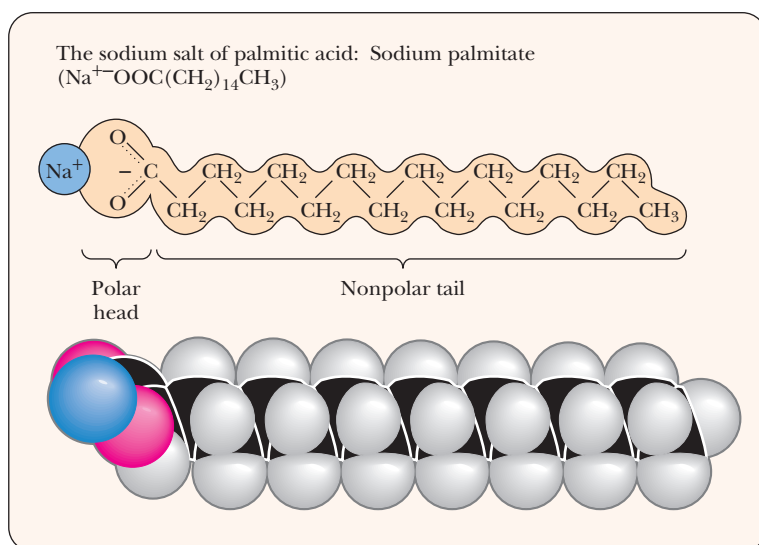## Why do oil and water mixed together separate into layers?

A single molecule may have both polar (hydrophilic) and nonpolar (hydrophobic) portions. Substances of this type are called **amphipathic.** A long-chain fatty acid having a polar carboxylic acid group and a long nonpolar hydrocarbon portion is a prime example of an amphipathic substance. The carboxylic acid group, the "head" group, contains two oxygen atoms in addition to carbon and hydrogen; it is very polar and can form a carboxylate anion at neutral pH. The rest of the molecule, the "tail," contains only carbon and hydrogen and is thus nonpolar (Figure 2.4). A compound such as this in the presence of water tends to form structures called *micelles,* in which the polar head groups are in contact with the aqueous environment and the nonpolar tails are sequestered from the water (Figure 2.5). A similar process is responsible for the separation of oil and water, such as you would see in Italian salad dressing. When shaken, initially the substances mix. Immediately thereafter you can see small spheres or oil droplets. As these float on water, they move to the top and coalesce into the oil layer.

Interactions between nonpolar molecules themselves are very weak and depend on the attraction between short-lived temporary dipoles and the dipoles they induce. A large sample of nonpolar molecules will always include some molecules with these temporary dipoles, which are caused by a momentary clumping of bonding electrons at one end of the molecule. A temporary dipole can induce another dipole in a neighboring molecule in the same way that a permanent dipole does. The interaction energy is low because the asso-

Table 2.2	
Examples of Hydrophobic and Hydrophilic Substances	
Hydrophilic	**Hydrophobic**
Polar covalent compounds (e.g., alcohols such as C_2H_5OH [ethanol] and ketones such as $(CH_3)_2C\!\!=\!\!O$ [acetone])	Nonpolar covalent compounds (e.g., hydrocarbons such as C_6H_{14} hexane])
Sugars	Fatty acids, cholesterol
Ionic compounds (e.g., KCl)	
Amino acids, phosphate esters	

The sodium salt of palmitic acid: Sodium palmitate
$(Na^{+-}OOC(CH_2)_{14}CH_3)$

Polar head

Nonpolar tail

■ **FIGURE 2.4 Sodium palmitate, an amphiphilic molecule.** Amphiphilic molecules are frequently symbolized by a ball and zigzag line structure, ●⌇⌇⌇, where the ball represents the hydrophilic polar head and the zigzag line represents the nonpolar hydrophobic hydrocarbon tail.
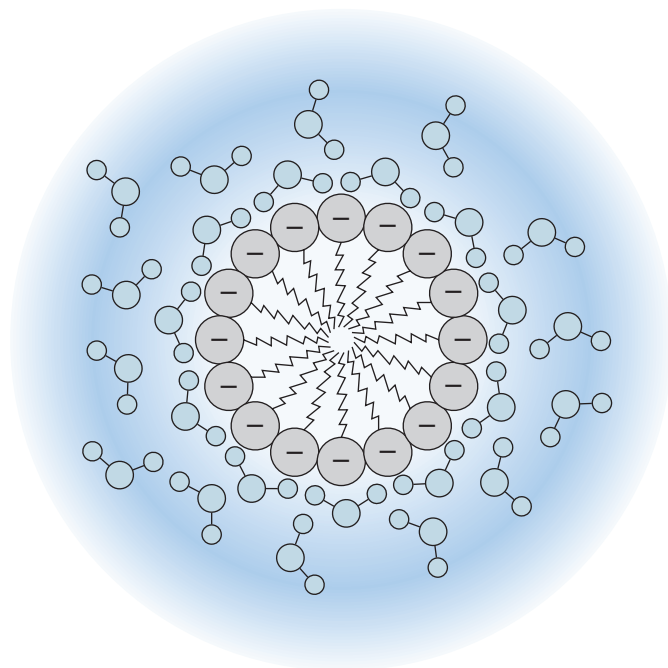
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■ **ACTIVE FIGURE 2.5 Micelle formation by amphipathic molecules in aqueous solution.** When micelles form, the ionized polar groups are in contact with the water, and the nonpolar parts of the molecule are protected from contact with the water. **Sign in at www.thomsonedu. com/login to explore an interactive version of this figure.**

ciation is so short-lived. It is called a **van der Waals interaction** (also referred to as a *van der Waals bond*). The arrangement of molecules in cells strongly depends on the molecules' polarity, as we saw with micelles.
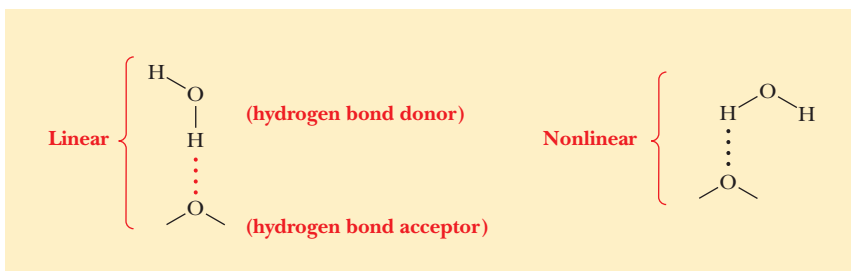
2.2 Hydrogen Bonds

In addition to the interactions discussed in Section 2.1, another important type of noncovalent interaction exists: **hydrogen bonding.** Hydrogen bonding is of electrostatic origin and can be considered a special case of dipole–dipole interaction. When hydrogen is covalently bonded to a very electronegative atom such as oxygen or nitrogen, it has a partial positive charge due to the polar bond, a situation that does not occur when hydrogen is covalently bonded to carbon. This partial positive charge on hydrogen can interact with an unshared (nonbonding) pair of electrons (a source of negative charge) on another electronegative atom. All three atoms lie in a straight line, forming a hydrogen bond. This arrangement allows for the greatest possible partial positive charge on the hydrogen and, consequently, for the strongest possible interaction with the unshared pair of electrons on the second electronegative atom (Figure 2.6). The group comprising the electronegative atom that is covalently bonded to hydrogen is called the *hydrogen-bond donor,* and the electronegative atom that contributes the unshared pair of electrons to the interaction is the *hydrogen-bond acceptor.* The hydrogen is not covalently bonded to the acceptor in the usual description of hydrogen bonding.

   Recent research has cast some doubt on this view, with experimental evidence to indicate some covalent character in the hydrogen bond. Some of this work is described in the article by Hellmans cited in the bibliography at the end of this chapter.

Why does water have such interesting and unique properties?

A consideration of the hydrogen-bonding sites in HF, H_2O, and NH_3 can yield some useful insights. Figure 2.7 shows that water constitutes an optimum situation in terms of the number of hydrogen bonds that each molecule can form. Water has two hydrogens to enter into hydrogen bonds and two unshared pairs of electrons on the oxygen to which other water molecules can be hydrogen-

■ **FIGURE 2.6** **A comparison of linear and nonlinear hydrogen bonds.** Nonlinear bonds are weaker than bonds in which all three atoms lie in a straight line.
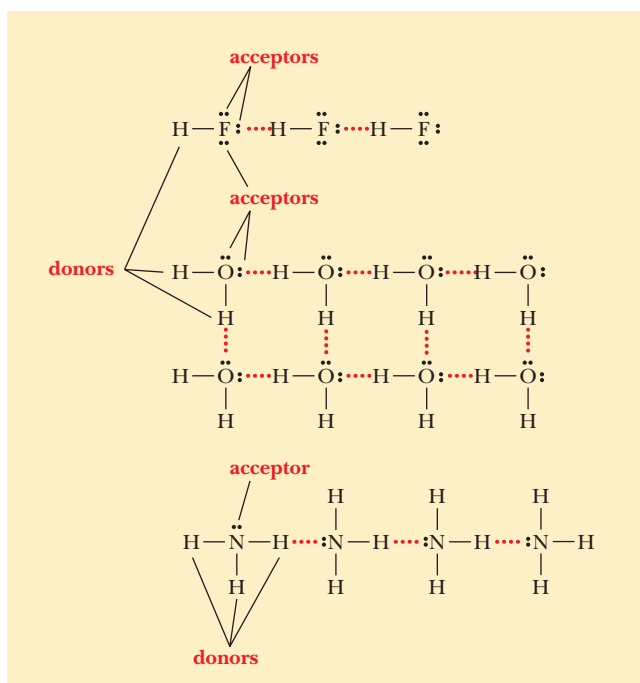
■ **FIGURE 2.7** **Hydrogen-bonding sites.** A comparison of the numbers of hydrogen-bonding sites in HF, H_2O, and NH_3. (Actual geometries are not shown.) Each HF molecule has one hydrogen-bond donor and three hydrogen-bond acceptors. Each H_2O molecule has two donors and two acceptors. Each NH_3 molecule has three donors and one acceptor.

bonded. Each water molecule is involved in four hydrogen bonds—as a donor in two and as an acceptor in two. Hydrogen fluoride has only one hydrogen to enter into a hydrogen bond as a donor, but it has three unshared pairs of electrons on the fluorine that could bond to other hydrogens. Ammonia has three hydrogens to donate to a hydrogen bond but only one unshared pair of electrons, on the nitrogen.

The geometric arrangement of hydrogen-bonded water molecules has important implications for the properties of water as a solvent. The bond angle in water is 104.3°, as was shown in Figure 2.1, and the angle between the unshared pairs of electrons is similar. The result is a tetrahedral arrangement of water molecules. Liquid water consists of hydrogen-bonded arrays that resemble ice crystals; each of these arrays can contain up to 100 water molecules. The hydrogen bonding between water molecules can be seen more clearly in the regular lattice structure of the ice crystal (Figure 2.8). There are several differences, however, between hydrogen-bonded arrays of this type in liquid water and the structure of ice crystals. In liquid water, hydrogen bonds are constantly breaking and new ones are constantly forming, with some molecules breaking off and others joining the cluster. A cluster can break up and re-form in 10^{-10} to 10^{-11} seconds in water at 25°C. An ice crystal, in contrast, has a more-or-less-stable arrangement of hydrogen bonds, and of course its number of molecules is many orders of magnitude greater than 100.

