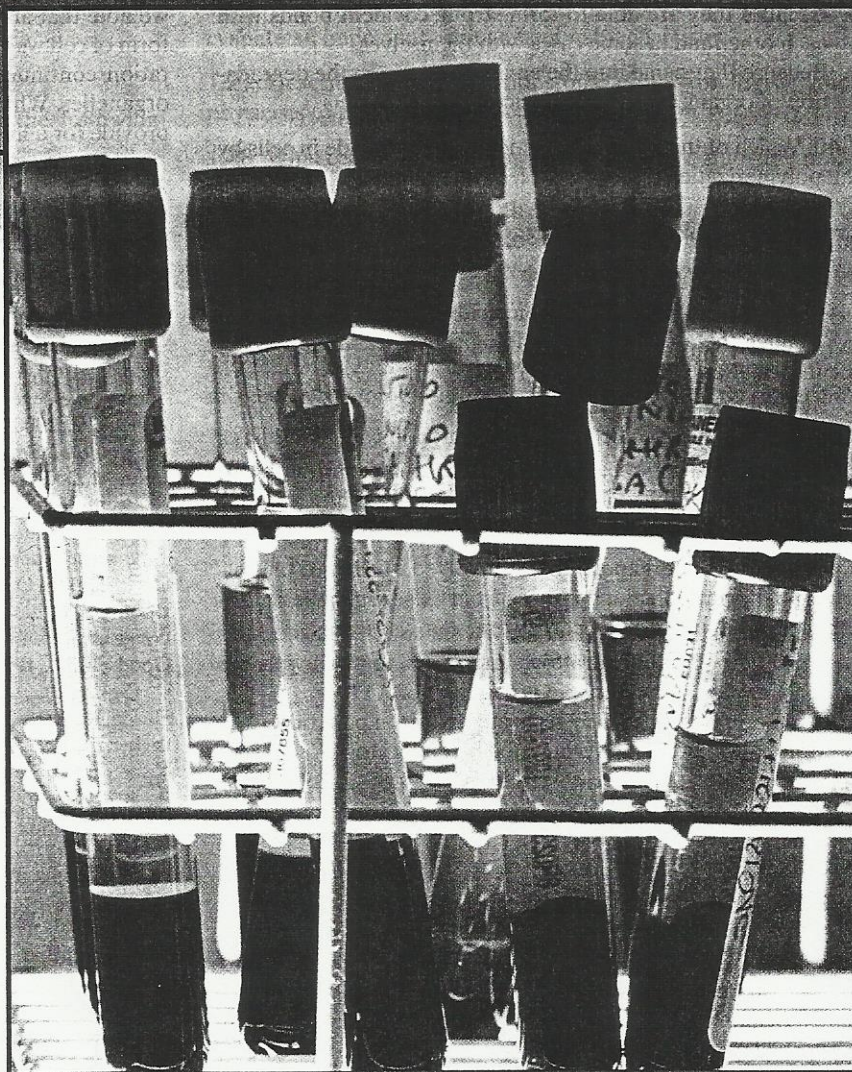


Blood, an aqueous-based solution, plays an essential role in the distribution of cells, proteins, nutrients, gases, and many other biological materials throughout organisms. Each of us has 5–6 liters of the complex solution coursing through our arteries and veins. Blood may be separated into its two parts by centrifugation. The sediment is composed of three types of blood cells: erythrocytes, leukocytes, and platelets (thrombocytes). The supernatant, the liquid portion called blood plasma, is a solution consisting of approximately 90% water and 10% dissolved solutes such as plasma proteins, glucose, hormones, cholesterol, vitamins, inorganic ions, and many other biomolecules. Collection of blood samples from an individual and clinical analysis of changes in the biochemical components is the most useful method for diagnosis of diseases.

(© Klaus Guldbrandsen/Photo Researchers, Inc.)



Biomolecules in Water

2.1 Water, the Biological Solvent

- Noncovalent Interactions in Biomolecules
- Examples of Noncovalent Interactions
- Characteristics of Noncovalent Interactions
- The Structure of Water
- The Importance of Hydrogen Bonds

2.2 Hydrogen Bonding and Solubility

- Physical Properties of Water
- Water as a Solvent

2.3 Cellular Reactions of Water

- Ionization of Water
- pH and pK
- Titration Curves
- The Henderson–Hasselbalch Equation
- Drug Efficacy Depends on Water Solubility

2.4 Buffer Systems

- Maintaining a Constant pH
- Effective Buffering Range
- Laboratory Buffers

Water! Although a seemingly simple and abundant substance containing only the atoms of hydrogen and oxygen, it has extraordinary physical, chemical, and biological properties. Water is vital to all forms of life and makes up about 70% to 85% of the weight of a typical cell (Table 2.1). In addition, extracellular fluids such as blood, cerebrospinal fluid, saliva, urine, and tears are aqueous-based solutions. Many scientists believe that life began in an aqueous environment and, during the early stages of evolutionary development, all living organisms resided in water. Although plants probably evolved first, many other forms of life developed lungs and were able to move to land. Some organisms (unicellular and multicellular) still require not just internal water but a constant extracellular environment that is aqueous. These organisms may live in rivers, lakes, and oceans or sheltered in the aqueous environment of another, larger cell.

The Biological Roles of Water

Water plays many roles in cells and organisms and has great influence on the structure and behavior of all biomolecules.

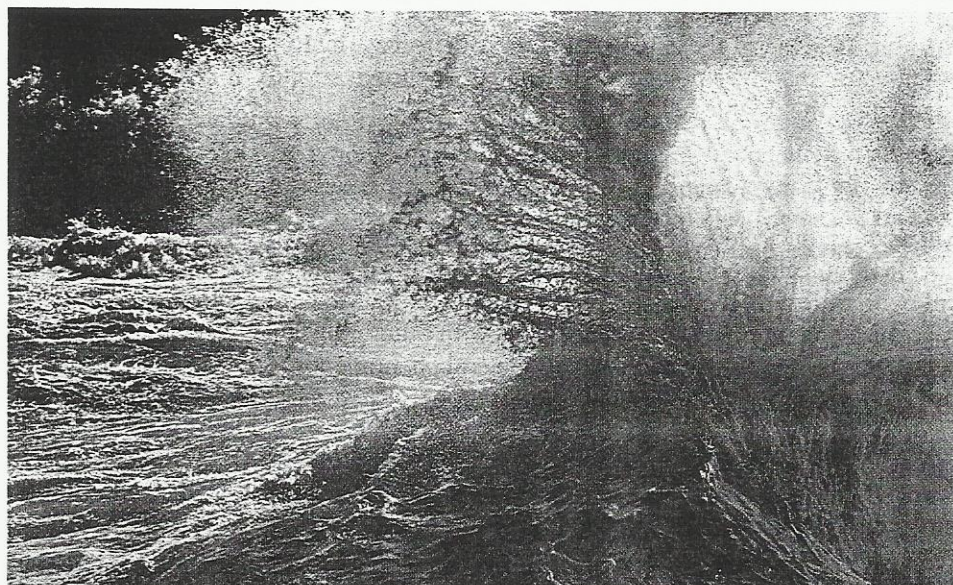
- *Water is important as a biological solvent.* A study of biomolecules is not complete without an understanding of the extraordinary properties of water as a solvent. Water provides a medium for metabolic reactions; it is literally the “matrix of life.” Because most biological fluids are aqueous-based, water provides for the delivery to the cell of nutrients for growth and removal of wastes for general cleansing of cells. The cellular fluids also assist in the absorption and action of pharmaceutical compounds by dissolving them and transporting them to target organs and cells. It is important to note that water, although a very effective solvent, is not a universal biological solvent as is sometimes declared. All biomolecules are not soluble in water and this, indeed, is fortunate. Because of this, organisms can develop compartmentation of cell structure and function by creating partitions (membranes) using molecules that are water insoluble. One of the newest developments in cellular biochemistry is the discovery of **aquaporins**, membrane proteins that form channels through which water molecules are transported in and out of cells (Section 9.3).
- *Water serves as an essential buffer to regulate temperature and pH.* With a high specific heat capacity, water is able to absorb large amounts of energy in the form of heat released from biochemical reactions. As a result, the water medium acts to maintain a constant cell temperature. Water also serves as a solvent to dissolve

