

**WATER** is the solvent of choice for biological systems

- Constitutes 70-85% of cell weight, typically
- Important as a **solvent** and a **reactant** in biochemical reactions
- Helps regulate **temperature** since it is able to absorb large amounts of heat
- Helps regulate **intracellular pH**
- Used for transport – delivers nutrients and removes waste from cells

Water is a unique **solvent** whose properties are

extremely important to biochemistry.

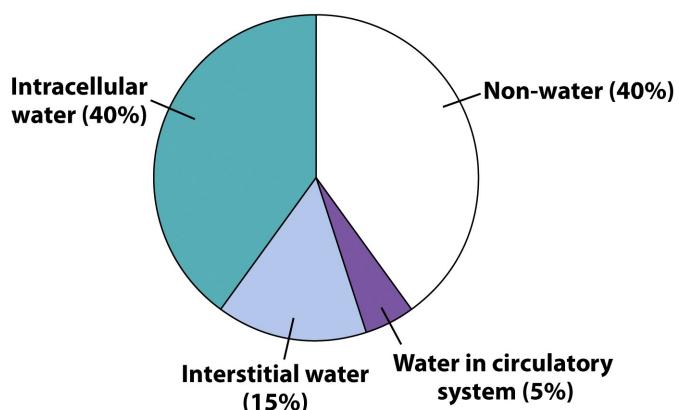
**Table 2.1**  
**Percent by weight of water in organs of the human body**

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

<sup>a</sup> In adults.

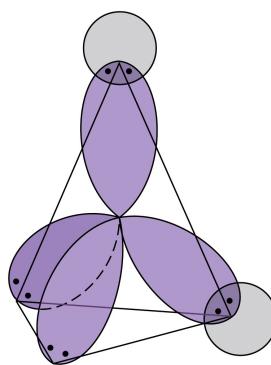
<sup>b</sup> Fat-free tissue.

Table 2-1 Concepts in Biochemistry, 3/e  
© 2006 John Wiley & Sons

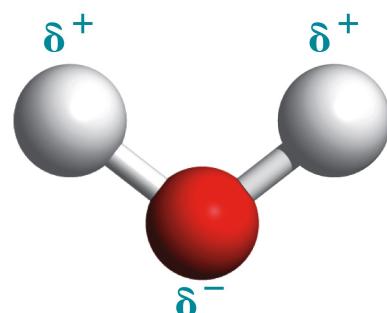


© John Wiley & Sons, Inc. All rights reserved.

- In water, the hydrogen atoms have a partial positive charge, and the oxygen atoms have a partial negative charge



© John Wiley & Sons, Inc. All rights reserved.



© John Wiley & Sons, Inc. All rights reserved.

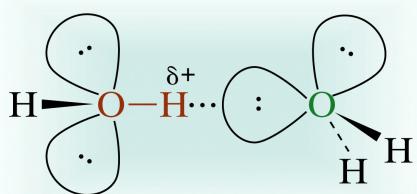
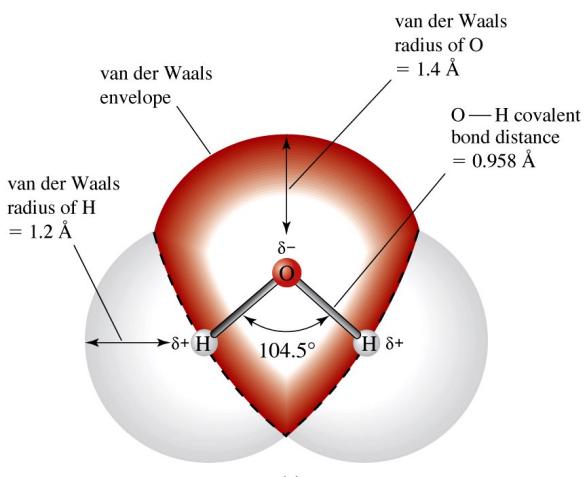
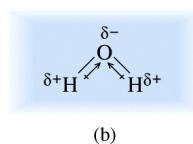


Figure 2-2 Concepts in Biochemistry, 3/e  
© 2006 John Wiley & Sons

Figure 2-1 Concepts in Biochemistry, 3/e  
© 2006 John Wiley & Sons



- The polar nature and geometry of the water molecule allows water molecules to form **hydrogen bonds** with each other and with dissolved **hydrophilic** substances.
- Hydrogen bonds between water molecules = electrostatic attraction between the oxygen atom of one water and the hydrogen of another
- Water can also form hydrogen bonds with functional groups of hydrophilic (polar or ionic) biomolecules and organic compounds.
  - **hydrogen bond donors**
  - **hydrogen bond acceptors**