Hydrogen bonds are much weaker than normal covalent bonds. Whereas the energy required to break the O—H covalent bond is 460 kJ mol^{-1} (110 kcal mol^{-1}), the energy of hydrogen bonds in water is about 20 kJ mol^{-1} (5 kcal mol^{-1}) (Table 2.3). Even this comparatively small amount of energy is enough to affect the properties of water drastically, especially its melting point, its boiling point, and its density relative to the density of ice. Both the melting point and the boiling point of water are significantly higher than would be predicted
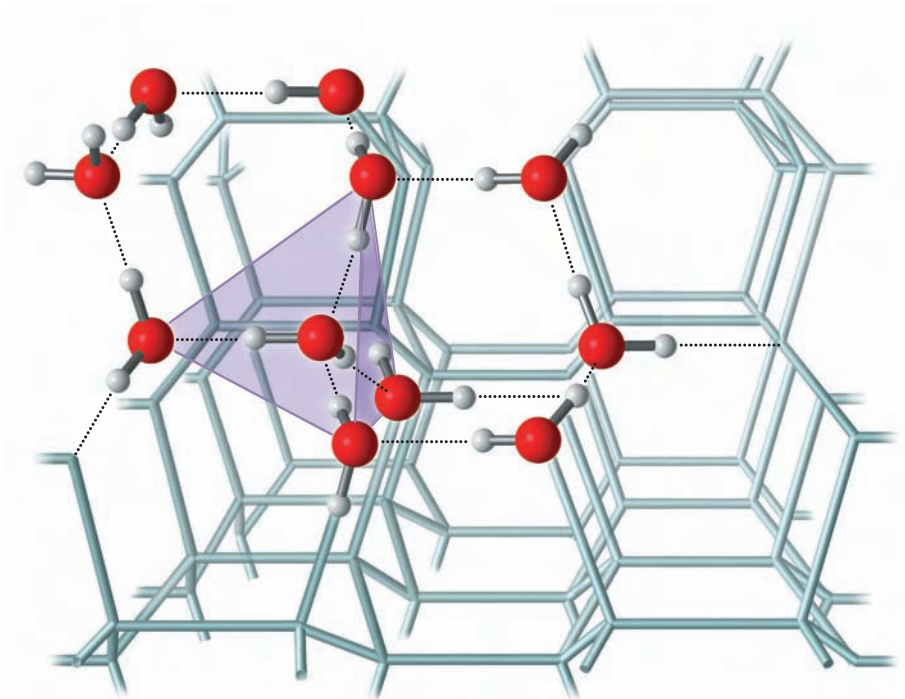
■ **FIGURE 2.8 Tetrahedral hydrogen bonding in H₂O.** In an array of H_2O molecules in an ice crystal, each H_2O molecule is hydrogen-bonded to four others.

for a molecule of this size (Table 2.4). Other substances of about the same molecular weight, such as methane and ammonia, have much lower melting and boiling points. The forces of attraction between the molecules of these substances are weaker than the attraction between water molecules, because of the number and strength of their hydrogen bonds. The energy of this attraction must be overcome to melt ice or boil water.

Ice has a lower density than liquid water because the fully hydrogen bonded array in an ice crystal is less densely packed than that in liquid water. Liquid water is less extensively hydrogen-bonded and thus is denser than ice. Thus, ice cubes and icebergs float. Most substances contract when they freeze, but the

Table 2.3			
Some Bond Energies			
		Energy	
	Type of Bond	**(kJ mol⁻¹)**	**(kcal mol⁻¹)**
Covalent Bonds	O—H	460	110
(Strong)	H—H	416	100
	C—H	413	105
Noncovalent Bonds	Hydrogen bond	20	5
(Weaker)	Ion–dipole interaction	20	5
	Hydrophobic interaction	4–12	1–3
	Van der Waals interactions	4	1

*Note that two units of energy are used throughout this text. The kilocalorie (kcal) is a commonly used unit in the biochemical literature. The kilojoule (kJ) is an SI unit and will come into wider use as time goes on. The kcal is the same as the "Calorie" reported on food labels.
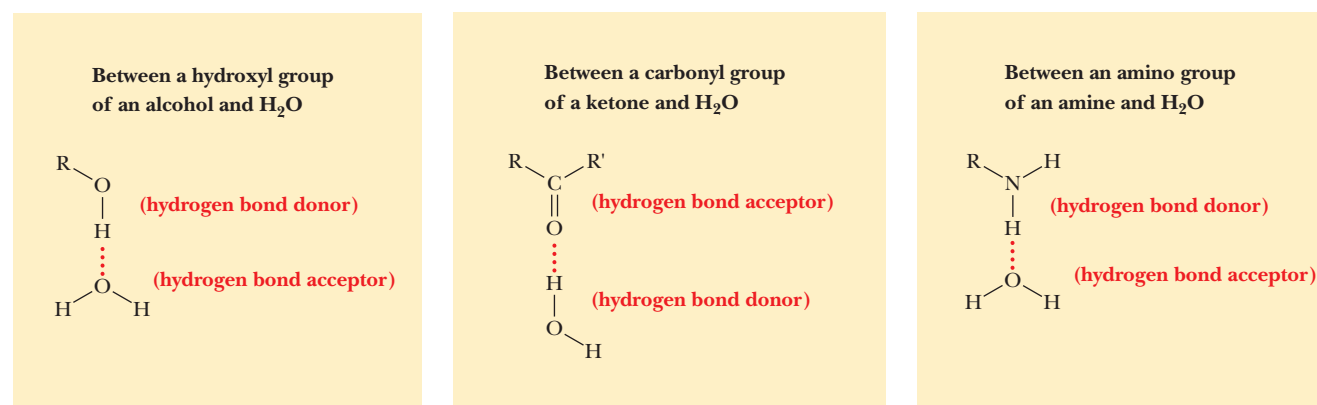
Table 2.4

Comparison of Properties of Water, Ammonia, and Methane

Substance	Molecular Weight	Melting Point (°C)	Boiling Point (°C)
Water (H_2O)	18.02	0.0	100.0
Ammonia (NH_3)	17.03	-77.7	-33.4
Methane (CH_4)	16.04	-182.5	-161.5

opposite is true of water. In cold weather, the cooling systems of cars require antifreeze to prevent freezing and expansion of the water, which could crack the engine block. In laboratory procedures for cell fractionation, the same principle is used in a method of disrupting cells with several cycles of freezing and thawing. Finally, aquatic organisms can survive in cold climates because of the density difference between ice and liquid water; lakes and rivers freeze from top to bottom rather than vice versa.

Hydrogen bonding also plays a role in the behavior of water as a solvent. If a polar solute can serve as a donor or an acceptor of hydrogen bonds, not only can it form hydrogen bonds with water but it can also be involved in nonspecific dipole−dipole interactions. Figure 2.9 shows some examples. Alcohols, amines, carboxylic acids, and esters, as well as aldehydes and ketones, can all form hydrogen bonds with water, so they are soluble in water. It is difficult to overstate the importance of water to the existence of life on the Earth, and it is difficult to imagine life based on another solvent. The following Biochemical Connections box explores some of the implications of this statement.

Other Biologically Important Hydrogen Bonds

Hydrogen bonds have a vital involvement in stabilizing the three-dimensional structures of biologically important molecules, including DNA, RNA, and proteins. The hydrogen bonds between complementary bases are one of the most striking characteristics of the double-helical structure of DNA (Section 9.3). Transfer RNA also has a complex three-dimensional structure characterized by hydrogen-bonded regions (Section 9.5). Hydrogen bonding in proteins gives rise to two important structures, the α-helix and β-pleated sheet conformations. Both types of conformation are widely encountered in proteins (Section 4.3). Table 2.5 summarizes some of the most important kinds of hydrogen bonds in biomolecules.

Between a hydroxyl group of an alcohol and H₂O

R—O—H (hydrogen bond donor)
O—H (hydrogen bond acceptor)

Between a carbonyl group of a ketone and H₂O

R—C—R' (hydrogen bond acceptor)
O
H—O—H (hydrogen bond donor)

Between an amino group of an amine and H₂O

R—N—H (hydrogen bond donor)
O—H (hydrogen bond acceptor)

■ **FIGURE 2.9** Hydrogen bonding between polar groups and water.

Table 2.5

Examples of Major Types of Hydrogen Bonds Found in Biologically Important Molecules

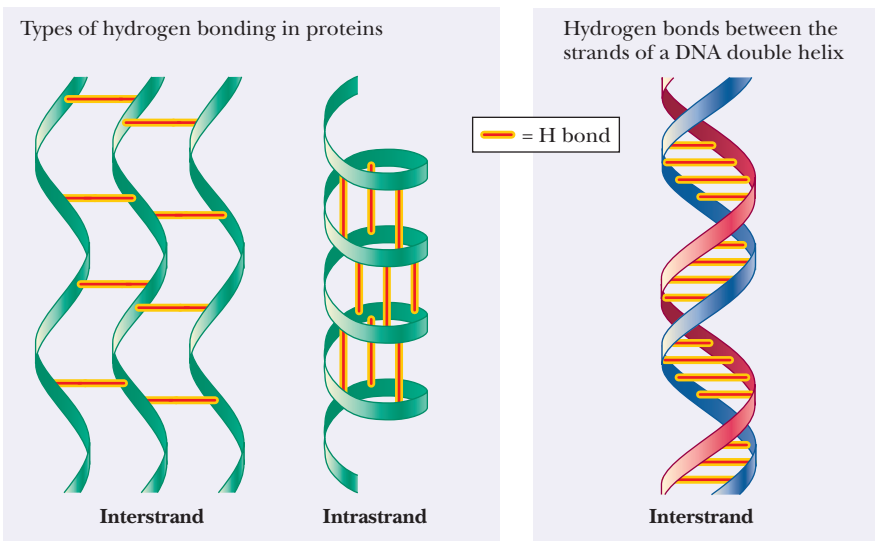| Bonding Arrangement	Molecules Where the Bond Occurs
—O—H••••••O— 　　　　　H	H bond formed in H_2O
—O—H••••••O=C<	Bonding of water to other molecules
N—H••••••O— 　　　　H N—H••••••O=C< N—H••••••N< >N—H••••••N　NH	Important in protein and nucleic acid structures

Biochemical Connections   *CHEMISTRY*

How Basic Chemistry Affects Life: The Importance of the Hydrogen Bond

Many noted biochemists have speculated that the hydrogen bond is essential to the evolution of life. Just like carbon, polymers, and stereochemistry, it is one of the criteria that can be used to search for extraterrestrial life. Even though the individual hydrogen bond (H bond) is weak, the fact that so many H bonds can form means that collectively they can exert a *very* strong force. Virtually all the unique properties of water (high melting and boiling points, ice and density characteristics, and solvent potency) are a result of its ability to form many hydrogen bonds per molecule.

If we look at the solubility of a simple ion like Na^+ or Cl^-, we find that water is attracted to these ions by polarity. In addition, other water molecules form H bonds with those surrounding water molecules, typically 20 or more water molecules per dissolved ion. When we consider a simple biomolecule such as glyceraldehyde, the H bonds start at the molecule itself. At least eight water molecules bind directly to the glyceraldehyde molecule, and then more water molecules bind to those eight.

