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Biochemical fundamentals of Life
(BCH 103)

Water and Buffers

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Water

Learning goals:

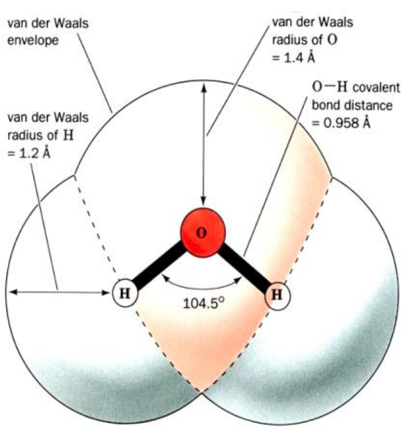
To answer the following questions?

- What kind of interactions occur between molecules?
- Why water is a good medium for life?
- Why nonpolar moieties aggregate in water?
- How dissolved molecules alter properties of water?
- How weak acids and bases behave in water?
- How buffers work and why we need them?
- How water participates in biochemical reactions?

The Solvent of Life

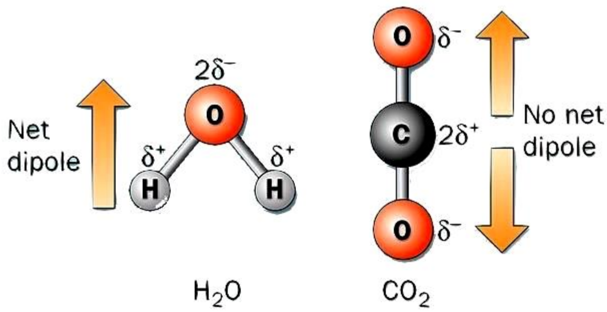
- We are ~ 70% Water
- H_2O is key to the behavior of macromolecules.
  - All life transformations occur in aqueous media
  - Most biochemical reactions take place in water
  - Water is a reactant in a number of reactions, usually in the form of H^+ and OH^-.
- Even water insoluble compounds such as lipid membranes derive their structure and function by interaction with H_2O
  - Biomolecules assume their shapes in response to the aqueous medium.

Structure of Water



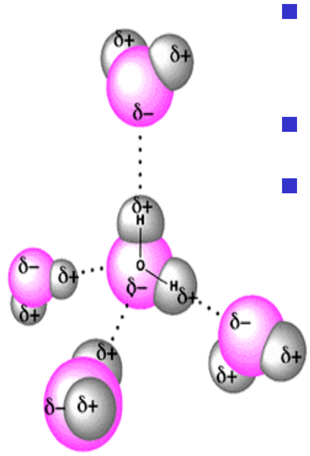
- The difference between O and H in electronegativity creates polar bonds
  - -OH is a very polar bond
  - H_2O can donate and accept hydrogen bonds
  - H_2O can function as an acid or a base
- Structure: water is a bent molecule (geometry & polarity)

Geometry Determines Polarity



While both bonds O-H and C-O are polar,
the sum of vectors in CO_2 is zero, and therefore,
CO_2 is nonpolar molecule
while H_2O is polar molecule

Hydrogen Bonding in Water



- Partial charges cause electrostatic attractions between O and H
- Each H_2O can bind 4 other H_2O 's.
- H-bonding among its molecules gives water:
  - a) high boiling point
  - b) high surface tension or capillary action
  - d) expansion upon freezing
  - e) solvent for polar molecules

Structure of the water molecule.

Two H_2O molecules joined by a hydrogen bond (designated by three blue lines) between the oxygen atom of the upper molecule and a hydrogen atom of the lower one.