Table 2.1
Percent by weight of water in
organs of the human body

Tissue or Organ	Percent by Weight of Water ^a
Skeletal muscle	79 ^b
Heart	83 ^b
Liver	71
Kidney	81
Spleen	79
Lung	79
Brain	77

^a In adults.

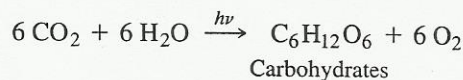
^b Fat-free tissue.



Water, which plays roles as a biological solvent, reactant molecule, and temperature regulator, is essential to life. (© Douglas Faulkner/Photo Researchers.)

substances that regulate hydrogen ion concentration (pH). Biological molecules will function properly only in an environment of constant pH. Buffering substances as simple as bicarbonate and as complex as proteins react with water to maintain a remarkably constant pH level in intracellular and extracellular fluids.

- *Water is a participant in many biochemical reactions.* One of the most common biological reactions is cleavage of a chemical bond by water (hydrolysis) as observed in the initial steps of digestion of proteins, carbohydrates, and nucleic acids. Water is also a principal reactant in the photosynthesis process:



Here water acts as a reducing agent, a source of electrons to reduce carbon dioxide for the manufacture of glucose. The process of respiration, the final stage of energy metabolism in animals, generates water from O_2 by oxidation–reduction reactions (see Chapter 17).

Clearly, water is not just an inert bystander in the cell, but a selectively reactive molecule with unique properties that greatly affect biochemical molecules and biological processes. In this chapter, we shall examine some of the unusual properties of water and learn how they influence structure and reactivity of biomolecules.

2.1

Water, the Biological Solvent

Learning Objective

Have a general knowledge of the chemical and biological properties of water and of the importance of weak, noncovalent interactions in biochemistry.

Noncovalent Interactions in Biomolecules

Before we look at the physical and chemical properties of water, we need to consider bonding arrangements that are important in water molecules and in how water interacts with other molecules. In Chapter 1, we discovered that biomolecules large and small are made by the covalent combining of elements forming C—C, C—H, C—O, C—N, and other strong bonds. These covalent bonds literally hold the atoms of a molecule together. Weaker interactions, called **noncovalent interactions**, bring together whole biomolecules for specific purposes. Four types of noncovalent interactions are important: **van der Waals forces**, **ionic bonds**, **hydrogen bonds**, and **hydrophobic interactions**. Properties and examples of these interactions are reviewed in Table 2.2. One of these types of stabilizing forces (hydrogen bonds) are at work when water dissolves polar molecules and when two strands of DNA are brought together in the double helix.



Examples of Noncovalent Interactions

Throughout our study of biochemistry we will encounter many examples where noncovalent molecular interactions bring together, in specific ways, two different molecules or different regions of the same molecule. Molecules have the ability to recognize and interact (bind) specifically with other molecules. We will use the term **molecular recognition** to describe this general phenomenon. The importance of these interactions in biology is that the combination of two molecules or the organized folding of a single molecule will lead to biological function not present in individual molecules or in unfolded, randomly arranged molecules.

The interactions important in molecular recognition are often between a small molecule (called a **ligand, L**) and a **macromolecule (M)**:

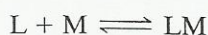
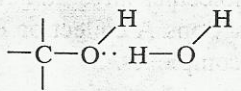
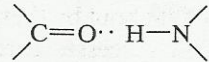
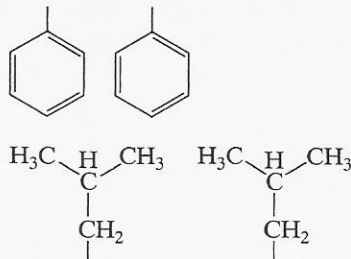


Table 2.2
Properties and examples of noncovalent interactions

Type	Brief Description and Example	Stabilization Energy (kJ/mol)	Length (nm)
Hydrogen bonds	<p>Between a hydrogen atom covalently bonded to an electronegative atom and a second electronegative atom (H-bond donor = red atoms, H-bond acceptor = green atoms)</p> <p>Between neutral groups</p>  <p>Between peptide bonds</p> 	10–30	0.18–0.30
van der Waals interactions	Between molecules with temporary dipoles induced by fluctuating electrons. May occur between any two atoms in close proximity	1–5	0.1–0.2
Hydrophobic interactions	<p>The tendency of nonpolar groups and molecules to stick together or cluster in aqueous solutions</p> 	5–30	—
Ionic bonds	<p>Attractive interactions that occur between oppositely charged atoms or groups</p> <p>Na^+Cl^-</p> <p>$\text{R}-\text{COO}^- + \text{NH}_3^+-\text{R}$</p>	20–30	0.25

LM represents a complex, held together by noncovalent interactions, with specialized biological function. The action of hormones is a good example. A hormone response is the consequence of weak, but specific, interactions between the hormone molecule and a receptor protein in the membrane of the target cell (Special Topic I).

Biochemical reactions also provide many examples of the importance of noncovalent interactions. Before a metabolic reaction can occur, a small substrate molecule must physically interact in a certain well-defined manner with a macro-molecular catalyst, an enzyme (Chapters 5 and 6). The biochemical action of a drug also depends on molecular interactions. The drug is first distributed throughout the body via the bloodstream. Drugs in the bloodstream are often noncovalently bound to plasma proteins, which act as carriers. When the drug molecules are transported to their site of action, a second molecular interaction is likely to occur. The drug will likely bind to a receptor protein or other proteins. Many drugs elicit their effects by then interfering with biochemical processes. This may take the form of enzyme inhibition, where the drug molecule binds to a specific enzyme and prohibits binding of normal reactant and, therefore, inhibits catalytic action. Intramolecular noncovalent interactions (those within a molecule) also play significant roles in biomolecular processes—for example, stabilizing the folding of protein, DNA, and RNA molecules into regular, three-dimensional arrangements.