The orderly and repetitive arrangement of hydrogen bonds in polymers determines their shape. The extended structures of cellulose and of peptides in a β-sheet allow for the formation of strong fibers through intrachain H bonding. Single helices (as in starch) and the α-helices of proteins are stabilized by intrachain H bonds. Double and triple helices, as in DNA and collagen, involve H bonds between the two or three respective strands. Collagen contains several special amino acids that have an extra hydroxyl group; these allow for additional hydrogen bonds, which provide stability.

Hydrogen bonding is also fundamental to the specificity of transfer of genetic information. The complementary nature of the DNA double helix is assured by hydrogen bonds. The genetic code, both its specificity and its allowable variation, is a result of H bonds. Indeed, many compounds that cause genetic mutations work by altering the patterns of H bonding. For example, fluorouracil is often prescribed by dentists for cold sores (viral sores of the lip and mouth) because it causes mutations in the herpes simplex virus that causes the sores.

Types of hydrogen bonding in proteins

Hydrogen bonds between the strands of a DNA double helix

━━ = H bond

Interstrand   **Intrastrand**   **Interstrand**

Section 2.2 Summary
- A hydrogen bond is a special example of a dipole–dipole bond.
- Water molecules are extensively hydrogen bonded.
- The ability to form strong hydrogen bonds is responsible for the many unique characteristics of water, such as its very high melting point and boiling point for a molecule of its size.
- The three-dimensional structures of many important biomolecules, including proteins and nucleic acids, are stabilized by hydrogen bonds.
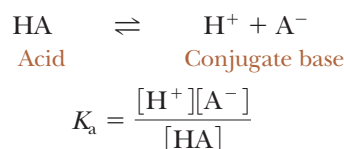

2.3 Acids, Bases, and pH

The biochemical behavior of many important compounds depends on their acid–base properties.
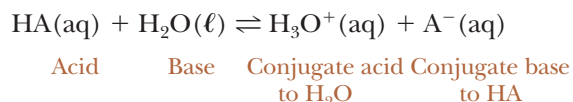
What are acids and bases?

A biologically useful definition of an acid is a molecule that acts as a proton (hydrogen ion) donor. A base is similarly defined as a proton acceptor. How readily acids or bases lose or gain protons depends on the chemical nature of the compounds under consideration. The degree of dissociation of acids in water, for example, ranges from essentially complete dissociation for a strong acid to practically no dissociation for a very weak acid, and any intermediate value is possible.

It is useful to derive a numerical measure of **acid strength,** which is the amount of hydrogen ion released when a given amount of acid is dissolved in water. Such an expression, called the **acid dissociation constant,** or K_a, can be written for any acid, HA, that reacts according to the equation

$$\text{HA} \rightleftharpoons \text{H}^+ + \text{A}^-$$
$$\text{Acid} \qquad\qquad \text{Conjugate base}$$

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

In this expression, the square brackets refer to molar concentration—that is, the concentration in moles per liter. For each acid, the quantity K_a has a fixed numerical value at a given temperature. This value is larger for more completely dissociated acids; thus the greater the K_a, the stronger the acid.

Strictly speaking, the preceding acid–base reaction is a proton-transfer reaction in which water acts as a base as well as the solvent. A more correct way to write the equation is as follows:

$$\text{HA(aq)} + \text{H}_2\text{O}(\ell) \rightleftharpoons \text{H}_3\text{O}^+(\text{aq}) + \text{A}^-(\text{aq})$$
$$\text{Acid} \qquad \text{Base} \quad \text{Conjugate acid} \; \text{Conjugate base}$$
$$\text{to H}_2\text{O} \qquad\quad \text{to HA}$$

The notation (aq) refers to solutes in aqueous solution, whereas (ℓ) refers to water in the liquid state. It is well established that there are no "naked protons" (free hydrogen ions) in solution; even the hydronium ion (H_3O^+) is an underestimate of the degree of hydration of hydrogen ion in aqueous solution. All solutes are extensively hydrated in aqueous solution. We will write the short form of equations for acid dissociation in the interest of simplicity, but the role of water should be kept in mind throughout our discussion.

What is pH?

The acid–base properties of water play an important part in biological processes because of the central role of water as a solvent. The extent of self-dissociation of water to hydrogen ion and hydroxide ion,

$$\text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{OH}^-$$
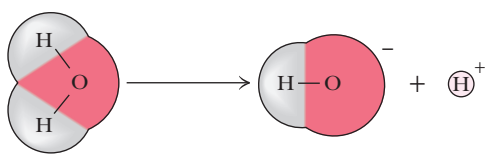
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■ **ACTIVE FIGURE 2.10** **The ionization of water. Sign in at www.thomsonedu.com/login to explore an interactive version of this figure.**
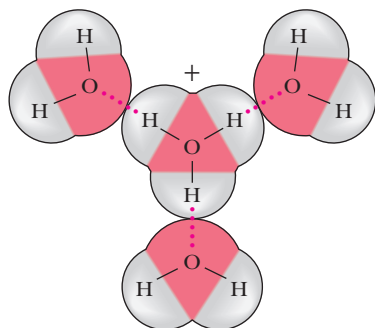
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■ **ANIMATED FIGURE 2.11** **The hydration of hydrogen ion in water. Sign in at www .thomsonedu.com/login to see an animated version of this figure.**

is small, but the fact that it takes place determines important properties of many solutes (Figure 2.10). Both the hydrogen ion (H^+) and the hydroxide ion (OH^-) are associated with several water molecules, as are all ions in aqueous solution, and the water molecule in the equation is itself part of a cluster of such molecules (Figure 2.11). It is especially important to have a quantitative estimate of the degree of dissociation of water. We can start with the expression

$$K_a = \frac{[H^+][OH^-]}{[H_2O]}$$

The molar concentration of pure water, $[H_2O]$, is quite large compared to any possible concentrations of solutes and can be considered a constant. (The numerical value is 55.5 M, which can be obtained by dividing the number of grams of water in 1 L, 1000 g, by the molecular weight of water, 18 g/mol; $1000/18 = 55.5$ M.) Thus,

$$K_a = \frac{[H^+][OH^-]}{55.5}$$

$$K_a \times 55.5 = [H^+][OH^-] = K_w$$

A new constant, K_w, the **ion product constant for water,** has just been defined, where the concentration of water has been included in its value.

The numerical value of K_w can be determined experimentally by measuring the hydrogen ion concentration of pure water. The hydrogen ion concentration is also equal, by definition, to the hydroxide ion concentration because water is a monoprotic acid (one that releases a single proton per molecule). At 25°C in *pure* water,

$$[H^+] = 10^{-7}\ M = [OH^-]$$

Thus, at 25°C, the numerical value of K_w is given by the expression

$$K_w = [H^+][OH^-] = (10^{-7})(10^{-7}) = 10^{-14}$$

This relationship, which we have derived for pure water, is valid for *any* aqueous solution, whether neutral, acidic, or basic.

The wide range of possible hydrogen ion and hydroxide ion concentrations in aqueous solution makes it desirable to define a quantity for expressing these concentrations more conveniently than by exponential notation. This quantity is called *pH* and is defined as

$$pH = -\log_{10}[H^+]$$

with the logarithm taken to the base 10. Note that, because of the logarithms involved, a difference of one pH unit implies a tenfold difference in hydrogen ion concentration, $[H^+]$. The pH values of some typical aqueous samples can be determined by a simple calculation.

Apply Your Knowledge

pH Calculations

From time to time you will find practice sessions such as these in the chapters. These give you the opportunity to practice what was learned immediately.

Because in pure water $[H^+] = 1 \times 10^{-7}\ M$ and pH = 7.0, you can calculate the pH of the following aqueous solutions:

a. $1 \times 10^{-3}\ M$ HCl
b. $1 \times 10^{-4}\ M$ NaOH

Assume that the self-ionization of water makes a negligible contribution to the concentrations of hydronium ions and of hydroxide ions, which will typically be true unless the solutions are extremely dilute.

Solution

The key points in the approach to this problem are the definition of pH, which needs to be used in both parts, and the self-dissociation of water, needed in the second part.

a. For 1×10^{-3} M HCl, $[H_3O^+] = 1 \times 10^{-3}$ M; therefore, pH = 3.

b. For 1×10^{-4} M NaOH, $[OH^-] = 1 \times 10^{-4}$ M. Because $[OH^-][H_3O^+] = 1 \times 10^{-14}$, $[H_3O^+] = 1 \times 10^{-10}$ M; therefore, pH = 10.0.

When a solution has a pH of 7, it is said to be *neutral,* like pure water. Acidic solutions have pH values lower than 7, and basic solutions have pH values higher than 7.

In biochemistry, most of the acids encountered are weak acids. These have a K_a well below 1. To avoid having to use numbers with large, negative exponents, a similar quantity, pK_a, has been defined by analogy with the definition of pH:

$$pK_a = -\log_{10} K_a$$

The pK_a is a more convenient numerical measure of acid strength. The smaller its value, the stronger the acid. This is the reverse of the situation with K_a, where larger values imply stronger acids (Table 2.6).

Why do we want to know the pH?

An equation connects the K_a of any weak acid with the pH of a solution containing both that acid and its conjugate base. This relationship has wide use in biochemistry, especially where it is necessary to control pH for optimum reaction conditions. Some reactions cannot take place if the pH varies from the optimum value. Important biological macromolecules lose activity at extremes of pH. Figure 2.12 shows how the activities of three enzymes are affected by pH. Note that each one has a peak activity that falls off rapidly as the pH is changed from the optimum. Also, some drastic physiological consequences can result from pH fluctuations in the body. Section 2.5 has more information about how pH can be controlled. To derive the involved equation, it is first necessary to take the logarithm of both sides of the K_a equation.