Hydrogen bonds are longer and weaker than covalent O—H bonds

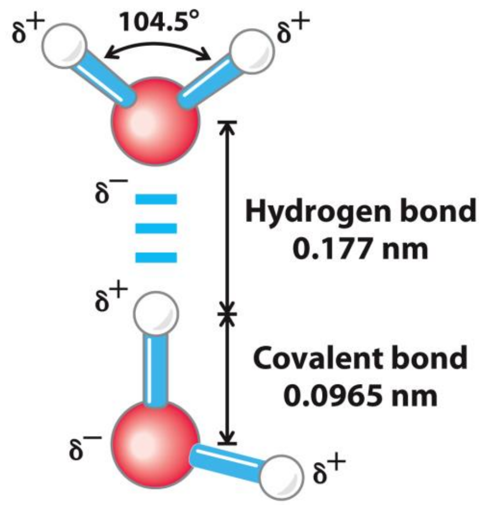
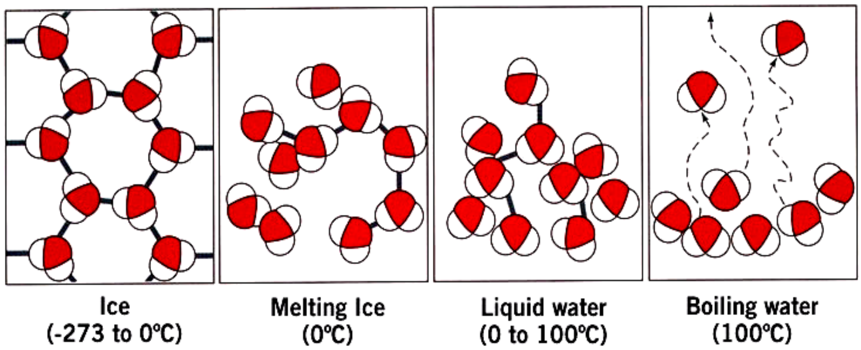


Figure 2-1b
Lehninger Principles of Biochemistry, Seventh Edition

Ice: Water in a Solid State

- Water has many different crystal forms; the hexagonal ice is the most common.
- Hexagonal ice forms an organized lattice and thus has a low entropy.
- Hexagonal ice contains maximal hydrogen bonds/ water molecules, forcing the water molecules into equidistant arrangement. Thus:
  - ice has lower density than liquid water
  - ice floats

Water: Ice, Liquid, and Vapor



	H_20	NH_3	CH_4	H_2S
Molecular weight	18	17	16	32
Boiling point (° C)	100	-33	-161	-60.7
Melting point(° C)	0	-78	-163	-65.5
Viscosity (centipoises)	1.01	0.35	0.10	0.15

- In ice, each water molecule forms four hydrogen bonds, the maximum possible for a water molecule, creating a regular crystal lattice.
- By contrast, in liquid water at room temperature and atmospheric pressure, each water molecule hydrogen-bonds with an average of 3.4 other water molecules.
- This crystal lattice structure makes ice less dense than liquid water, and thus ice floats on liquid water.

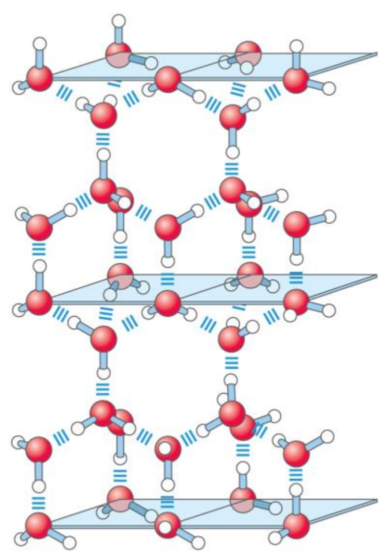


Figure 2-2
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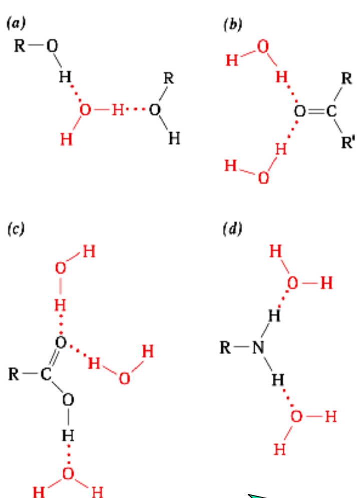
Water as a Solvent

- Water is a good solvent for charged and polar substances:
  - amino acids and peptides
  - small alcohols
  - carbohydrates
- Water is a poor solvent for nonpolar substances:
  - nonpolar gases
  - aromatic moieties
  - aliphatic chains

Water as a solvent: H-bonding

Based on their interaction with H_2O, Molecules are divided into two types:

- Hydrophobic molecules: do not interact with H_2O
- Hydrophilic molecules: able to interact with H_2O via polar functional groups or charged groups

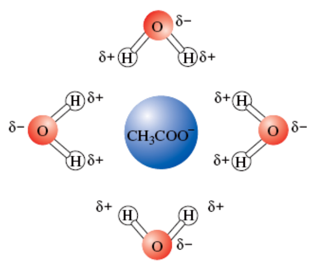


Name each of these compounds?