Characteristics of Noncovalent Interactions

All molecular interactions that are the basis of molecular recognition have at least three common characteristics:

- **First, the forces that are the basis of these interactions are noncovalent and relatively weak.** The strengths of these interactions are in the range of 1–30 kJ/mol compared to about 350 kJ/mol for a carbon–carbon single bond, a typical covalent bond. A single noncovalent bond is usually insufficient to hold two molecules together. DNA, RNA, and protein molecules have numerous functional groups that participate in noncovalent interactions. A collection of many of these interactions will lead to greatly stabilized complexes.
- **Second, noncovalent interactions are reversible.** Noncovalent interactions are initiated when diffusing (wandering or moving) molecules or regions of a molecule come into close contact. Diffusion is brought about by thermal motions. An initial close encounter may not always result in the successful formation of a complex. A few weak bonds may form but may be disrupted by thermal motion, causing the molecules to dissociate. Therefore, bonds may constantly form and break until enough bonds have accumulated to result in an intermediate with a transient but significant existence. The complex can then initiate a specific biological process. An intermediate rarely lasts longer than a few seconds. Eventually, thermal motions cause the complex to dissociate to the individual molecules. Reversibility is an important characteristic of these interactions so that a static, gridlock situation does not occur. The biological process initiated by the complex LM must have a starting time and an ending.
- **Third, the binding between molecules is specific.** Imagine that the interactions bring together two molecular surfaces. The two surfaces will be held together if noncovalent interactions occur. If on one surface there is a nonpolar molecular group (phenyl ring, hydrophobic alkyl chain, etc.), the adjacent region on the other surface must also be hydrophobic and nonpolar. If a positive charge exists on one surface, there may be a neutralizing negative charge on the other surface. A hydrogen bond donor on one surface can interact with a hydrogen bond acceptor on another. Simply stated, the two molecules must be compatible or complementary in a physical and chemical sense so the development of stabilizing forces can hold molecules together. The concept of molecular recognition will take on many forms in our continuing studies of biochemistry.

The Structure of Water

The arrangement of hydrogen and oxygen atoms in the water molecule is nonlinear with an H—O—H bond angle of 104.5° (Figure 2.1a). The electronegativity value for the oxygen atom (3.5) is approximately one and one-half times that of hydrogen (2.1). Therefore, the electrons of the two covalent bonds are not shared equally; the oxygen atom has a stronger pull on the electrons and takes on a partial negative charge (δ^-). The hydrogen atoms are left with a partial positive charge (δ^+), because they do not have equal access to the bonding electrons. This gives rise to a molecule with a dipolar structure: a negative end sometimes called the “head” (oxygen) and positive ends sometimes called “tails” (hydrogens) (Figure 2.1b). The water molecule is electrically neutral (no net charge) but has a relatively large dipole moment because of its bent geometry. Water can be contrasted with another molecule of biochemical significance, CO_2 , which also has polar bonds caused by electronegativity differences between the carbon and oxygen atoms but no dipole moment because it is linear (Figure 2.2).

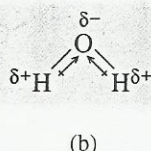
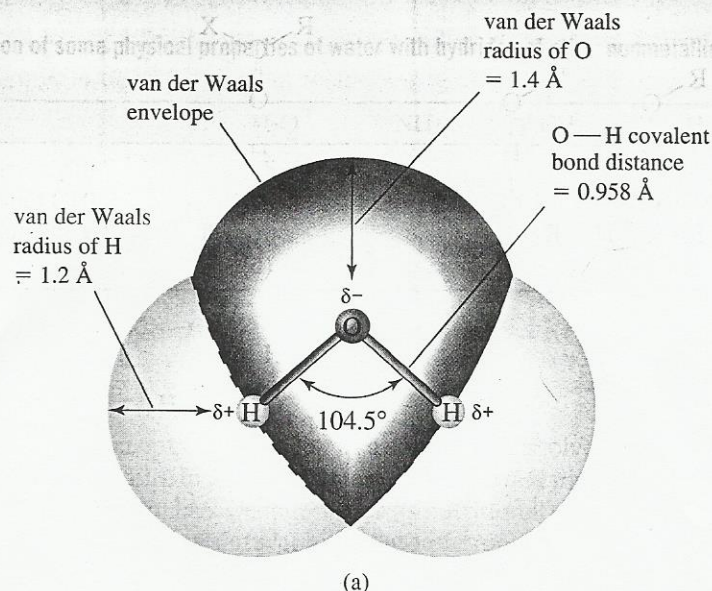


Figure 2.1 (a) The structure of the water molecule showing the relative size of each atom by the van der Waals radius. Covalent bonds hold together oxygen and hydrogen atoms. The polar character, which is the result of electronegativity differences between oxygen and hydrogen, is indicated by the partial charges ($\delta+$ and $\delta-$) on atoms. (b) Water has a dipole moment because of its bent geometry. The arrows pointing to the more electronegative atom are used to show bond polarity.

The Importance of Hydrogen Bonds

The characteristics of water as described here have profound consequences for its structure and interactions with biomolecules. Water molecules can interact with each other by attraction of a positive tail (hydrogen) with a negative head (oxygen) as shown in Figure 2.3. This favorable interaction results in a **hydrogen bond** (see Table 2.2). This type of bond, which may be represented as $X-H\cdots A$, is formed when an electronegative atom (A), such as oxygen or nitrogen, interacts with a hydrogen atom that is slightly positive or acidic as in $X-H^{\delta+}$, where X may be nitrogen, oxygen, or sulfur. The electronegative atom A is defined as the **hydrogen bond acceptor** and the $X-H$ group as the **hydrogen bond donor**. The hydrogen bond is strongest when the three atoms, $X-H\cdots A$, are in a straight line (180°) with the hydrogen atom interacting directly with a lone pair electron cloud of A. The hydrogen bond distance in water is about 0.18 nm (1.8 Å) and has a bond energy of about 20 kJ/mol (5 kcal/mol) compared to 0.096 nm (0.96 Å) and 460 kJ/mol (110 kcal/mol) for an O—H covalent bond.

Water structure has great significance in biochemistry because many biomolecules have atoms that can hydrogen bond with water, with themselves, and with other molecules. Some biochemical examples of hydrogen bonding are shown in Figure 2.4. Functional groups that participate in hydrogen bonding include (1) the hydroxyl groups in alcohols, organic acids, and carbohydrates; (2) carbonyl groups in aldehydes, ketones, acids, amides, and esters; and (3) N—H groups in amines and amides. The specific hydrogen bonding that occurs between complementary base pairs in DNA and RNA (Figure 2.4d) is discussed in Chapter 10. Although the strength of a single hydrogen bond may be small, the enormous number of potential hydrogen bonding groups in biomolecules more than makes up for their individual weaknesses.

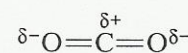


Figure 2.2 The CO_2 molecule, although composed of polar bonds, has no dipole moment because it is linear. The electronegativity differences between C and O atoms are indicated by the partial charges (δ).