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

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

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

We then use the definitions of pH and pK_a:

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

This relationship is known as the **Henderson–Hasselbalch equation** and is useful in predicting the properties of buffer solutions used to control the

Table 2.6

Dissociation Constants of Some Acids

Acid	HA	A^-	K_a	pK_a
Pyruvic acid	$CH_3COCOOH$	$CH_3C\!-\!COO^-$	3.16×10^{-3}	2.50
Formic acid	$HCOOH$	$HCOO^-$	1.78×10^{-4}	3.75
Lactic acid	$CH_3CHOHCOOH$	$CH_3CH\!-\!HCOO^-$	1.38×10^{-4}	3.86
Benzoic acid	C_6H_5COOH	$C_6H_5COO^-$	6.46×10^{-5}	4.19
Acetic acid	CH_3COOH	CH_3COO^-	1.76×10^{-5}	4.76
Ammonium ion	NH_4^+	NH_3	5.6×10^{-10}	9.25
Oxalic acid (1)	$HOOC\!-\!COOH$	$HOOC\!-\!COO^-$	5.9×10^{-2}	1.23
Oxalic acid (2)	$HOOC\!-\!COO^-$	$^-OOC\!-\!COO^-$	6.4×10^{-5}	4.19
Malonic acid (1)	$HOOC\!-\!CH_2\!-\!COOH$	$HOOC\!-\!CH_2\!-\!COO^-$	1.49×10^{-3}	2.83
Malonic acid (2)	$HOOC\!-\!CH_2\!-\!COO^-$	$^-OOC\!-\!CH_2\!-\!COO^-$	2.03×102^{-6}	5.69
Malic acid (1)	$HOOC\!-\!CH_2\!-\!CHOH\!-\!COOH$	$HOOC\!-\!CH_2\!-\!CHOH\!-\!COO^-$	3.98×10^{-4}	3.40
Malic acid (2)	$HOOC\!-\!CH_2\!-\!CHOH\!-\!COO^-$	$^-OOC\!-\!CH_2\!-\!CHOH\!-\!COO^-$	5.5×10^{-6}	5.26
Succinic acid (1)	$HOOC\!-\!CH_2\!-\!CH_2O\!-\!OOH$	$HOOC\!-\!CH_2\!-\!CH_2\!-\!COO^-$	6.17×10^{-5}	4.21
Succinic acid (2)	$HOOC\!-\!CH_2\!-\!CH_2\!-\!COO^-$	$^-OOC\!-\!CH_2\!-\!CH_2\!-\!COO^-$	2.3×10^{-6}	5.63
Carbonic acid (1)	H_2CO_3	HCO_3^-	4.3×10^{-7}	6.37
Carbonic acid (2)	HCO_3^-	CO_3^{2-}	5.6×10^{-11}	10.20
Citric acid (1)	$HOOC\!-\!CH_2\!-\!C(OH)$ $(COOH)\ OCH_2\!-\!COOH$	$HOOC\!-\!CH_2\!-\!C(OH)$ $(COOH)\ -\!CH_2\!-\!COO^-$	8.14×10^{-4}	3.09
Citric acid (2)	$HOOC\!-\!CH_2\!-\!C(OH)\ (COOH)$ $OCH_2\!-\!COO^-$	$^-OOC\!-\!CH_2\!-\!C(OH)$ $(COOH)\ -\!CH_2\!-\!COO^-$	1.78×10^{-5}	4.75
Citric acid (3)	$^-OOC\!-\!CH_2\!-\!C(OH)\ (COOH)$ $OCH_2\!-\!COO^-$	$^-OOC\!-\!CH_2\!-\!C(OH)\ (COO^-)\!-$ $CH_2\!-\!COO^-$	3.9×10^{-6}	5.41
Phosphoric acid (1)	H_3PO_4	$H_2PO^-_4$	7.25×10^{-3}	2.14
Phosphoric acid (2)	$H_2PO_4^-$	HPO_4^{2-}	6.31×10^{-8}	7.20
Phosphoric acid (3)	HPO_4^{2-}	PO_4^{3-}	3.98×10^{-13}	12.40
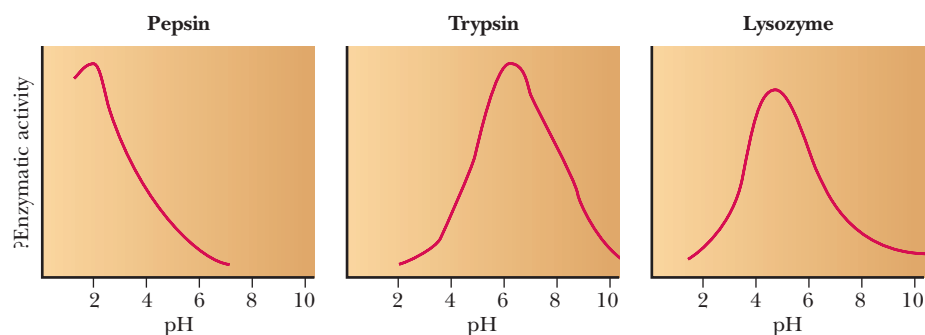
■ **FIGURE 2.12 pH versus enzymatic activity.** Pepsin, trypsin, and lysozyme all have steep pH optimum curves. Pepsin has maximum activity under very acidic conditions, as would be expected for a digestive enzyme that is found in the stomach. Lysozyme has its maximum activity near pH 5, while trypsin is most active near pH 6.

pH of reaction mixtures. When buffers are discussed in Section 2.5, we will be interested in the situation in which the concentration of acid, [HA], and the concentration of the conjugate base, $[A^-]$, are equal ($[HA] = [A^-]$). The ratio $[A^-]/[HA]$ is then equal to 1, and the logarithm of 1 is equal to zero. Therefore, when a solution contains equal concentrations of a weak acid and its conjugate base, the pH of that solution equals the pK_a value of the weak acid.
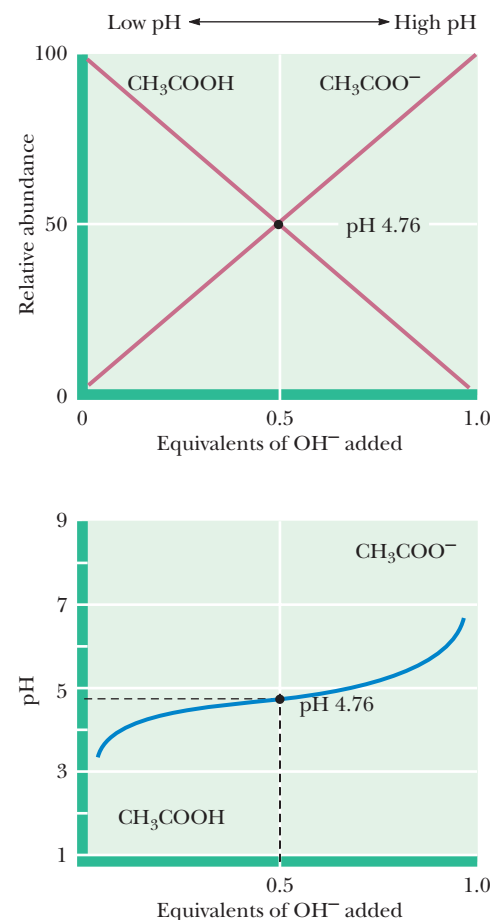
2.4 Titration Curves

When base is added to a sample of acid, the pH of the solution changes. A **titration** is an experiment in which measured amounts of base are added to a measured amount of acid. It is convenient and straightforward to follow the course of the reaction with a pH meter. The point in the titration at which the acid is exactly neutralized is called the **equivalence point.**

If the pH is monitored as base is added to a sample of acetic acid in the course of a titration, an inflection point in the titration curve is reached when the pH equals the pK_a of acetic acid (Figure 2.13). As we saw in our discussion of the Henderson–Hasselbalch equation, a pH value equal to the pK_a corresponds to a mixture with equal concentrations of the weak acid and its conjugate base—in this case, acetic acid and acetate ion, respectively. The pH at the inflection point is 4.76, which is the pK_a of acetic acid. The inflection point occurs when 0.5 mol of base has been added for each mole of acid present. Near the inflection point, the pH changes very little as more base is added.

When 1 mol of base has been added for each mole of acid, the equivalence point is reached, and essentially all the acetic acid has been converted to acetate ion. (See Question 44 at the end of this chapter.) Figure 2.13 also plots the relative abundance of acetic acid and acetate ion with increasing additions of NaOH. Notice that the percentage of acetic acid plus the percentage of acetate ion adds up to 100%. The acid (acetic acid) is progressively converted to its conjugate base (acetate ion) as more NaOH is added and the titration proceeds. It can be helpful to keep track of the percentages of a conjugate acid and base in this way to understand the full significance of the reaction taking place in a titration. The form of the curves in Figure 2.13 represents the behavior of any monoprotic weak acid, but the value of the pK_a for each individual acid determines the pH values at the inflection point and at the equivalence point.

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■ **ANIMATED FIGURE 2.13** **Titration curve for acetic acid.** Note that there is a region near the pK_a at which the titration curve is relatively flat. In other words, the pH changes very little as base is added in this region of the titration curve. **Sign in at www.thomsonedu.com/login to see an animated version of this figure.**

Apply Your Knowledge

Calculating pH Values for Weak Acids and Bases

Calculate the relative amounts of acetic acid and acetate ion present at the following points when 1 mol of acetic acid is titrated with sodium hydroxide. Also use the Henderson–Hasselbalch equation to calculate the values of the pH at these points. Compare your results with Figure 2.13.

a. 0.1 mol of NaOH is added
b. 0.3 mol of NaOH is added
c. 0.5 mol of NaOH is added
d. 0.7 mol of NaOH is added
e. 0.9 mol of NaOH is added

Solution

We approach this problem as an exercise in stoichiometry. There is a 1:1 ratio of moles of acid reacted to moles of base added. The difference between the original number of moles of acid and the number reacted is the number of moles of acid remaining. These are the values to be used in the numerator and denominator, respectively, of the Henderson–Hasselbalch equation.

a. When 0.1 mol of NaOH is added, 0.1 mol of acetic acid reacts with it to form 0.1 mol of acetate ion, leaving 0.9 mol of acetic acid. The composition is 90% acetic acid and 10% acetate ion.

$$pH = pK_a + \log \frac{0.1}{0.9}$$

$$pH = 4.76 + \log \frac{0.1}{0.9}$$

$$pH = 4.76 - 0.95$$

$$pH = 3.81$$

b. When 0.3 mol of NaOH is added, 0.3 mol of acetic acid reacts with it to form 0.3 mol of acetate ion, leaving 0.7 mol of acetic acid. The composition is 70% acetic acid and 30% acetate ion.

$$pH = pK_a + \log \frac{0.3}{0.7}$$

$$pH = 4.39$$

c. When 0.5 mol of NaOH is added, 0.5 mol of acetic acid reacts with it to form 0.5 mol of acetate ion, leaving 0.5 mol of acetic acid. The composition is 50% acetic acid and 50% acetate ion.

$$pH = pK_a + \log \frac{0.5}{0.5}$$

$$pH = 4.76$$

Note that this one is possible without doing much math. We know that when the $[HA] = [A^-]$, the $pH = pK_a$. Therefore, the minute we saw that we added 0.5 mol of NaOH to 1 mol of acetic acid, we knew that we had added enough NaOH to convert half of the acid to the conjugate base form. Therefore the pH has to be equal to the pK_a.

d. When 0.7 mol of NaOH is added, 0.7 mol of acetic acid reacts with it to form 0.7 mol of acetate ion, leaving 0.3 mol of acetic acid. The composition is 30% acetic acid and 70% acetate ion.

$$pH = pK_a + \log \frac{0.7}{0.3}$$

$$pH = 5.13$$

e. When 0.9 mol of NaOH is added, 0.9 mol of acetic acid reacts with it to form 0.9 mol of acetate ion, leaving 0.1 mol of acetic acid. The composition is 10% acetic acid and 90% acetate ion.

$$pH = pK_a + \log \frac{0.9}{0.1}$$

$$pH = 5.71$$

Table 2.6 lists values for the acid dissociation constant, K_a, and for the pK_a for a number of acids. Note that these acids are categorized into three groups. The first group consists of monoprotic acids, which release one hydrogen ion and have a single K_a and pK_a. The second group consists of diprotic acids, which can release two hydrogen ions and have two K_a values and two pK_a values. The third group consists of polyprotic acids, which can release more than two hydrogen ions. The two examples of polyprotic acids given here, citric acid and phosphoric acid, can release three hydrogen ions and have three K_a values and three pK_a values. Amino acids and peptides, the subject of Chapter 3, behave as diprotic and polyprotic acids; we shall see examples of their titration curves later. Here is a way to keep track of protonated and deprotonated forms of acids and their conjugate bases, and this can be particularly useful with diprotic and polyprotic acids. When the pH of a solution is less than the pK_a of an acid, the protonated form predominates. (Remember that the definition of pH includes a negative logarithm.) When the pH of a solution is greater than the pK_a of an acid, the deprotonated (conjugate base) form predominates.

$$pH < pK_a$$

H^+ on, substance protonated

$$pH > pK_a$$

H^+ off, substance deprotonated

Section 2.4 Summary

- In aqueous solution, the relative concentrations of a weak acid and its conjugate base can be related to the titration curve of the acid.
- In the region of the titration curve in which the pH changes very little upon addition of acid or base, the acid/base ratio varies within a narrow range (10:1 at one extreme and 1:10 at the other extreme).