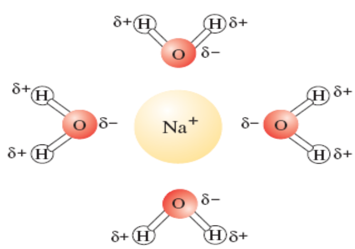
Which chemical groups are hydrophilic????

- All charged groups are hydrophilic
- Uncharged polar molecules have functional groups that form H-bonds with H_2O.
- Examples: Alcohols, amines, carbonyls (aldehydes & ketones)

Water As a Solvent: Ionic Interactions



- Water can solvate charged molecules (both cations & anions)
- Water projects its partially positive hydrogens towards negatively charged ions.
- Water projects its partially negative oxygen towards positively charged ions



- Notice the opposite orientation of water molecules around a cation versus anion.
- This type of interaction is called ion–dipole interactions.

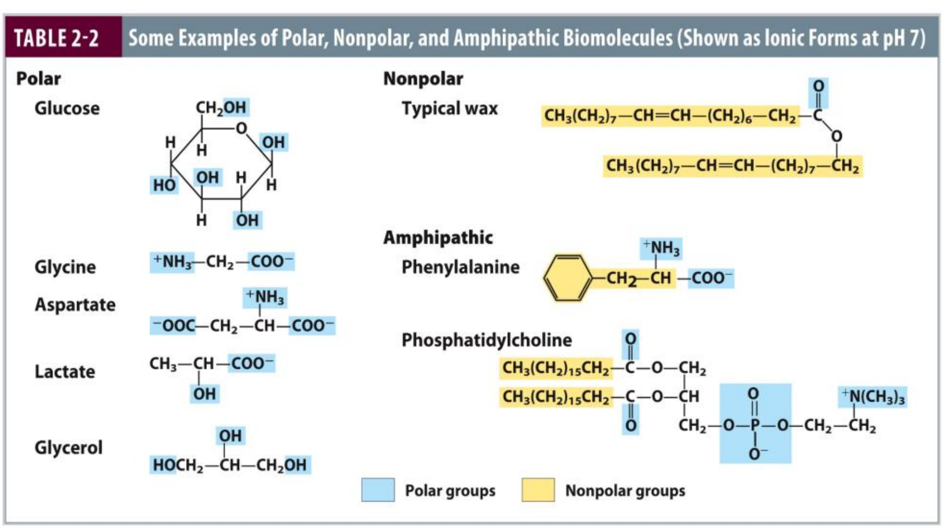


Table 2-2
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Osmotic Pressure

- Water moves from areas of high water concentration (low solute concentration) to areas of low water concentration (high solute concentration).
- Osmotic pressure (π) is the force necessary to resist the movement.
- Osmotic pressure is influenced by the concentration of each solute in solution.
- Dissociated components of a solute individually influence the osmotic pressure.

Osmotic Pressure

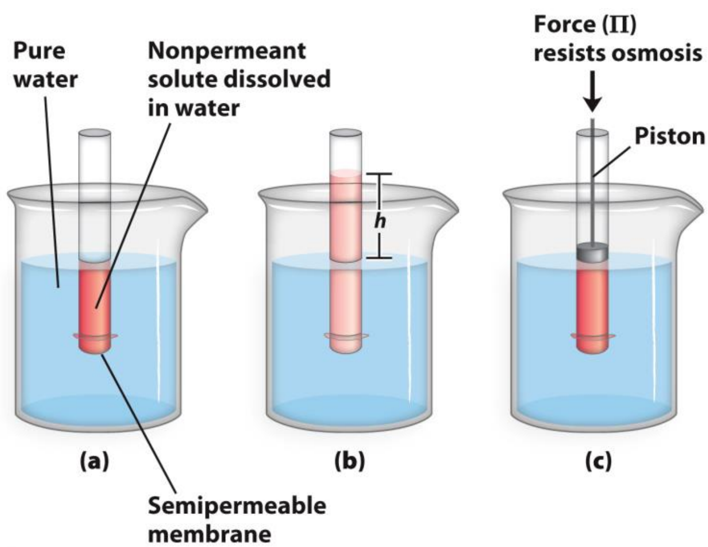

Figure 2-12
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Effect of Osmotic Pressure on Cells