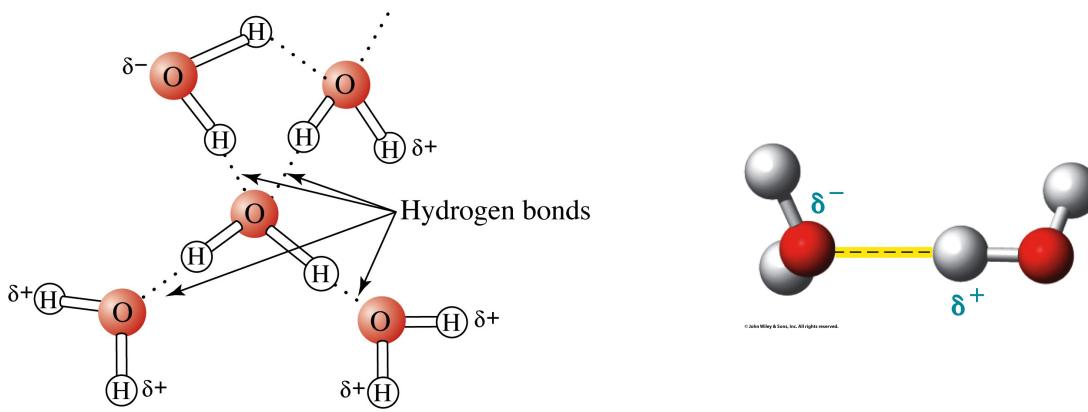


Figure 2.5a Concepts in Biochemistry, 3/e  
© 2006 John Wiley & Sons

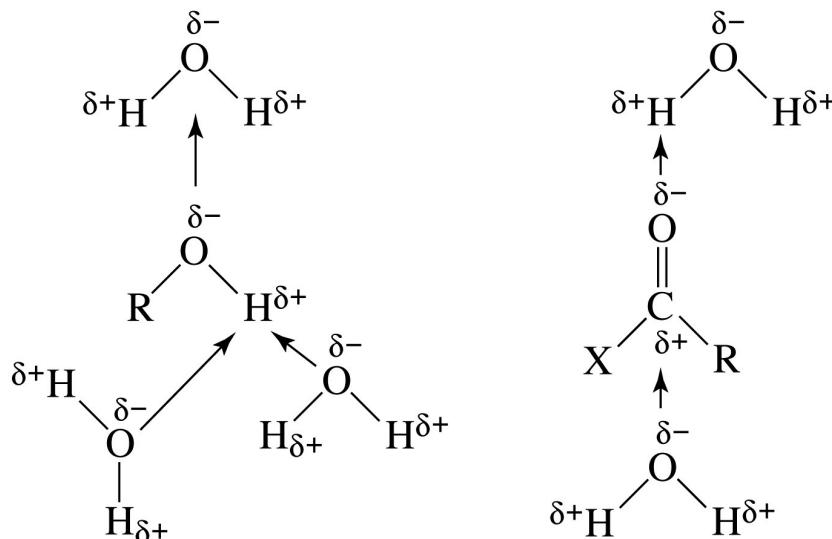
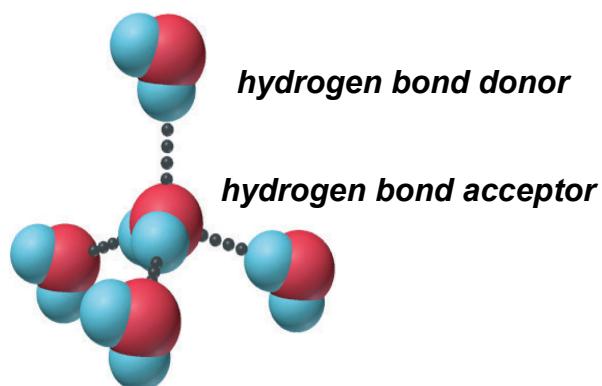


Figure 2.6a Concepts in Biochemistry, 3/e  
© 2006 John Wiley & Sons

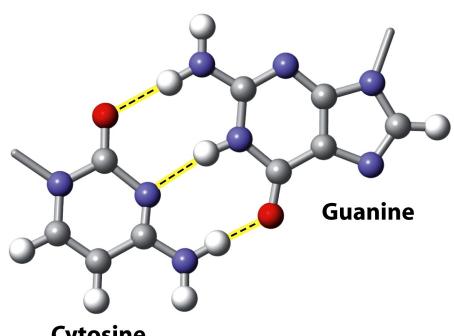
H-bonding is especially strong in water because:

1. the O—H bond is very polar
2. there are 2 lone pairs on the O atom
3. Each H<sub>2</sub>O molecule can form **four** H bonds to other molecules, resulting in a tetrahedral arrangement.