2.5 Buffers

A **buffer** is something that resists change. In terms of acid and base chemistry, a **buffer solution** tends to resist change in pH when small to moderate amounts of a strong acid or strong base are added. A buffer solution consists of a mixture of a weak acid and its conjugate base.

How do buffers work?

Let us compare the changes in pH that occur on the addition of equal amounts of strong acid or strong base to pure water at pH 7 and to a buffer solution at pH 7. If 1.0 mL of 0.1 M HCl is added to 99.0 mL of pure water, the pH drops drastically. If the same experiment is conducted with 0.1 M NaOH instead of 0.1 M HCl, the pH rises drastically (Figure 2.14).

Apply Your Knowledge

How Buffers Work

Calculate the pH value obtained when 1.0 mL of 0.1 M HCl is added to 99.0 mL of pure water. Also, calculate the pH observed when 1.0 mL of 0.1 M NaOH is added to 99.0 mL of pure water. *Hint:* Be sure to take the dilution of both acid and base to the final volume of 100 mL into account.

Solution

Because HCl is a strong acid, we will assume that 0.1 M HCl dissociates completely to give 0.1 $M\,H_3O^+$. If we have 1 mL of the acid, we calculate the amount of H_3O^+ as follows:

$$1\,mL = 1 \times 10^{-3}\,L$$

$$1 \times 10^{-3}\,L \times 0.1\,mol/L = 1 \times 10^{-4}\,mol\,H_3O^+$$

Therefore, 1×10^{-4} mol of H_3O^+ are diluted to a final volume of 100 mL or 0.1 L, because 1 mL was added to 99 mL. The final concentration of H_3O^+ is calculated as follows:

$$1 \times 10^{-4}\,mol\,H_3O^+/0.1\,L = 1 \times 10^{-3}\,M$$

The pH is then calculated based on the definition:

$$pH = -\log\,[H_3O^+] = -\log\,(1 \times 10^{-3}) = 3$$

For the added base the calculations are done similarly to generate the concentration of $[OH^-]$, which also equals 1×10^{-3} M because we used the same concentration and same volume of base.
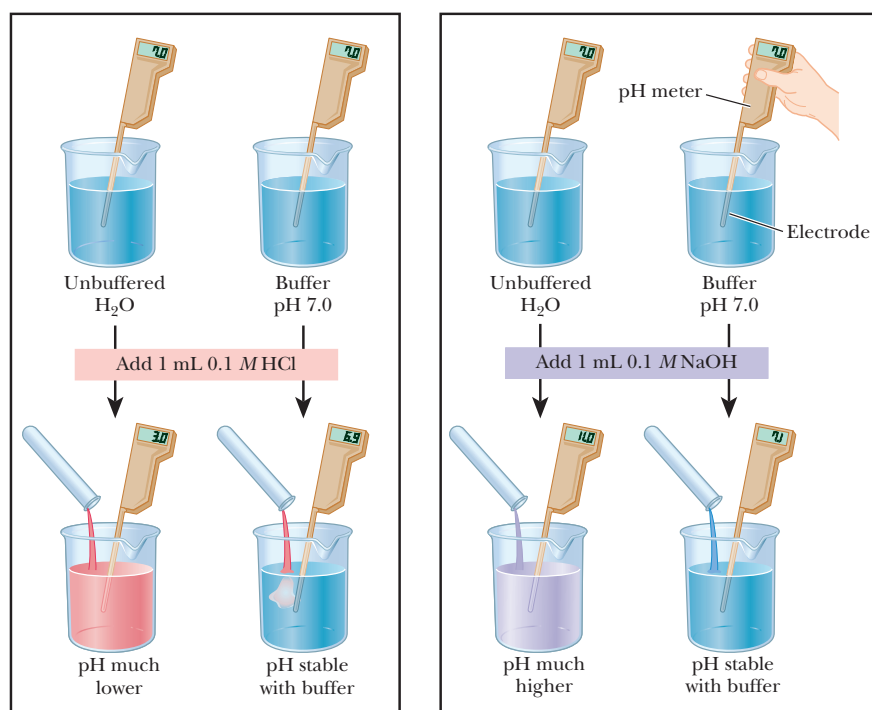
The $[H_3O^+]$ is then calculated using the concentration of OH^- and the water equation:

$$[OH^-][H_3O^+] = 1 \times 10^{-14}$$

$$[H_3O^+] = 1 \times 10^{-14}/[OH^-] = 1 \times 10^{-14}/1 \times 10^{-3} = 1 \times 10^{-11}$$

Finally, the pH is calculated:

$$pH = -\log\,(1 \times 10^{-11}) = 11$$

■ **FIGURE 2.14 Buffering.** Acid is added to the two beakers on the left. The pH of unbuffered H_2O drops dramatically while that of the buffer remains stable. Base is added to the two beakers on the right. The pH of the unbuffered water rises drastically while that of the buffer remains stable.

The results are different when 99.0 mL of buffer solution is used instead of pure water. A solution that contains the monohydrogen phosphate and dihydrogen phosphate ions, HPO_4^{2-} and $H_2PO_4^-$, in suitable proportions can serve as such a buffer. The Henderson–Hasselbalch equation can be used to calculate the $HPO_4^{2-}/H_2PO_4^-$ ratio that corresponds to pH 7.0.

Apply Your Knowledge

Using the Henderson–Hasselbalch Equation

First, convince yourself that the proper ratio of A^-/HA for pH 7.00 is 0.63 parts HPO_4^{2-} to 1 part $H_2PO_4^-$ by doing the calculation.

Solution

Use the Henderson–Hasselbalch equation with pH $= 7.00$ and $pK_a = 7.20$.

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

$$7.00 = 7.20 + \log \frac{[HPO_4^{2-}]}{[H_2PO_4^-]}$$

$$-0.20 = \log \frac{[HPO_4^{2-}]}{[H_2PO_4^-]}$$

$$\frac{[HPO_4^{2-}]}{[H_2PO_4^-]} = \text{antilog} - 0.20 = 0.63$$

For purposes of illustration, let us consider a solution in which the concentrations are $[HPO_4^{2-}] = 0.063\ M$ and $[H_2PO_4^-] = 0.10\ M$; this gives the conjugate base/weak acid ratio of 0.63 seen previously. If 1.0 mL of 0.10 M HCl is added to 99.0 mL of the buffer, the reaction

$$[HPO_4^{2-}] + H^+ \rightleftharpoons H_2PO_4^-$$

takes place, and almost all the added H^+ will be used up. The concentrations of $[HPO_4^{2-}]$ and $[H_2PO_4^-]$ will change, and the new concentrations can be calculated.

Concentrations (mol/L)			
	$[HPO_4^{2-}]$	$[H^+]$	$[H_2PO_4^-]$
Before addition of HCl	0.063	1×10^{-7}	0.10
HCl added—no reaction yet	0.063	1×10^{-3}	0.10
After HCl reacts with HPO_4^{2-}	0.062	To be found	0.101

The new pH can then be calculated using the Henderson−Hasselbalch equation and the phosphate ion concentrations. The appropriate pK_a is 7.20 (Table 2.6).

$$ph = pK_a + \log \frac{[HPO_4^{2-}]}{[H_2PO_4^-]}$$

$$pH = 7.20 + \log \frac{0.062}{0.101}$$

$$pH = 6.99$$

The new pH is 6.99, a much smaller change than in the unbuffered pure water (Figure 2.14). Similarly, if 1.0 mL of 0.1 M NaOH is used, the same reaction takes place as in a titration:

$$H_2PO_4^- + OH^- \rightleftharpoons HPO_4^{2-}$$

Almost all the added OH^- is used up, but a small amount remains. Because this buffer is an aqueous solution, it is still true that $K_w = [H^+][OH^-]$. The increase in hydroxide ion concentration implies that the hydrogen ion concentration decreases and that the pH increases. Use the Henderson–Hasselbalch equation to calculate the new pH and to convince yourself that the result is pH = 7.01, again a much smaller change in pH than took place in pure water (Figure 2.14). Many biological reactions will not take place unless the pH remains within fairly narrow limits, and, as a result, buffers have great practical importance in the biochemistry laboratory.

How do we choose a buffer?

A consideration of titration curves can give insight into how buffers work (Figure 2.15a). The pH of a sample being titrated changes very little in the vicinity of the inflection point of a titration curve. Also, at the inflection point, half the amount of acid originally present has been converted to the conjugate base. The second stage of ionization of phosphoric acid,

$$H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{2-}$$

was the basis of the buffer just used as an example. The pH at the inflection point of the titration is 7.20, a value numerically equal to the pK_a of the dihydrogen phosphate ion. At this pH, the solution contains equal concentrations of the dihydrogen phosphate ions and monohydrogen phosphate ions, the acid and base forms. Using the Henderson–Hasselbalch equation, we can calculate the ratio of the conjugate base form to the conjugate acid form for any pH when we know the pK_a. For example, if we choose a pH of 8.2 for a buffer composed of $H_2PO_4^-$ and HPO_4^{2-}, we can solve for the ratio

$$ph = pK_a + \log \frac{[HPO_4^{2-}]}{[H_2PO_4^-]}$$

$$8.2 = 7.2 + \log \frac{[HPO_4^{2-}]}{[H_2PO_4^-]}$$

$$1 = \log \frac{[HPO_4^{2-}]}{[H_2PO_4^-]}$$

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

Thus, when the pH is one unit higher than the pK_a, the ratio of the conjugate base form to the conjugate acid form is 10. When the pH is two units higher than the pK_a, the ratio is 100, and so on. Table 2.7 shows this relationship for several increments of pH value.

A buffer solution can maintain the pH at a relatively constant value because of the presence of appreciable amounts of both the acid and its conjugate base. This condition is met at pH values at or near the pK_a of the acid. If OH^- is added, an appreciable amount of the acid form of the buffer is present in solution to react with the added base. If H^+ is added, an appreciable amount of the basic form of the buffer also is present to react with the added acid.