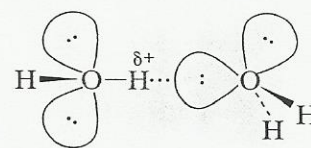
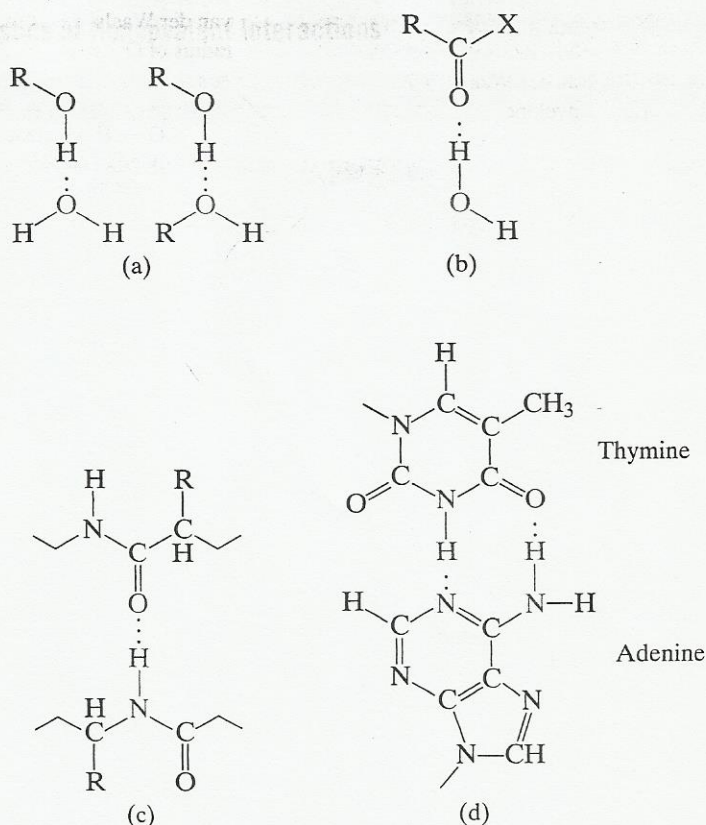


Figure 2.3 Hydrogen bond between two water molecules. The hydrogen atom (partially charged) of one water molecule interacts with a lone pair of electrons in an orbital of the oxygen atom of another water molecule.

Figure 2.4 Hydrogen bonds of biological importance: (a) between an alcohol and water or between alcohol molecules; (b) between a carbonyl group and water ($X = H, R, OH, OR,$ or NH_2); (c) between two peptide chains, the carbonyl group of one peptide bonds to an $N-H$ of another; (d) between complementary base pairs in DNA. Atoms involved as hydrogen bond donors are in red; hydrogen bond acceptors are in green.



2.2

Hydrogen Bonding and Solubility

Learning Objective

Be able to explain how water functions as a solvent.

Physical Properties of Water

The unusual physical properties of water are best understood by comparison to substances of similar structure and molecular weight (Table 2.3). Water has a higher boiling point, melting point, and viscosity than any other hydride of a nonmetallic element. These peculiar properties are the result of water's unusually high internal cohesiveness or the tendency of water molecules to "stick together," which is due to an extensive network of hydrogen bonds. Each water molecule theoretically can hydrogen bond with four neighboring water molecules (Figure 2.5a). In reality, the average number of hydrogen bonds to each molecule in liquid water at 10°C is about three. The number of hydrogen bonds decreases with increasing temperature. The theoretical number of four interacting neighbors for each water molecule is approached in crystalline ice (Figure 2.5b).

A close examination of water structure by X-ray and neutron diffraction techniques reveals more detailed features. The network of hydrogen bonds as shown in Figure 2.5 is a snapshot representing an instant in time. The actual structure is dynamic, with water molecules undergoing constant geometrical reorientations and forming new hydrogen bonds with other neighboring water molecules. This change happens for each molecule about once every 10^{-12} s. The term "flickering clusters" has sometimes been used to describe the constantly changing network of hydrogen bonds in liquid water. Using powerful X-rays generated at the European Synchrotron Radiation Facility in Grenoble, France, it has been shown that the hydrogen bonds that hold water molecules together in ice have substantial covalent character, so they are a lot stronger than previously thought.



Table 2.3 A comparison of some physical properties of water with hydrides of other nonmetallic elements: N, C, and S

Property	H ₂ O	NH ₃	CH ₄	H ₂ S
Molecular weight	18	17	16	34
Boiling point (°C)	100	-33	-161	-60.7
Freezing point (°C)	0	-78	-183	-85.5
Viscosity ^a	1.01	0.25	0.10	0.15

^aUnits are centipoise.

Water as a Solvent

Water displays an exceptional capacity as a solvent to dissolve many of the biomolecules found in living cells. In addition, water is an important medium for dissolving and transporting other chemicals we ingest such as nutritional supplements and pharmaceutical compounds. Many natural and synthetic molecules that are ionic as well as some that are polar and uncharged are soluble in water. Here we will examine the interactions of water with these compounds.