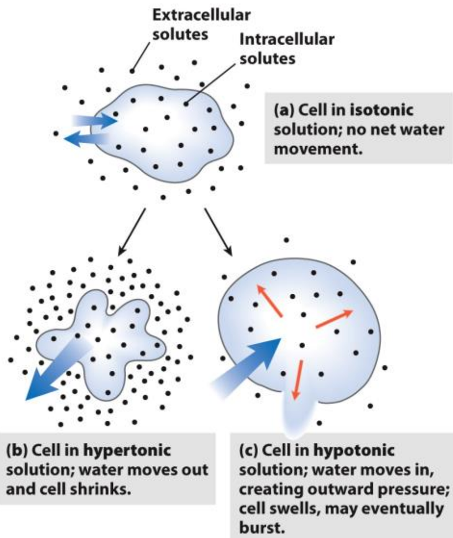

Figure 2-13
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Ionization of Water

$$H_2O \rightleftarrows H^+ + OH^-$$

- O-H bonds are polar and can dissociate heterolytically.
- Products are a proton (H^+) and a hydroxide ion (OH^-).
- Dissociation of water is a rapid reversible process.
- Most water molecules remain un-ionized, thus pure water has very low electrical conductivity (resistance: 18 MΩ•cm).
- The equilibrium is strongly to the left (low K_{eq}).
- The extent of dissociation depends on the temperature.

Ionization of Water

Concentrations of participating species in an equilibrium process are not independent but are related via the equilibrium constant:

$$H_2O \rightleftarrows H^+ + OH^- \qquad K_{eq} = \frac{[H^+] \bullet [OH^-]}{[H_2O]}$$

K_{eq} can be determined experimentally, it is 1.8 • 10^{-16} M at 25°C.
$[H_2O]$ can be determined from water density, it is 55.5 M.

- Ionic product of water:

$$K_w = K_{eq} \cdot [H_2O] = [H^+][OH^-] = 1 \cdot 10^{-14} M^2$$

- In pure water, $[H^+] = [OH^-] = 10^{-7}$ M.

Ionization of Water: pH Scale

$$H_2O \Leftrightarrow H^+ + OH^-$$

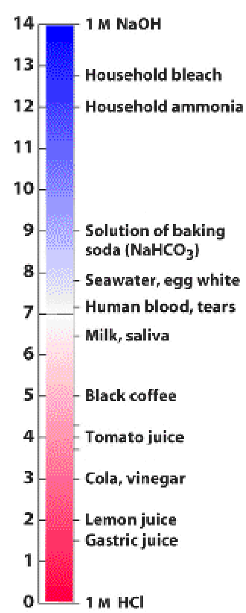
- Pure water has equal concentrations of H^+ and HO^-

$[H^+]=[HO^-] = 10^{-7}$ M for a neutral solution

If $[H^+] > 10^{-7}$ M, then the solution is acidic

If $[H^+] < 10^{-7}$ M, then the solution is basic

pH = -log[H^+]



Bronsted-Lowry Acids

$$HA + H_2O \rightleftharpoons H_3O^+ + A^-$$

- An acid is a substance that can donate a proton
- A base is a substance that can accept a proton
- In the above equation, HA is the acid and H_2O is the base
- A^- is the conjugate base of HA, and H_3O^+ is the conjugate acid of H_2O

$$CH_3COOH \rightleftharpoons H^+ + CH_3COO^-$$

Acid — Conjugate base

Proton Hydration

- Protons do not exist free in solution.
- They are immediately hydrated to form hydronium ions (H_3O^+).