The H_2PO_4/HPO_4^- pair is suitable as a buffer near pH 7.2, and the CH_3COOH/CH_3COO^- pair is suitable as a buffer near pH 4.76. At pH values below the pK_a, the acid form predominates, and at pH values above the pK_a,
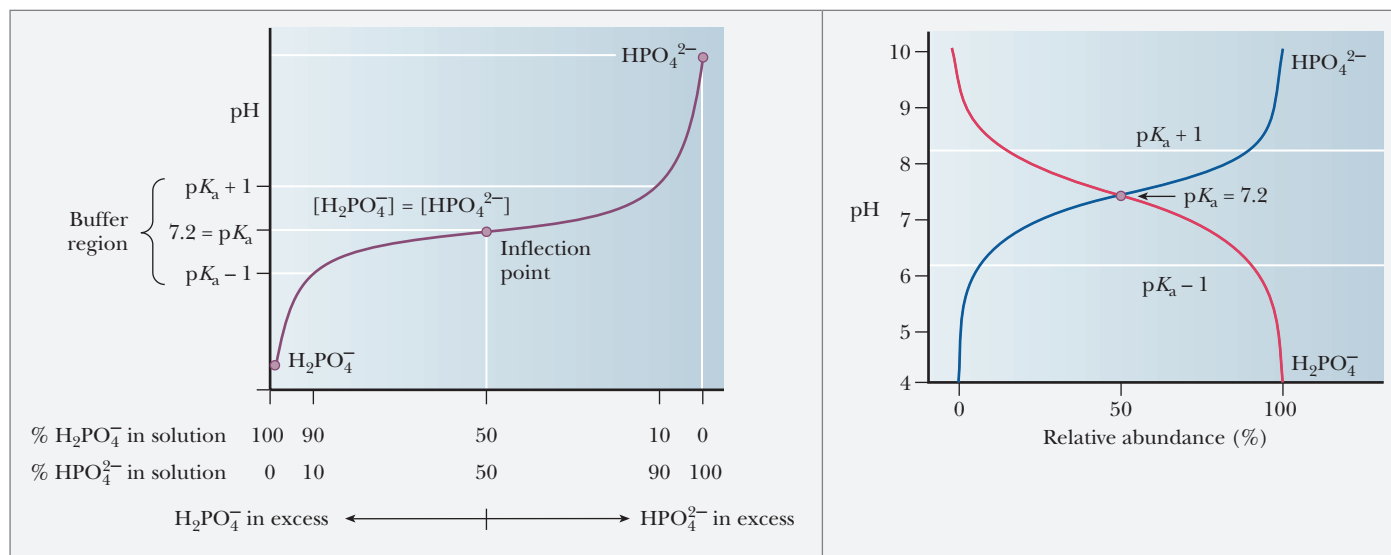
A   The titration curve of $H_2PO_4^-$, showing the buffer region for the $H_2PO_4^-/HPO_4^{2-}$ pair.

B   Relative abundance of $H_2PO_4^-$ and HPO_4^{2-}.

■ **FIGURE 2.15** The relationship between the titration curve and buffering action in $H_2PO_4^-$.

the basic form predominates. The plateau region in a titration curve, where the pH does not change rapidly, covers a pH range extending approximately one pH unit on each side of the pK_a. Thus, the buffer is effective within a range of about two pH units (Figure 2.15b).

In many biochemical studies, a strict pH range must be maintained in order for the experiment to be successful. Using our knowledge of the range of an effective buffer compared to its pK_a, we can select an appropriate buffer. If we were doing an experiment and needed the pH to be 7.2, we might select the H_2PO_4/HPO_4^- pair to be our buffer. If we wanted a pH near 9.0, we would look at tables of buffers to find one with a pK_a close to nine. The following Biochemical Connections box goes into greater detail on buffer selection.

The condition that a buffer contains appreciable amounts of both a weak acid and its conjugate base applies both to the ratio of the two forms and to the absolute amount of each present in a given solution. If a buffer solution contained a suitable ratio of acid to base, but very low concentrations of both,

Table 2.7

pH Values and Base/Acid Ratios for Buffers

If the pH equals	The ratio of base form/acid form equals
$pK_a - 3$	1/1000
$pK_a - 2$	1/100
$pK_a - 1$	1/10
pK_a	1/1
$pK_a + 1$	10/1
$pK_a + 2$	100/1
$pK_a + 3$	1000/1

Titration curve
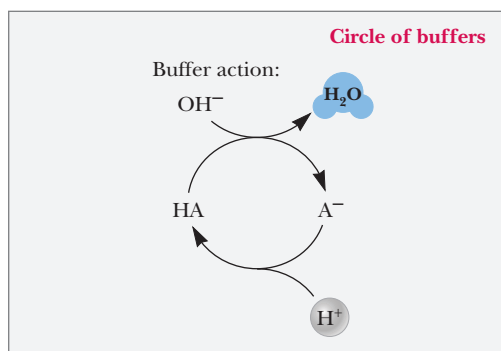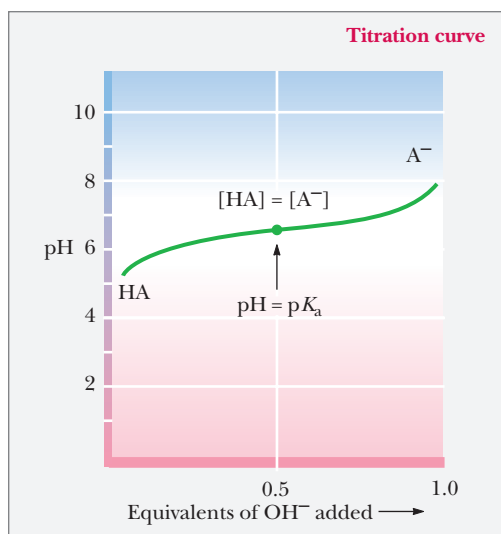
Circle of buffers

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■ **ACTIVE FIGURE 2.16** **Two ways of looking at buffers.** In the titration curve, we see that the pH varies only slightly near the region in which [HA] = [A⁻]. In the circle of buffers, we see that adding OH⁻ to the buffer converts HA to A⁻. Adding H⁺ converts A⁻ to HA. **Sign in at www.thomsonedu.com/login to explore an interactive version of this figure.**

it would take very little added acid to use up all of the base form, and vice versa. A buffer solution with low concentrations of both the acid and base forms is said to have a low **buffering capacity.** A buffer that contains greater amounts of both acid and base has a higher buffering capacity.

How do we make buffers in the laboratory?

When we study buffers in theory, we often use the Henderson−Hasselbalch equation and do many calculations concerning ratios of conjugate base form to conjugate acid form. In practice, however, making a buffer is much easier. To have a buffer, all that is necessary are the two forms of the buffer present in the solution at reasonable quantities. This situation can be obtained by adding predetermined amounts of the conjugate base form (A⁻) to the acid form (HA), or we could start with one and create the other, which is how it is usually done in practice. Remember that HA and A⁻ are interconverted by adding strong acid or strong base (Figure 2.16). To make a buffer, we could start with the HA form and add NaOH until the pH is correct, as determined by a pH meter. We could also start with A⁻ and add HCl until the pH is correct. Depending on the relationship of the pH we desire to the pK_a of the buffer, it may be more convenient to start with one than the other. For example, if we are making an acetic acid/acetate buffer at pH 5.7, it would make more sense to start with the A⁻ form and to add a small amount of HCl to bring the pH down to 5.7, rather than to start with HA and to add much more NaOH to bring the pH up past the pK_a.

Are naturally occurring pH buffers present in living organisms?

Up until now, we have been considering buffers from the perspective of a chemist trying to control an experiment. However, the real importance of buffers is that they are critical to life. Buffer systems in living organisms and in the laboratory are based on many types of compounds. Because physiological pH in most organisms stays around 7, it might be expected that the phosphate buffer system would be widely used in living organisms. This is the case where phosphate ion concentrations are high enough for the buffer to be effective, as in most intracellular fluids.
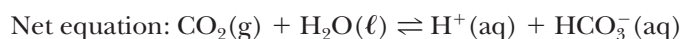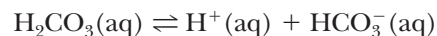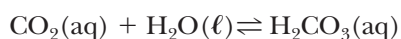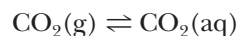
The H_2PO_4/HPO_4^- pair is the principal buffer in cells. In blood, phosphate ion levels are inadequate for buffering, and a different system operates.

The buffering system in blood is based on the dissociation of carbonic acid (H_2CO_3):

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

where the pK_a of H_2CO_3 is 6.37. The pH of human blood, 7.4, is near the end of the buffering range of this system, but another factor enters into the situation.

Carbon dioxide can dissolve in water and in water-based fluids, such as blood. The dissolved carbon dioxide forms carbonic acid, which, in turn, reacts to produce bicarbonate ion:

$$CO_2(g) \rightleftharpoons CO_2(aq)$$

$$CO_2(aq) + H_2O(\ell) \rightleftharpoons H_2CO_3(aq)$$

$$H_2CO_3(aq) \rightleftharpoons H^+(aq) + HCO_3^-(aq)$$

$$\text{Net equation: } CO_2(g) + H_2O(\ell) \rightleftharpoons H^+(aq) + HCO_3^-(aq)$$

Biochemical Connections *BUFFER CHEMISTRY*

Buffer Selection

Much of biochemistry is studied by carrying out enzymatic reactions in a test tube or *in vitro* (literally, in glass). Such reactions are usually buffered to maintain a constant pH. Similarly, virtually all methods for enzyme isolation, and even for growth of cells in tissue culture, use buffered solutions. The following criteria are typical for selecting a buffer for a biochemical reaction.

1. Suitable pK_a for the buffer.
2. No interference with the reaction or with the assay.
3. Suitable ionic strength of the buffer.
4. No precipitation of reactants or products due to presence of the buffer.
5. Nonbiological nature of the buffer.

The rule of thumb is that the pK_a should be ±1 pH unit from the pH of the reaction; ±½ pH unit is even better. Although the perfect generic buffer would have a pH equal to its pK_a, if the reaction is known to produce an acidic product, it is advantageous if the pK_a is below the reaction pH, because then the buffer capacity increases as the reaction proceeds.

Sometimes a buffer can interfere with a reaction or with the assay method. For example, a reaction that requires or produces phosphate or CO_2 may be inhibited if too much phosphate or carbonate is present in the reaction mixture. Even the counterion may be important. Typically a phosphate or carbonate buffer is prepared from the Na^+ or K^+ salt. Because many enzymes that react with nucleic acids are activated by one of these two ions and inhibited by the other, the choice of Na^+ or K^+ for a counterion could be critical. A buffer can also affect the spectrophotometric determination of a colored assay product.

If a buffer has a poor buffering capacity at the desired pH, its efficiency can often be increased by increasing the concentration; however, many enzymes are sensitive to high salt concentration. Beginning students in biochemistry often have difficulty with enzyme isolations and assays because they fail to appreciate the sensitivity of many enzymes. Fortunately, to minimize this problem, most beginning biochemistry laboratory manuals call for the use of enzymes that are very stable.

A buffer may cause precipitation of an enzyme or even of a metallic ion that may be a cofactor for the reaction. For example, many phosphate salts of divalent cations are only marginally soluble.

Finally, it is often desirable to use a buffer that has no biological activity at all, so it can never interfere with the system being studied. TRIS is a very desirable buffer, because it rarely interferes with a reaction. Special buffers, such as HEPES and PIPES (Table 2.8), have been developed for growing cells in tissue culture.