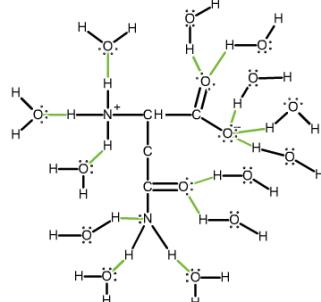
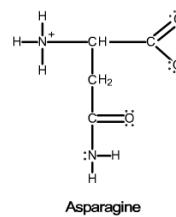
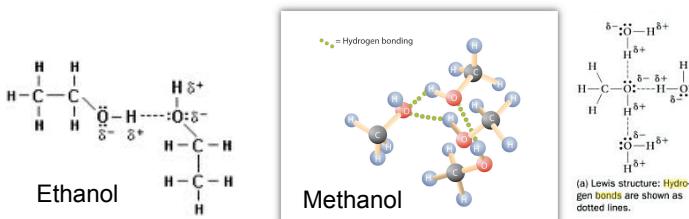
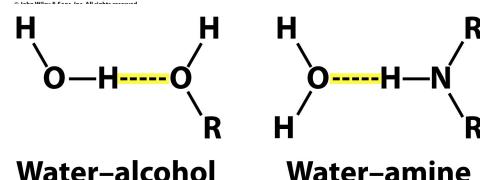
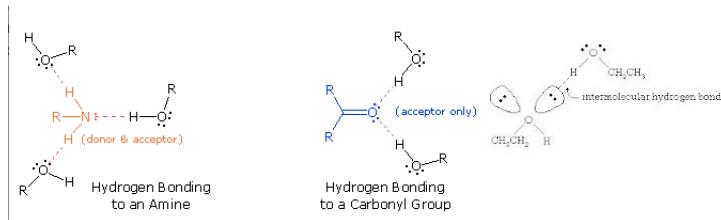


## Noncovalent Interactions: Relatively weak and reversible

1. **Hydrogen Bonds:** Special dipole-dipole interaction (electronegative atom (e.g. O or N) interacts with H atom that is partially positive (i.e. attached to N, O, F))  
Very important for protein and DNA structure.



# More Hydrogen Bonding

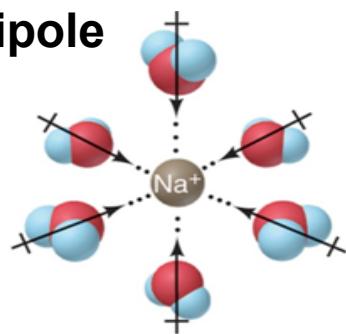


## **2. Van Der Waals interactions**

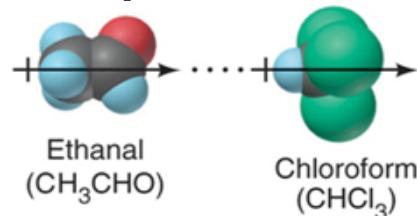
- a. Dispersion Forces (London Forces) (induced dipoles in non-polar molecules)
  - b. Dipole-dipole forces

3. **Ionic Bonds:** Electrostatic interaction between two oppositely charged ions.

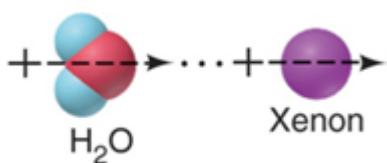
## Ion-dipole



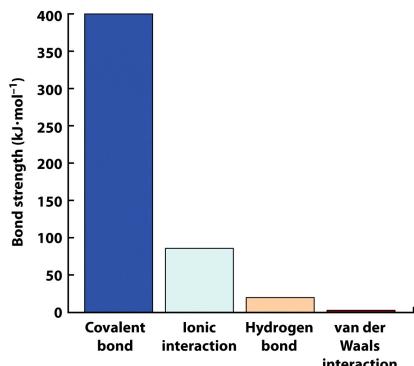
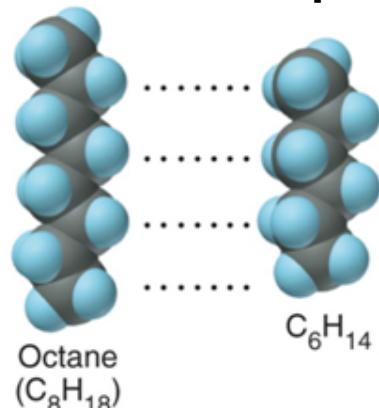
# Dipole-dipole