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

- A hydronium ion is a water molecule with a proton associated with one of the nonbonding electron pairs.
- Hydronium ions are solvated by nearby water molecules.
- The covalent and hydrogen bonds are interchangeable. This allows for an extremely fast mobility of protons in water via "proton hopping."

Proton Hopping

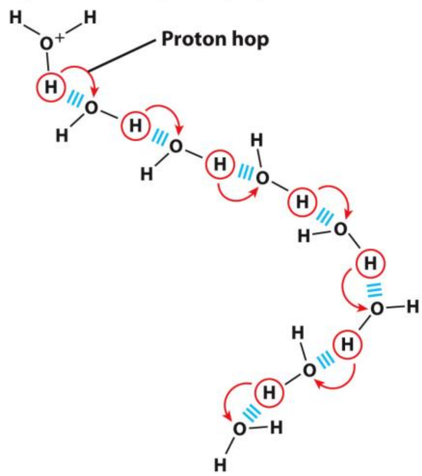


Figure 2-14
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What Is pH?

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

$$K_w = [H^+][OH^-] = 1 \cdot 10^{-14} M^2$$

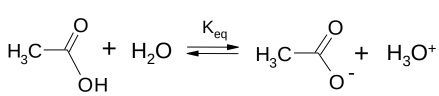
$$-\log[H^+] - \log[OH^-] = +14$$

$$pH + pOH = 14$$

- pH is defined as the negative logarithm of the hydrogen ion concentration.
- Simplifies equations
- The pH and pOH must always add up to 14.
- In neutral solution, $[H^+] = [OH^-]$ and the pH is 7.
- pH can be negative ($[H^+] = 6$ M).

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

Dissociation of Weak Electrolytes: Principle

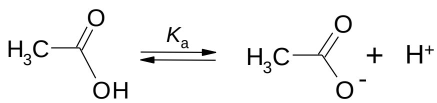


$$K_a = \frac{[H^+][CH_3COO^-]}{[CH_3COOH]} = 1.74 \cdot 10^{-5}\,M$$

- Weak electrolytes dissociate only partially in water.
- The extent of dissociation is determined by the acid dissociation constant K_a.
- We can calculate the pH if the K_a is known. But some algebra is needed!

Dissociation of Weak Electrolytes: Example

What is the final pH of a solution when 0.1 moles of acetic acid is added to water to a final volume of 1L?



0.1 – x x x

$$K_a = \frac{[x][x]}{[0.1 - x]} = 1.74 \cdot 10^{-5}\,\mathrm{M}$$

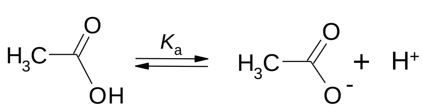
$$x^2 = 1.74 \cdot 10^{-6} - 1.74 \cdot 10^{-5} x$$

$$x^2 + 1.74 \cdot 10^{-5} x - 1.74 \cdot 10^{-6} = 0$$

x = 0.001310, pH = 2.883

- We assume that the only source of H^+ is the weak acid.
- To find the $[H^+]$, a quadratic equation must be solved.

Dissociation of Weak Electrolytes: Simplification



0.1 – *x* *x* *x*

0.1 *x* *x*

$$K_a = \frac{[x][x]}{[0.1]} = 1.74 \times 10^{-5}\,\mathrm{M}$$

$$x^2 = 1.74 \times 10^{-6}$$

x = 0.00132, pH = 2.880

- The equation can be simplified if the amount of dissociated species is much less than the amount of undissociated acid.
- Approximation works for sufficiently weak acids and bases.
- Check that $x <$ [total acid].