At the pH of blood, which is about one unit higher than the pK_a of carbonic acid, most of the dissolved CO_2 is present as HCO_3^-. The CO_2 being transported to the lungs to be expired takes the form of bicarbonate ion. A direct relationship exists between the pH of the blood and the pressure of carbon dioxide gas in the lungs. The properties of hemoglobin, the oxygen-carrying protein in the blood, also enter into the situation (see the Biochemical Connections box in Chapter 4).

The phosphate buffer system is common in the laboratory (*in vitro,* outside the living body) as well as in living organisms *(in vivo)*. The buffer system based on TRIS [*tris*(hydroxymethyl)aminomethane] is also widely used in vitro. Other buffers that have come into wide use more recently are **zwitterions,** which are compounds that have both a positive charge and a negative charge. Zwitterions are usually considered less likely to interfere with biochemical reactions than some of the earlier buffers (Table 2.8).

Most living systems operate at pH levels close to 7. The pK_a values of many functional groups, such as the carboxyl and amino groups, are well above or well below this value. As a result, under physiological conditions, many important biomolecules exist as charged species to one extent or another. The practical consequences of this fact are explored in the following Biochemical Connections box.

Table 2.8

Acid and Base Form of Some Useful Biochemical Buffers

| Acid Form		Base Form	pK_a
TRIS—H$^+$ (protonated form) $(HOCH_2)_3CNH_3^+$	N—*tris*[hydroxymethyl]aminomethane (TRIS) $\rightleftharpoons$	TRIS (free amine) $(HOCH_2)_3CNH_2$	8.3
$^-$TES—H$^+$ (zwitterionic form) $(HOCH_2)_3C\overset{+}{N}H_2CH_2CH_2SO_3^-$	N—*tris*[hydroxymethyl]methyl-2-aminoethane sulfonate (TES) $\rightleftharpoons$	$^-$TES (anionic form) $(HOCH_2)_3CNHCH_2CH_2SO_3^-$	7.55
$^-$HEPES—H$^+$ (zwitterionic form) $HOCH_2CH_2N^+\ NCH_2CH_2SO_3^-$ H	N—2—hydroxyethylpiperazine-N′-2-ethane sulfonate (HEPES) $\rightleftharpoons$	$^-$HEPES (anionic form) $HOCH_2CH_2N\ NCH_2CH_2SO_3^-$	7.55
$^-$MOPS—H$^+$ (zwitterionic form) $O\ ^+NCH_2CH_2CH_2SO_3^-$ H	3—[N—morpholino]propane-sulfonic acid (MOPS) $\rightleftharpoons$	$^-$MOPS (anionic form) $O\ NCH_2CH_2CH_2SO_3^-$	7.2
$^{2-}$PIPES—H$^+$ (protonated dianion) $^-O_3SCH_2CH_2N\ ^+NCH_2CH_2SO_3^-$ H	Piperazine—N,N′-*bis*[2-ethanesulfonic acid] (PIPES) $\rightleftharpoons$	$^{2-}$PIPES (dianion) $^-O_3SCH_2CH_2N\ NCH_2CH_2SO_3^-$	6.8

Biochemical Connections   *CHEMISTRY OF THE BLOOD*

Some Physiological Consequences of Blood Buffering

The process of respiration plays an important role in the buffering of blood. In particular, an increase in H$^+$ concentration can be dealt with by raising the rate of respiration. Initially, the added hydrogen ion binds to bicarbonate ion, forming carbonic acid.

$$H^+(aq) + HCO_3^-(aq) \rightleftharpoons H_2CO_3(aq)$$
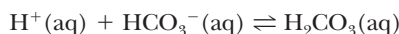
An increased level of carbonic acid raises the levels of dissolved carbon dioxide and, ultimately, gaseous carbon dioxide in the lungs.

$$H_2CO_3(aq) \rightleftharpoons CO_2(aq) + H_2O(\ell)$$

$$CO_2(aq) \rightleftharpoons CO_2(g)$$
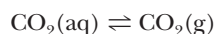
A high respiration rate removes this excess carbon dioxide from the lungs, starting a shift in the equilibrium positions of all the foregoing reactions. The removal of gaseous CO$_2$ decreases the amount of dissolved CO$_2$. Hydrogen ion reacts with HCO$_3$, and, in the process, lowers the H$^+$ concentration of blood back to its original level. In this way, the blood pH is kept constant.

In contrast, *hyperventilation* (excessively deep and rapid breathing) removes such large amounts of carbon dioxide from the lungs that it raises the pH of blood, sometimes to dangerously high levels that bring on weakness and fainting. Athletes, however, have learned how to use the increase in blood pH caused by hyperventilation. Short bursts of strenuous exercise produce high levels of lactic acid in the blood as a result of the anaerobic breakdown of glycogen. The presence of so much lactic acid tends to lower the pH of the blood, but a brief (30-second) period of hyperventilation before a short-distance event (say, a 400-m dash, 100-m swim, 1-km bicycle race, or any event that lasts between 30 seconds and about a minute) counteracts the effects of the added lactic acid and maintains the pH balance.

An increase in H$^+$ in blood can be caused by large amounts of any acid entering the bloodstream. Aspirin, like lactic acid, is an acid, and extreme acidity resulting from the ingestion of large doses of aspirin can cause *aspirin poisoning*. Exposure to *high altitudes* has an effect similar to hyperventilation at sea level. In response to the tenuous atmosphere, the rate of respiration increases. As with hyperventilation, more carbon dioxide is expired from the lungs, ultimately lowering the H$^+$ level in blood and raising the pH. When people who normally live at sea level are suddenly placed at a high elevation, their blood pH rises temporarily, until they become acclimated.

Biochemical Connections   *ACIDS AND SPORTS*

Lactic Acid—Not Always the Bad Guy

If you ask anybody who knows something of sports about lactic acid, you will likely hear that it is the acid that causes muscle pain and muscle fatigue. This has been the dogma since 1929, when the first papers were written about accumulation of lactic acid in muscle tissue under anaerobic conditions. However, lactic acid is not biochemically all bad and, in fact, recent evidence suggests that there are some benefits we did not previously know about.

In the first place, we should distinguish between the hydrogen ion dissociated from the lactic acid and the conjugate base, lactate. The H^+ ion released is the ion that is the reactive species and most likely accounts for the pain we associate with lactic acid buildup in the muscles. The lowering of the cellular pH would have effects on a variety of enzyme and muscular systems. However, the conjugate base, lactate, is removed by the blood and makes its way to the liver. Once in the liver, it is converted to glucose via a process called gluconeogenesis that we will see in Chapter 18. Hospital patients are often given intravenous lactate solutions to indirectly help keep their blood glucose levels up.

Until recently, any athlete would have proclaimed the evils of lactic acid on muscle performance. However, recent evidence suggests that lactic acid actually has a positive effect on fatiguing muscles (see the articles by Allen and Westerblad and by Pedersen et al. cited in the bibliography of this chapter). The results of this study suggested that in a situation where muscles were becoming fatigued, the lactic acid actually maintained the muscle membrane's ability to depolarize and repolarize longer, allowing the muscles to continue to contract even though they were fatigued. When isolated muscle cells were stripped of lactic acid, they fatigued even faster. The same evidence was seen with cases of humans that suffered from a disease where they lacked the enzyme that breaks down muscle glycogen. Without the muscle glycogen breakdown, anaerobic metabolism is impossible and no lactic acid accumulates. The results—these patients' muscle fibers fatigued even faster. Some of the "knowledge" about the undesirable effect of lactic acid on muscle fatigue may be explained by the fact that muscle pain seems linked to decreased muscle performance. Lactic acid, as the putative cause of the muscle pain, was therefore assumed to also be the cause of the fatigue. This is an active area of research and we still have a lot to learn about it. Despite decades of study and popular myth, we still do not really know exactly what causes muscle fatigue.

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Section 2.5 Summary

- Buffer solutions are characterized by their tendency to resist pH change when small amounts of strong acid or strong base are added.
- Buffers work because the concentration of the weak acid and base is kept in the narrow window of the acid titration curve.
- Many experiments must have a buffered system to keep a stable pH.
- Many physiological buffers, such as the bicarbonate blood buffer or the phosphate buffer, help maintain physiological pH.

Summary

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What is polarity? When two atoms with the same electronegativity form a bond, the electrons are shared equally between the two atoms. However, if atoms with differing electronegativity form a bond, the electrons are not shared equally and more of the negative charge is found closer to one of the atoms.

Why do some chemicals dissolve in water while others do not? The polar nature of water largely determines its solvent properties. Ionic compounds with full charges and polar compounds with partial charges tend to dissolve in water. The underlying physical principle is electrostatic attraction between unlike charges. The negative end of a water dipole attracts a positive ion or the positive end of another dipole. The positive end of a water molecule attracts a negative ion or the negative end of another dipole.

Why do oil and water mixed together separate into layers? Oil molecules are amphipathic—having both polar (hydrophilic) heads and nonpolar (hydrophobic) tail portions. When oil and water separate into layers, the polar head groups of the oil molecules are in contact with the aqueous environment and the nonpolar tails are sequestered from the water. Van der Waals interactions between nonpolar

molecules provide the energetic basis for this spontaneous molecular arrangement.

Why does water have such interesting and unique properties? Water has unique properties for a molecule its size, such as a very high boiling point and melting point. This is due to the extensive hydrogen bonding possible between water molecules. Each water molecule has two sources of partial positive charge and two of partial negative charge. This allows water to form an array in a solid form and to bond with many other water molecules in liquid form. The extensive hydrogen bonding requires large amounts of energy to disrupt, and therefore it melts and boils at higher temperatures than other molecules of its relative size.

What are acids and bases? Acids are compounds that release hydrogen ions (protons) when dissolved in aqueous solution. In other words, they are proton donors. Bases are compounds that are proton acceptors.

What is pH? The mathematical definition of pH is the negative of the logarithm of the hydrogen ion concentration. It is a measure of the acidity of the solution. The lower the pH, the more acidic the solution. Because of the log term, a pH change of one unit means a tenfold change in hydrogen ion concentration.

Why do we want to know the pH? It is important to know the pH because many biological reactions require a very tight range of pH values. For example, an enzyme that is active at pH 7.0 may be completely inactive at pH 8.0. Solutions used in science often must have their pH controlled in order to have an experiment function correctly. While local variations in pH may occur in certain subcellular organelles, a cell must maintain a pH near neutrality in order to stay alive.

How do buffers work? Buffers work based on the nature of weak acids and their conjugate bases that compose the buffer. If a source of extra hydrogen ion is added to a buffer solution, it reacts with the conjugate base to form the weak acid. If a source of hydroxide ion is added to the buffer, it reacts with the weak acid to form water and the conjugate base. In this way, either added H^+ or OH^- is "used up" by adding it to a buffer. This keeps the pH much more stable than if the same acid or base had been added to an unbuffered system.

How do we choose a buffer? We choose a buffer primarily by knowing the pH that we wish to maintain. For example, if we are performing an experiment and we want the solution to stay at pH 7.5, we look for a buffer that has a pK_a of 7.5 because buffers are most effective when the pH is close to the buffer pK_a.

How do we make buffers in the laboratory? The most efficient way to make a buffer in the laboratory is to add either the weak acid form or the weak base form of the buffer compound to a container, add water, and then measure the pH with a pH meter. The pH will be either too low or too high. We then add strong acid or strong base until the pH is the desired buffer pH. Then we bring the solution up to the final volume so that concentration is correct.