## Dipole-induced dipole



## Dispersion/Induced Dipole



### Bond Strength Comparison

4. **Hydrophobic Interactions/Hydrophobic Effect:** Relations between water and hydrophobic molecules (low water-soluble molecules). Nonpolar substances tend to aggregate in aqueous solution and exclude water molecules. Thermodynamically unfavorable to dissolve hydrophobic substance in water. Entropy-driven process.  $\Delta G = \Delta H - T\Delta S$

Water molecules align themselves around non-polar molecule and lose freedom to form hydrogen bonds. Entropy lost in system results in thermodynamic barrier.

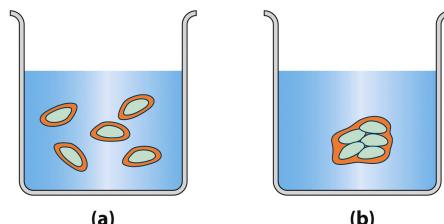
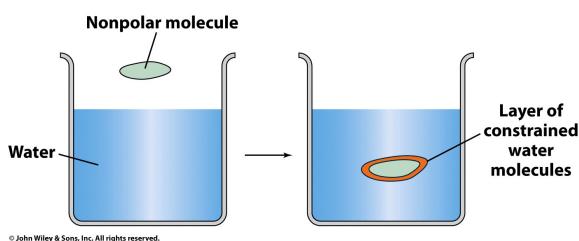
Multiple molecules aggregate – increase the entropy of the system because fewer water molecules needed to surround the aggregate than to hydrate each dispersed molecule.

The **hydrophobic effect** is the observed tendency of nonpolar substances to aggregate in aqueous solution and exclude water molecules.

Mostly **entropy** driven:  
Water more ordered at interface.  
Entropy of water molecules increases upon exclusion of non-polar species



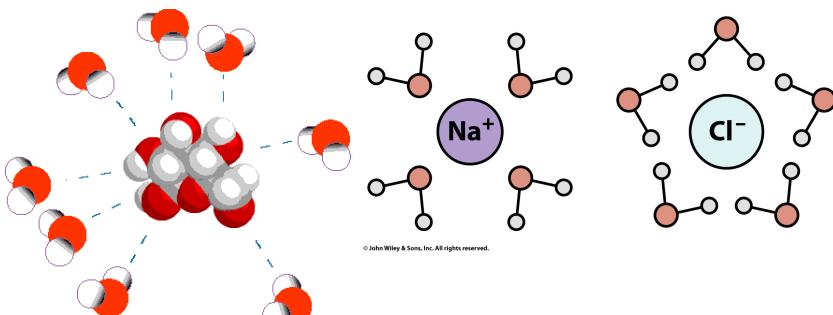
SCIENCEPHOTOLIBRARY



Exclusion of nonpolar substances from aqueous solution = **HYDROPHOBIC EFFECT**

Important concept in biochemistry – governs protein folding (hydrophobic amino acids are on the interior of the protein), formation of membranes (hydrophobic lipid tails sequestered in bilayer).

**Water soluble** compounds are those in which the interactions between the solute and water are greater than those between solute molecules. i.e. salts, biological molecules that have polar or ionic groups (e.g. glucose, ethanol)



### H-BONDING IN DRUG DESIGN

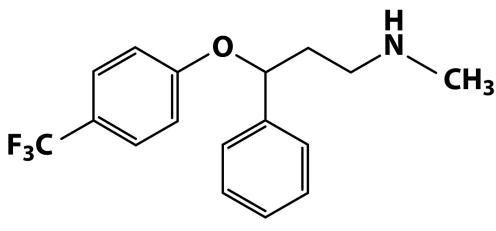
25% of drugs contain Fluorine! Why?

Takes the place of H in a chemical structure.

Very electronegative – thus electron withdrawing.