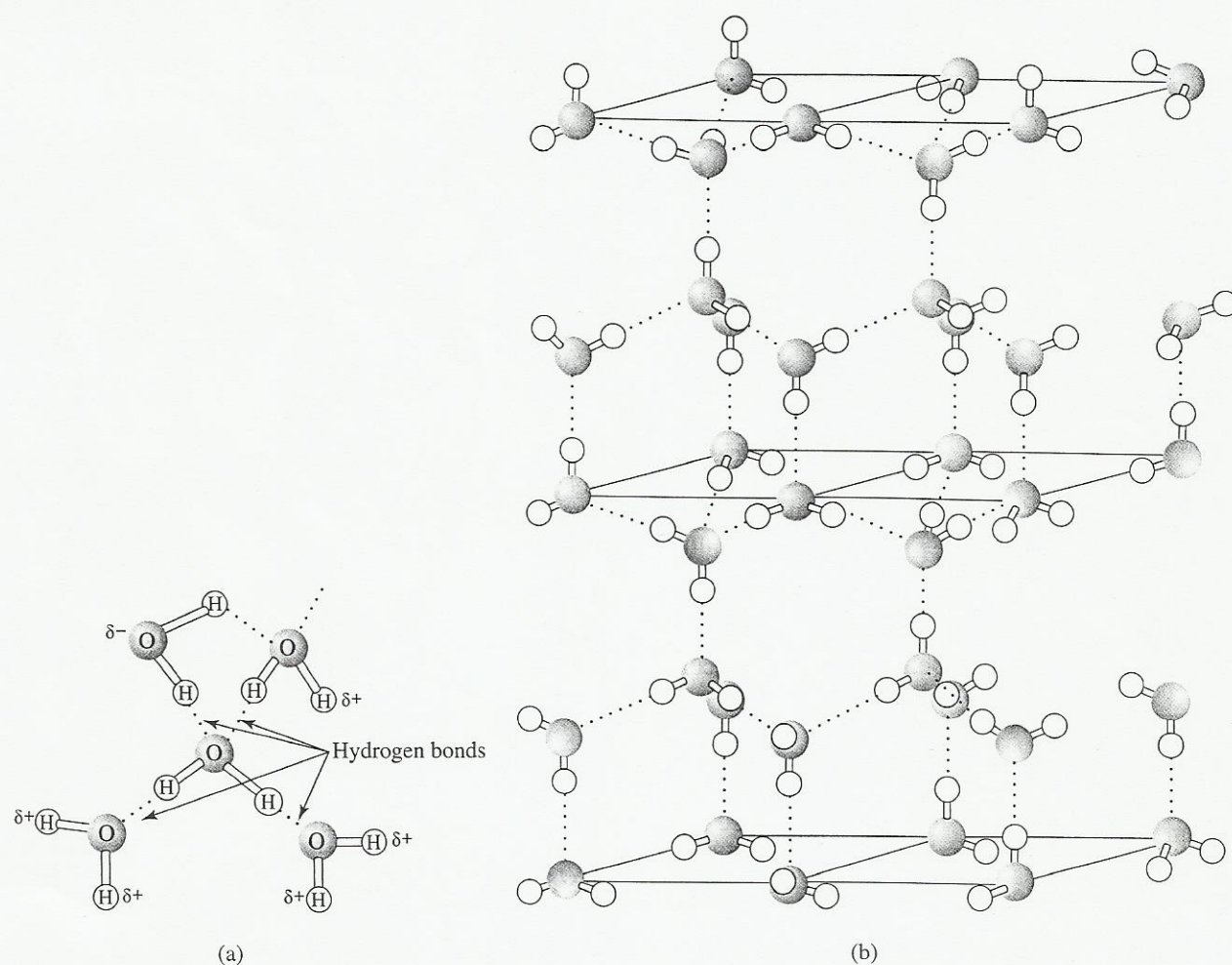
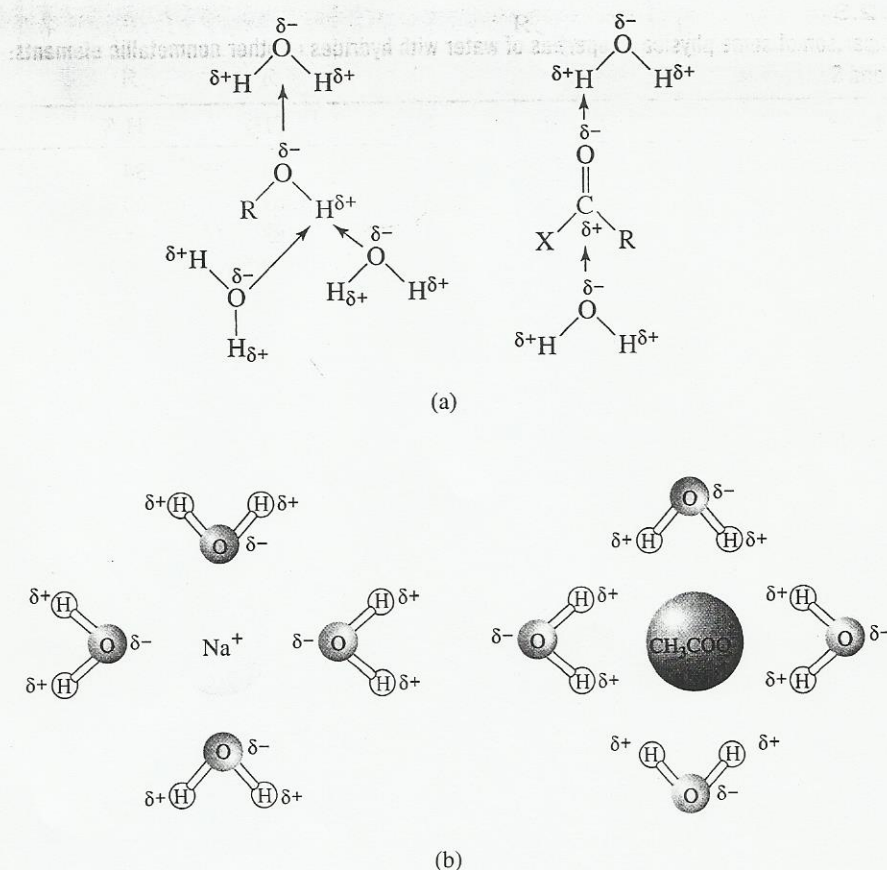


Figure 2.5 The network of potential hydrogen bonds in water. (a) The center water molecule may form hydrogen bonds with up to four neighboring molecules, but the average is about three. The network structure is constantly changing, with water molecules undergoing geometrical reorientations and forming new hydrogen bonds with other neighboring water molecules. (b) In ice, hydrogen bonding leads to the formation of a crystalline lattice.

Figure 2.6 Chemicals are made soluble in water by noncovalent interactions. (a) Dipole–dipole interactions. The partially charged positive atoms (hydrogen) of water and alcohol are attracted to oxygen atom dipoles of water and alcohol. The carbonyl group of an aldehyde, ketone, or acid can also be solvated by water. (b) Ion–dipole interactions. The positively charged sodium ion is surrounded by water molecules projecting their partially negative oxygen atoms (dipoles). The acetate ion interacts with the partially positive hydrogen atoms (dipoles) of water.



Polar Compounds Many uncharged biomolecules readily dissolve in water because they have polar functional groups that form favorable dipole–dipole interactions. A few examples of these compounds, including alcohols, amines, amides, and esters, are featured in Figure 2.4. Further examples are shown in Figure 2.6a. Extensive hydrogen bond networks are possible where the atoms of the polar functional groups combine with identical molecules, similar molecules, and/or water. Because of a favorable attraction to water molecules, ionic and polar compounds are said to be **hydrophilic**, a word of Greek origin translated as water (*hydro*) and loving (*philic*). Not all compounds containing polar functional groups are water soluble. Those with a relatively large hydrocarbon component (usually greater than four carbon atoms) are usually insoluble unless an ionic group or several polar groups are present. Cyclohexane is insoluble in water; but if one of the carbon–hydrogen groups is converted to an aldehyde or ketone and a hydroxyl group is substituted on each of the remaining five carbon atoms, the molecule becomes similar to a carbohydrate such as glucose and is water soluble.

Ionic Compounds Ionic compounds such as sodium acetate ($\text{CH}_3\text{COO}^-\text{Na}^+$) and monopotassium phosphate ($\text{K}^+\text{H}_2\text{PO}_4^-$) dissolve in water because their individual ions can be solvated (hydrated) by polar water molecules (Figure 2.6b). The negative dipole (oxygen atom) of water binds favorably with the Na^+ or K^+ forming a dipole–ion interaction. The acetate and phosphate anions are hydrated also by dipole–ion interactions. In this case, the partially positive hydrogen ends of water associate with the negative charges on the anion. The attraction between most ions and polar water molecules is strong enough to overcome the tendency of anions and cations to recombine. The great ion-solvating ability of water is illustrated by the Na^+ , K^+ , and Cl^- concentrations in human blood of 0.14 M, 0.004 M, and 0.10 M,

respectively. Hundreds of other ionic substances are dissolved in blood. Even macromolecules, including proteins, nucleic acids, some lipids, and some carbohydrates, under in vivo conditions exist as hydrated ions.