Are naturally occurring pH buffers present in living organisms? Buffers are not just an artificial system used in the laboratory. Living systems are buffered by naturally occurring compounds. Naturally occurring phosphate and carbonate buffers help maintain physiological pH near 7.0.

# Review Exercises

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2.1 Water and Polarity

1. **Reflect and Apply** Why is water necessary for life?
2. **Reflect and Apply** Contemplate biochemistry if atoms did not differ in electronegativity.

2.2 Hydrogen Bonds

3. **Recall** What are some macromolecules that have hydrogen bonds as a part of their structures?
4. **Biochemical Connections** How are hydrogen bonds involved in the transfer of genetic information?
5. **Reflect and Apply** Rationalize the fact that hydrogen bonding has not been observed between CH_4 molecules.
6. **Reflect and Apply** Draw three examples of types of molecules that can form hydrogen bonds.
7. **Recall** What are the requirements for molecules to form hydrogen bonds? (What atoms must be present and involved in such bonds?)
8. **Reflect and Apply** Many properties of acetic acid can be rationalized in terms of a hydrogen-bonded dimer. Propose a structure for such a dimer.
9. **Reflect and Apply** How many water molecules could hydrogen-bond *directly* to the molecules of glucose, sorbitol, and ribitol, shown here?

Glucose

Sorbitol   Ribitol
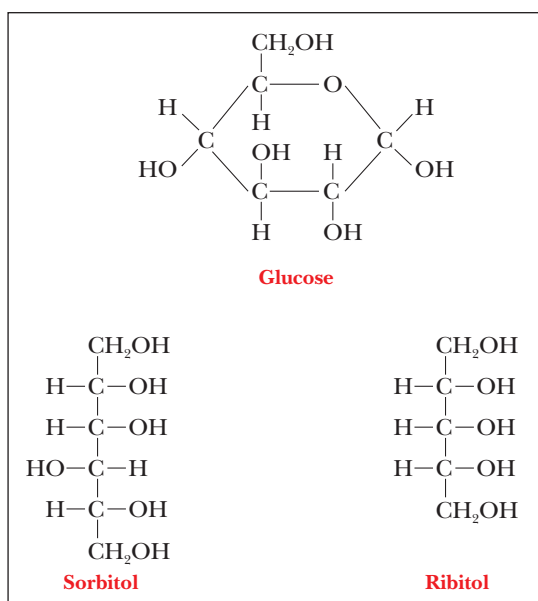
10. **Reflect and Apply** Both RNA and DNA have negatively charged phosphate groups as part of their structure. Would you expect ions that bind to nucleic acids to be positively or negatively charged? Why?

2.3 Acids, Bases, and pH

11. **Recall** Identify the conjugate acids and bases in the following pairs of substances:
    (a) $(CH_3)_3NH^+/(CH_3)_3N$
    (b) $^+H_3N—CH_2COOH/^+H_3N—CH_2—COO^-$
    (c) $^+H_3N—CH_2—COO^-/H_2N—CH_2—COO^-$
    (d) $^-OOC—CH_2—COOH/^-OOC—CH_2—COO^-$
    (e) $^-OOC—CH_2—COOH/HOOC—CH_2—COOH$

12. **Recall** Identify conjugate acids and bases in the following pairs of substances:
    (a) $(HOCH_2)_3 CNH_3^+$ $(HOCH_2)_3 CNH_2$

    (b) $HOCH_2 CH_2 N$ ⬡ $N CH_2 CH_2 SO_3^-$

    $HOCH_2 CH_2 N^+$ ⬡ $N CH_2 CH_2 SO_3^-$
    | H

    (c) $O_3^- SCH_2 CH_2 N$ ⬡ $N^+CH_2 CH_2 SO_3^-$
                              | H
    $O_3^- SCH_2 CH_2 N$ ⬡ $N CH_2 CH_2 SO_3^-$

13. **Reflect and Apply** Aspirin is an acid with a pK_a of 3.5; its structure includes a carboxyl group. To be absorbed into the bloodstream, it must pass through the membrane lining the stomach and the small intestine. Electrically neutral molecules can pass through a membrane more easily than can charged molecules. Would you expect more aspirin to be absorbed in the stomach, where the pH of gastric juice is about 1, or in the small intestine, where the pH is about 6? Explain your answer.

14. **Recall** Why does the pH change by one unit if the hydrogen ion concentration changes by a factor of 10?

15. **Mathematical** Calculate the hydrogen ion concentration, $[H^+]$, for each of the following materials:
    (a) Blood plasma, pH 7.4
    (b) Orange juice, pH 3.5

(c) Human urine, pH 6.2
(d) Household ammonia, pH 11.5
(e) Gastric juice, pH 1.8

16. **Mathematical** Calculate the hydrogen ion concentration, $[H^+]$, for each of the following materials:
    (a) Saliva, pH 6.5
    (b) Intracellular fluid of liver, pH 6.9
    (c) Tomato juice, pH 4.3
    (d) Grapefruit juice, pH 3.2

17. **Mathematical** Calculate the hydroxide ion concentration, $[OH^-]$, for each of the materials used in Question 16.

2.4 Titration Curves

18. **Recall** Define the following:
    (a) Acid dissociation constant
    (b) Acid strength
    (c) Amphipathic
    (d) Buffering capacity
    (e) Equivalence point
    (f) Hydrophilic
    (g) Hydrophobic
    (h) Nonpolar
    (i) Polar
    (j) Titration

19. **Reflect and Apply** Look at Figure 2.15 and Table 2.8. Which compound in the table would give a titration curve the most similar to the one shown in the figure? Why?

20. **Reflect and Apply** Look at Figure 2.15. If you did this titration using TRIS instead of phosphate, how would the titration curve look compared to the figure? Explain.

2.5 Buffers

21. **Biochemical Connections** List the criteria used to select a buffer for a biochemical reaction.

22. **Biochemical Connections** What is the relationship between pK_a and the useful range of a buffer?

23. **Mathematical** What is the $[CH_3COO^-]/[CH_3COOH]$ ratio in an acetate buffer at pH 5.00?

24. **Mathematical** What is the $[CH_3COO^-]/[CH_3COOH]$ ratio in an acetate buffer at pH 4.00?

25. **Mathematical** What is the ratio of TRIS/TRIS-H$^+$ in a TRIS buffer at pH 8.7?

26. **Mathematical** What is the ratio of HEPES/HEPES-H$^+$ in a HEPES buffer at pH 7.9?

27. **Mathematical** How would you prepare 1 L of a 0.050 M phosphate buffer at pH 7.5 using crystalline K_2HPO_4 and a solution of 1.0 M HCl?

28. **Mathematical** The buffer needed for Question 27 can also be prepared using crystalline NaH_2PO_4 and a solution of 1.0 M NaOH. How would you do this?

29. **Mathematical** Calculate the pH of a buffer solution prepared by mixing 75 mL of 1.0 M lactic acid (see Table 2.6) and 25 mL of 1.0 M sodium lactate.

30. **Mathematical** Calculate the pH of a buffer solution prepared by mixing 25 mL of 1.0 M lactic acid and 75 mL of 1.0 M sodium lactate.

31. **Mathematical** Calculate the pH of a buffer solution that contains 0.10 M acetic acid (Table 2.6) and 0.25 M sodium acetate.

32. **Mathematical** A catalog in the lab has a recipe for preparing 1 L of a TRIS buffer at 0.0500 M and with pH 8.0: dissolve 2.02 g of TRIS (free base, MW = 121.1 g/mol) and 5.25 g of TRIS hydrochloride

(the acidic form, MW = 157.6 g/mol) in a total volume of 1 L. Verify that this recipe is correct.

33. **Mathematical** If you mixed equal volumes of 0.1 M HCl and 0.20 M TRIS (free amine form; see Table 2.8), is the resulting solution a buffer? Why or why not?

34. **Mathematical** What would be the pH of the solution described in Question 33?

35. **Mathematical** If you have 100 mL of a 0.10 M TRIS buffer at pH 8.3 (Table 2.8) and you add 3.0 mL of 1 M HCl, what will be the new pH?

36. **Mathematical** What would be the pH of the solution in Question 35 if you were to add 3.0 mL more of 1 M HCl?

37. **Mathematical** Show that, for a pure weak acid in water, pH = (pK_a − log [HA])/2.

38. **Mathematical** What is the ratio of concentrations of acetate ion and undissociated acetic acid in a solution that has a pH of 5.12?

39. **Biochemical Connections** You need to carry out an enzymatic reaction at pH 7.5. A friend suggests a weak acid with a pK_a of 3.9 as the basis of a buffer. Will this substance and its conjugate base make a suitable buffer? Why or why not?

40. **Mathematical** If the buffer suggested in Question 39 were made, what would be the ratio of the conjugate base/conjugate acid?

41. **Biochemical Connections** Suggest a suitable buffer range for each of the following substances:
    (a) Lactic acid (pK_a = 3.86) and its sodium salt
    (b) Acetic acid (pK_a = 4.76) and its sodium salt
    (c) TRIS (pK_a = 8.3; see Table 2.8) in its protonated form and its free amine form
    (d) HEPES (pK_a = 7.55; see Table 2.8) in its zwitterionic form and its anionic form

42. **Biochemical Connections** Which of the buffers shown in Table 2.8 would you choose to make a buffer with a pH of 7.3? Explain why.

43. **Mathematical** The solution in Question 27 is called 0.050 M, even though the concentration of neither the free base nor the conjugate acid is 0.050 M. Why is 0.050 M the correct concentration to report?

44. **Reflect and Apply** In Section 2.4 we said that at the equivalence point of a titration of acetic acid, *essentially all* the acid has been converted to acetate ion. Why do we not say that *all* the acetic acid has been converted to acetate ion?

45. **Mathematical** Define buffering capacity. How do the following buffers differ in buffering capacity? How do they differ in pH?
    Buffer a: 0.01 M Na_2HPO_4 and 0.01 M NaH_2PO_4
    Buffer b: 0.10 M Na_2HPO_4 and 0.10 M NaH_2PO_4
    Buffer c: 1.0 M Na_2HPO_4 and 1.0 M NaH_2PO_4

46. **Biochemical Connections** If you wanted to make a HEPES buffer at pH 8.3, and you had both HEPES acid and HEPES base available, which would you start with, and why?

47. **Biochemical Connections** We usually say that a perfect buffer has its pH equal to its pK_a. Give an example of a situation in which it would be advantageous to have a buffer with a pH 0.5 unit higher than its pK_a.

48. **Recall** What quality of zwitterions makes them desirable buffers?

49. **Reflect and Apply** Many of the buffers used these days, such as HEPES and PIPES, were developed because they have desirable characteristics, such as resisting pH change with dilution. Why would resisting pH change with dilution be advantageous?

50. **Reflect and Apply** Another characteristic of modern buffers such as HEPES is that their pH changes little with changes in temperature. Why is this desirable?

51. **Reflect and Apply** Identify the zwitterions in the list of substances in Question 11.

52. **Biochemical Connections** A frequently recommended treatment for hiccups is to hold one's breath. The resulting condition, hypoventilation, causes buildup of carbon dioxide in the lungs. Predict the effect on the pH of blood.

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