pK_a Measures Acidity

$$pK_a = -\log K_a$$

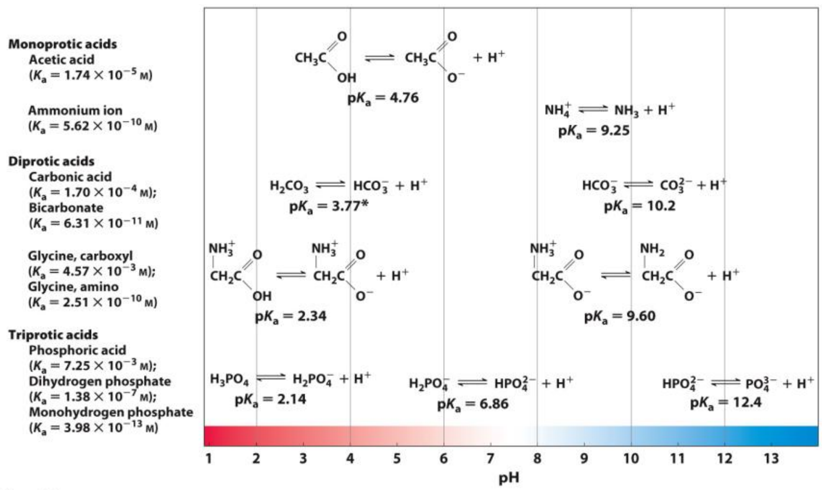


Figure 2-16
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Buffers

- Buffers are solutions that resist change in pH after addition of small quantity of weak acid or base.
- Buffers are mixtures of weak acids and their conjugate base
- Each acid has a conjugate base
- For example B^- is the conjugate base of the acid HB

$$NH_4^+ + H_2O \rightleftharpoons H_3O^+ + NH_3$$

$$HCl + H_2O \rightleftharpoons H_3O^+ + Cl^-$$

$$HSO_4 + H_2O \rightleftharpoons H_3O^+ + SO_4^-$$

$$HBr + H_2O \rightleftharpoons H_3O^+ + Br^-$$

$$H_2O \quad + \quad H_2O \rightleftharpoons H_3O^+ \quad + \quad OH^-$$

Weak acids dissociate weakly because they have strong conjugate base that bind hydrogen.
Strong acids ionize easily to give H^- because they have weak conjugate base
Acids are proton donors
Bases are proton acceptors

At pH = pK_a, there is a 50:50 mixture of acid and anion forms of the compound.

Buffering capacity of acid/anion system is greatest at pH = pK_a.

Buffering capacity is lost when the pH differs from pK_a by more than 1 pH unit.

Henderson–Hasselbalch Equation: Derivation

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

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

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

↓

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

↓

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

Henderson–Hasselbalch Equation: Example

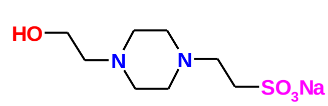
A buffer is comprised of 0.1 M Acetic acid (CH_3COOH, pKa=4.76) and 0.05 M Sodium acetate (CH_3COONa). What is the final pH of the buffer?

$$\text{pH} = 4.76 + log\frac{[0.05]}{[0.1]}$$

Final buffer pH = 4.45

Biological Buffer Systems

- Maintenance of intracellular pH is vital to all cells.
  - Enzyme-catalyzed reactions have optimal pH.
  - Solubility of polar molecules depends on H-bond donors and acceptors.
  - Equilibrium between CO_2 gas and dissolved HCO_3^- depends on pH.
- Buffer systems *in vivo* are mainly based on:
  - phosphate, concentration in millimolar range
  - bicarbonate, important for blood plasma
  - histidine, efficient buffer at neutral pH
- Buffer systems *in vitro* are often based on sulfonic acids of cyclic amines.
  - HEPES
  - PIPES
  - CHES



Quiz

Answer by marking true (T) or False (F)

1. The most abundant elements in cell are C H N O P S .... (   )
2. Molybdenum, bromide and boron are examples of trace elements .... (   )
3. Water represents 50% of the living cell .... (   )
4. Covalent bond is the strongest bond in biochemistry .... (   )
5. Hydrogen bond can be dissociated by heating or changing pH .... (   )
6. The difference between O and H in electronegativity creates covalent bonds .... (   )
7. Most of the water soluble compounds have polar or charged groups .... (   )
8. Each H_2O molecule can bind 3 other H_2O to form complex of $4H_2O$ .... (   )
9. The Oxygen in water molecule has one partial negative charge .... (   )

Quiz

Answer by marking true (T) or False (F)

1.H_2O can function as an acid or a base .... (   )

2.The structure of H_2O is a linear molecule .... (   )

3.Phenols act as a weak acid as it gives protons to a strong base .... (   )

4.Sulpher is less electronegative than Oxygen .... (   )

5.An acid is a substance that can accept a proton .... (   )