Nonpolar Compounds Nonpolar compounds are usually not water soluble because they contain neither ions nor polar functional groups that can interact favorably with water molecules. Hence, they are called **hydrophobic** (water fearing). Decane and benzene are examples of hydrophobic molecules (Figure 2.7). Some significant biochemicals have dual properties; they have both nonpolar and ionic characteristics. They are classified as **amphiphilic** (*amphi*, on both sides or ends, and *philic*, loving). This class of compounds is best illustrated by metal salts of long-chain carboxylic acids (Figure 2.8). A specific example, sodium stearate, has an ionic side or end (the carboxylate anion associated with the sodium cation) and a nonpolar hydrocarbon end. This molecule must be confused when placed in water solution. The observed result of this experiment is not the formation of a true solution but the self-assembly of the acid molecules into aggregates called **micelles**. The amphiphilic molecules avoid water contact in the hydrophobic region by pointing their hydrocarbon chains ("tails") to the water-free interior of the aggregate. The surface of each micelle is composed of the ionic "heads" stabilized by electrostatic interaction with metal cations and water. The favorable association of nonpolar hydrocarbon tails inside the micelle is defined as a **hydrophobic interaction** (see Table 2.2). In simplified terms, this situation is favorable because less energy is required to form the micelle than if the hydrocarbon chains were allowed to point out into the water, disrupting the network of hydrogen bonds. One important practical application of micellar solutions of sodium stearate and other similar compounds is their use as soaps to "solubilize" oil and grease in water. Owing to their dual chemical character, soaps are able to trap oil in the nonpolar region of the micelle and yet remain dispersed in aqueous solutions by hydration of the ionic region. Micelle formation is also the key to construction of biological membranes (see Chapter 9).

Much of this section has focused on how the structure of water is affected by the presence of solute molecules. It is also important to note changes in the structures of biomolecules brought about by the presence of water. Because of their dual character, long-chain carboxylic acid salts and other amphiphilic lipids take on a special arrangement in water. Proteins and nucleic acids also contain hydrophobic regions and ionic functional groups; therefore, these are amphiphilic substances. As we shall discover in later chapters, these biomolecules in water solution fold into conformations that bury hydrophobic regions in water-free areas and expose ionic and polar functional groups to water molecules. Important consequences result because it is often found that these complex and ordered arrangements of biomolecules are the only ones with biological activity.

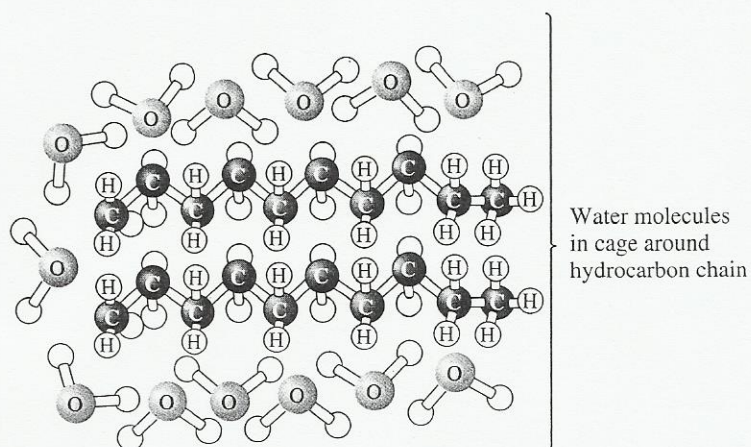
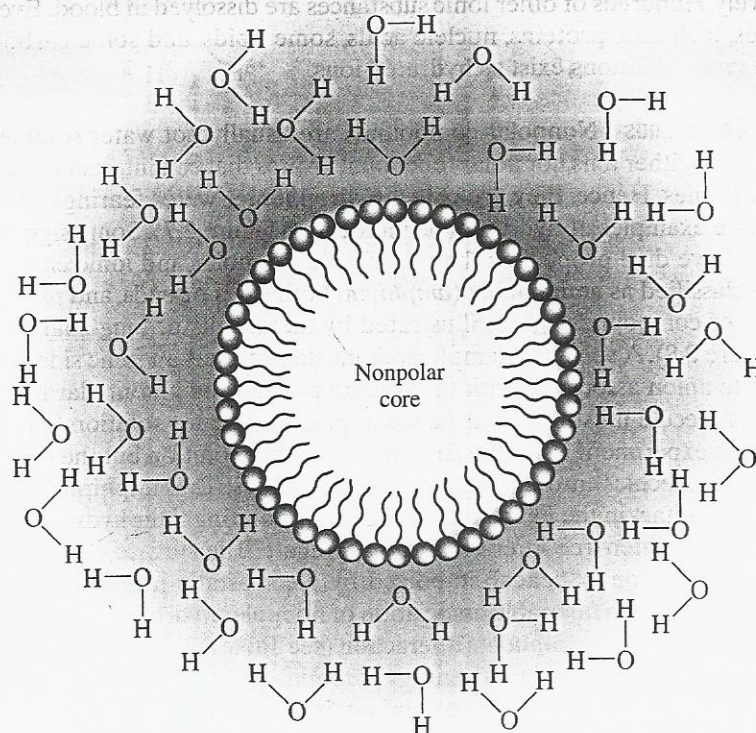


Figure 2.7 Because hydrophobic molecules have no polar groups to interact with water, they have to be surrounded by a boundary of water molecules. The formation of this highly ordered cage of water requires much energy, which comes from hydrophobic interactions.

e 2.8 Formation of a micelle
the sodium salt of a long-chain
xylic acid. The nonpolar
carbon tails of the acid arrange
selves to avoid contact with water.
egatively charged carboxyl groups
act with water by forming
lipole interactions.



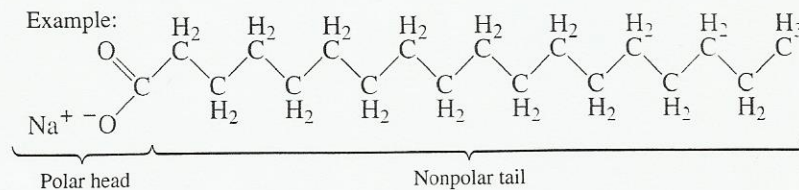
Sodium Stearate Micelle

Key: Polar head of sodium stearate



Nonpolar tail of sodium stearate

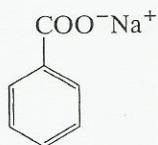
Example:



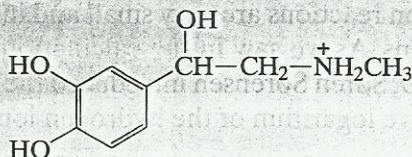
Amphiphilic Compound

Before You Go On...

1. Show examples of all the possible types of hydrogen bonding that would exist between the molecules in an ethanol–water solution (50:50).
2. Show how the functional groups of the amino acid glycine, $\text{H}_3\text{N}^+\text{CH}_2\text{COO}^-$, may be solvated in an aqueous solution.
3. Show how the chemical salt sodium benzoate may be solvated in an aqueous solution. Sodium benzoate is an inhibitor of fungal growth that is often used as a food preservative.



4. The structure for the neurotransmitter and hormone epinephrine (also called adrenaline) is shown below. Show how the functional groups could be solvated by water.



2.3

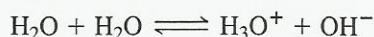
Cellular Reactions of Water

Learning Objective

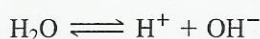
Understand how the ionization of water affects the structures and actions of biomolecules in the cell.

Ionization of Water

It may be easy to view water as just a background material without much dynamic activity in the cell and organism; however, this is not a realistic picture. Although water is not usually considered a substance with robust chemical reactivity, it does display features of selective reactivity. Several examples of water as a participant in biochemical processes are presented in later chapters. Perhaps the most important reaction of water is its reversible self-dissociation or **ionization** to generate the **hydronium ion** (H_3O^+) and the **hydroxide ion** (OH^-):



Although not as correct, because free H^+ does not exist in aqueous solution, the equation is often abbreviated as



The extent to which this ionization reaction takes place is of special interest because it helps characterize the internal medium of cells. The more water molecules dissociated, the more ionic the medium. We can use the law of mass action to obtain a quantitative measure of the equilibrium point for the dissociation reaction:

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

K_{eq} represents the **equilibrium constant** for the reaction, and brackets for each chemical entity indicate concentration units in moles per liter (M). If the K_{eq} for the ionization of pure water is determined from experimental measurements, it is possible to calculate a quantity for $[\text{H}^+]$ and $[\text{OH}^-]$ and, therefore, to estimate the extent of self-dissociation. K_{eq} for pure water at 25°C has been determined to be $1.8 \times 10^{-16} M$. A value for $[\text{H}_2\text{O}]$ can be estimated by dividing the weight of water in one liter (1000 g) by the molecular weight of water (18). This yields $[\text{H}_2\text{O}] = 55.5 M$. Therefore,

$$\begin{aligned} [\text{H}^+][\text{OH}^-] &= K_{\text{eq}}[\text{H}_2\text{O}] \\ [\text{H}^+][\text{OH}^-] &= (1.8 \times 10^{-16} M)(55.5 M) \\ [\text{H}^+][\text{OH}^-] &= 1.0 \times 10^{-14} M^2 \end{aligned}$$

Because according to the chemical equation for dissociation H^+ and OH^- must have equal concentrations in pure water, then

$$[\text{H}^+] = [\text{OH}^-] = \sqrt{1.0 \times 10^{-14} M^2} = 1.0 \times 10^{-7} M$$

Hydrogen ion concentrations expressed in exponential form are difficult to work with. A more useful terminology is **pH**, defined as the negative logarithm of the $[\text{H}^+]$ (see Just in Time Review 2-1).



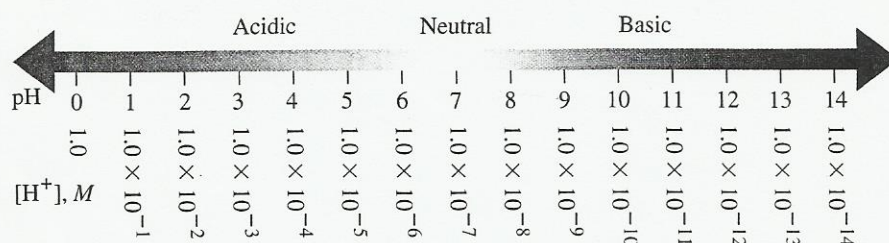
Using pH to Define Hydrogen Ion Concentration

Hydrogen ion concentrations calculated for ionization reactions are very small and in the exponential form are cumbersome for use in mathematical expressions. As we saw in the calculation for the ionization of water above, the $[H^+]$ for water is $1 \times 10^{-7} M$. In 1909, Søren Sørensen introduced the term **pH** to more conveniently express $[H^+]$. He defined pH as the negative logarithm of the hydrogen ion concentration:

$$pH = -\log[H^+]$$

The $[H^+]$ of $1 \times 10^{-7} M$ becomes a pH of 7. The entire pH scale from 0 to 14 is defined in the figure below. The logarithmic feature of the pH scale is an important characteristic as it allows for its use over a very wide range of hydrogen ion concentrations. Note that each digit increase or decrease of pH represents a 10-fold change in $[H^+]$. A solution at pH 7 has 10 times greater $[H^+]$ than a solution at pH 8.

The determination of pH is one of the most frequent measurements made in the biochemistry laboratory. The structures of biomolecules and the efficiency of biochemical processes are dependent on $[H^+]$. A pH measurement is usually taken by immersing a glass or plastic combination electrode into a solution and reading the pH directly from a digital readout or meter. The electrode is calibrated with buffer solutions of known pH values.



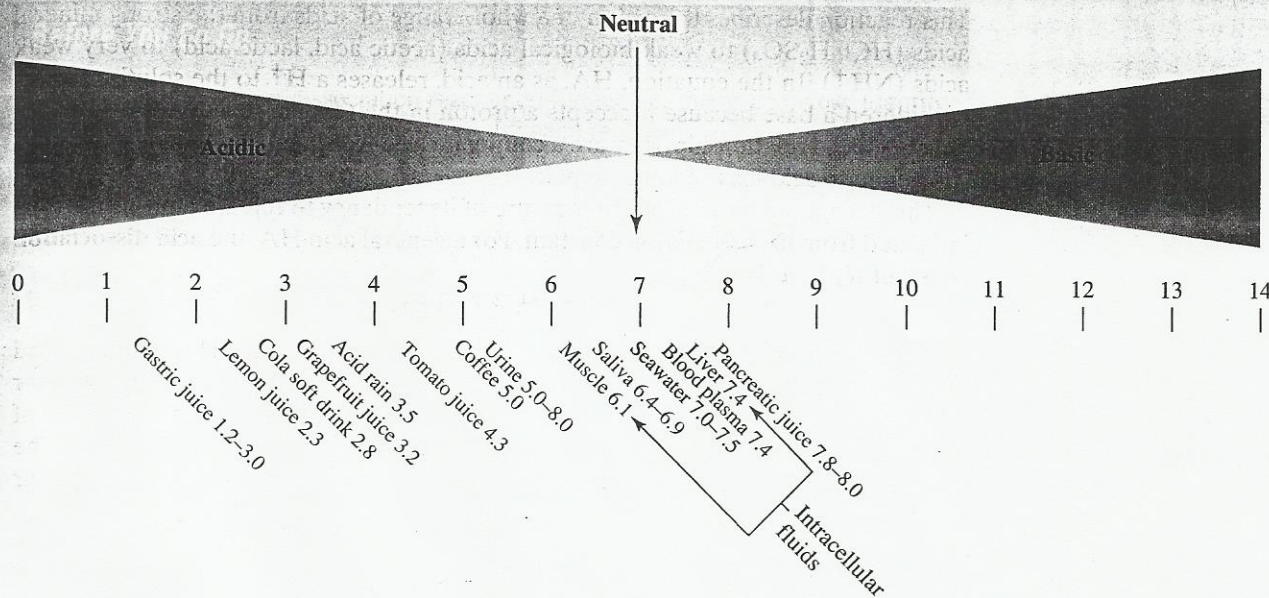


Figure 2.9 The pH values of some substances. Note that many natural fluids, but not all, are grouped around a neutral pH of 7.

As evident from the small size of $[H^+]$ ($1 \times 10^{-7} M$) the extent of water self-dissociation is slight, but it does influence the ionic character of water-based matrices in cells and other aqueous-based biological fluids. The ionic environment and the presence of H^+ and OH^- promote the ionization of dissolved acidic and basic biomolecules. The pH values for several fluids are compared in Figure 2.9.

Before You Go On...

1. Calculate the $[H^+]$ in each of the following fluids.

- a. Coffee, pH = 5.0

Solution:

$$pH = -\log[H^+]$$

$$\log[H^+] = -5.0$$

$$[H^+] = 1 \times 10^{-5} M$$

- b. Urine, pH = 6.0

- c. Saliva, pH = 6.6

- d. Cola, pH = 2.8

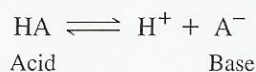
2. Calculate the pH for each of the fluids below.

- a. Gastric juice, $[H^+] = 1 \times 10^{-2} M$

- b. Acid rain, $[H^+] = 3.2 \times 10^{-3} M$

pH and pK

Calculations and measurements of pH as described above can be carried out for all aqueous solutions. The pH of a solution will depend little on the hydrogen ions generated by the self-dissociation of water, but rather on the presence of other substances (acids or bases) that increase or decrease the H^+ concentration. Acids and bases are chemical substances that change the ionic properties of solutions. A useful definition of an **acid** is a substance that releases a proton (H^+). A **base** is a substance that accepts a proton. An acid HA dissociates in aqueous solution accordingly:



This reaction describes the action of a whole range of acids from the strong mineral acids (HCl , H_2SO_4) to weak biological acids (acetic acid, lactic acid) to very weak acids (NH_4^+). In the equation, HA , as an acid, releases a H^+ to the solution. A^- is considered a base because it accepts a proton in the reverse reaction. HA and A^- have a special relationship; they are a **conjugate acid–conjugate base pair**, sometimes shortened to acid–base conjugate pair.

The strength of an acid, or the measure of its tendency to release a proton, can be indicated from its dissociation constant. For a general acid HA , the **acid dissociation constant** K_a is defined by

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

Acids common in biochemistry have a wide range of K_a values. Hydrochloric acid (HCl) is a strong acid with an immeasurably large K_a ; acetic acid is much weaker with a K_a at 25°C of $1.74 \times 10^{-5} \text{ M}$; and NH_4^+ , a very weak acid, has a K_a at 25°C of $5.62 \times 10^{-10} \text{ M}$. Note that the larger the K_a , the stronger the acid, and hence the greater the dissociation. Several acids of biochemical importance are listed with their dissociation constants in Table 2.4.

Dissociation constants written as exponentials are not convenient for everyday use. These numbers are modified for easier use by the following definition:

$$\text{p}K_a = -\log K_a$$

The negative logarithm of the dissociation constant K_a is defined as $\text{p}K_a$ (pH has a similar definition, the negative logarithm of $[\text{H}^+]$). Like K_a , the $\text{p}K_a$ value is a quantitative measure of acid strength. The most common range of $\text{p}K_a$ values for biochemical acids is from 2 to about 13 or 14. The smaller the value of $\text{p}K_a$, the stronger the acid. Note that this is the opposite of K_a , where a large value indicates a strong acid. Table 2.4 lists $\text{p}K_a$ values along with K_a values for several biochemical acids.

Table 2.4
Acids of biochemical importance

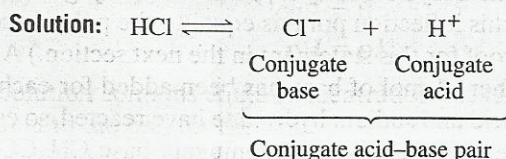
Acid	Structure ^a	K_a	$\text{p}K_a$
Formic acid	HCOOH	1.78×10^{-4}	3.75
Acetic acid	CH_3COOH	1.74×10^{-5}	4.76
Pyruvic acid	CH_3COCOOH	3.16×10^{-3}	2.50
Lactic acid	$\text{CH}_3\text{CHOHCOOH}$	1.38×10^{-4}	3.86
Malic acid	$\text{HOOC}-\text{CH}_2-\text{CHOH}-\text{COOH}$	(1) 3.98×10^{-4} (2) 5.50×10^{-6}	3.40 5.26
Citric acid	$\text{HOOC}-\text{CH}_2-\text{C}(\text{OH})(\text{COOH})-\text{CH}_2-\text{COOH}$	(1) 8.14×10^{-4} (2) 1.78×10^{-5} (3) 3.92×10^{-6}	3.09 4.75 5.41
Carbonic acid	$\text{HO}-\overset{\text{O}}{\underset{\text{O}}{\text{C}}}-\text{OH}$	(1) 4.31×10^{-7} (2) 5.62×10^{-11}	6.37 10.26
Phosphoric acid	$\text{HO}-\overset{\text{O}}{\underset{\text{OH}}{\text{P}}}-\text{OH}$	(1) 7.25×10^{-3} (2) 6.31×10^{-8} (3) 3.98×10^{-13}	2.14 7.20 12.40
Ammonium ion	$\text{H}-\overset{\text{H}}{\underset{\text{H}}{\text{N}^+}}-\text{H}$	5.62×10^{-10}	9.25

^a Acidic protons are in red.

Before You Go On...

1. Write out the proton dissociation reaction(s) for each of the acids below. Identify the conjugate acid and conjugate base for each reaction.

a. HCl, hydrochloric acid



b. CH_3COOH , acetic acid

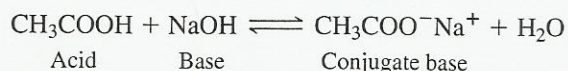
c. NH_4^+ , ammonium ion

2. The K_a for lactic acid is 1.38×10^{-4} . What is the value of the $\text{p}K_a$?

Titration Curves

Values of $\text{p}K$ (and dissociation constants) for acids can be determined experimentally by the procedure of **titration**. This consists of adding, with a buret, incremental amounts of a base to an acid sample dissolved in water and monitoring changes in the pH of the solution with a pH meter. A graph is constructed by plotting the pH on the vertical axis and the amount of base added on the horizontal axis. This yields a titration curve from which the $\text{p}K_a$ can be determined (Figure 2.10).

Monoprotic Acids We will illustrate the construction of a titration curve using acetic acid, a weak **monoprotic** biochemical acid, and sodium hydroxide, a strong base. The equation representing the chemistry of titration is



The data from the experiment are shown in Figure 2.10. The general shape of the curve, which is obtained for all weak acids, reveals useful information about the acid. The information is both structural and quantitative. The beginning pH of the acid solution, before addition of base, can be used to calculate the concentration of H^+ in

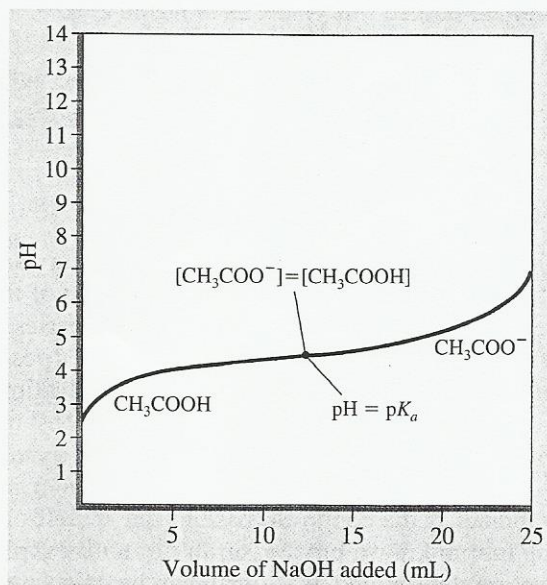


Figure 2.10 A titration curve. The experimental curve obtained from the titration of acetic acid with sodium hydroxide. The predominant structure for acetic acid is shown in two pH ranges, up to 4.76, and above 4.76. Note that at the inflection point the two forms CH_3COOH and CH_3COO^- are present in equal molar concentrations. The $\text{p}K_a$ (4.76) for the acid–base conjugate pair is measured here.