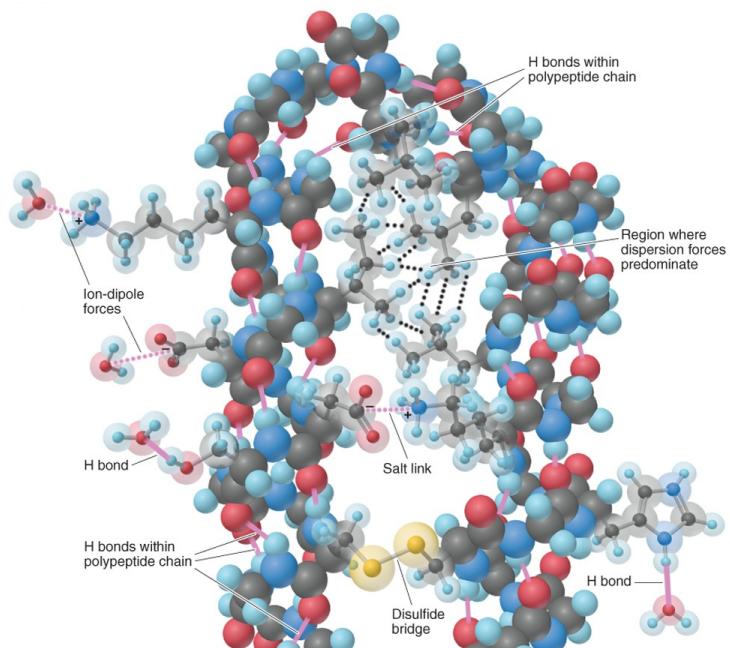
Gives different properties to the group.

- $\text{CF}_3$  – Electron withdrawing, can decrease basicity of nearby amino groups – fewer positive charges – can penetrate cells better.
- C – F bond can form hydrogen bonds and other dipole-dipole interactions, potentially increasing binding to target molecule in the body. May be more effective at lower concentrations.



### NON-COVALENT INTERACTIONS IN PROTEINS:

- Non-covalent interactions also form between two biomolecules (e.g. proteins & DNA)
- The 3-dimensional structure of many biological molecules (eg. proteins) and macromolecular structures (eg. membranes, DNA) is determined by hydrogen bonding, hydrophobic interactions, ionic interactions and van der Waals interactions.
- Hydrogen bonds are weak but their high abundance makes them important!



**ACIDS, BASES and BUFFERS****REACTIONS OF WATER:**

- Reversible self-dissociation = ionization
  - o Generates  $\text{H}^+$  and  $\text{OH}^-$
  - o Can be described by the following equilibrium:



\*Note hydrogen atoms do not exist as free  $\text{H}^+$  in solution.

Actually are hydronium ions ( $\text{H}_3\text{O}^+$ ). For simplicity, we just write  $\text{H}^+$ .

- Express extent of ionization quantitatively:  
Use law of mass action to define the equilibrium point of the dissociation reaction:

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

$K_{\text{eq}}$  is defined as the **ratio** of the **concentrations** of the **products** and **reactants**.

Units used to define concentration are **Molarity (M) = moles/L**

- $K_{\text{eq}}$  for pure water determined experimentally to be  **$1.8 \times 10^{-16} \text{ M}$**  at  **$25^\circ\text{C}$**
- **Concentration of pure  $\text{H}_2\text{O} = 55.5\text{M}$**  (weight of water in 1 L (1000 g) divided by mw of 18)

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

$$[\text{H}^+][\text{OH}^-] = (1.8 \times 10^{-16})(55.5)$$

$$[\text{H}^+][\text{OH}^-] = 1.0 \times 10^{-14} \text{ M} \quad (\text{M}^2)$$

Because according to the chemical equation for dissociation  $\text{H}^+$  and  $\text{OH}^-$  must have equal concentrations in pure water, then:

$$[\text{H}^+] = [\text{OH}^-] = \sqrt{1.0 \times 10^{-14} \text{ M}} = 1.0 \times 10^{-7} \text{ M} \quad (\text{under square root should read M}^2)$$

- This is the basis of the pH scale!
- These numbers are very small and difficult to work with, so in 1909 Soren Sorenson introduced the term pH to more conveniently express  $[\text{H}^+]$ .
- **Defined pH as the negative logarithm of the hydrogen ion concentration:**

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

- Also the same as  $\log 1/[\text{H}^+]$
- “p” is an operator – means to “take the negative log of”
- Example:  $\text{pOH} = -\log[\text{OH}^-]$ ; pH of pure water?  $[\text{H}^+] = 1 \times 10^{-7}$ ,  $\text{pH} = -\log(1 \times 10^{-7}) = 7$
- Back to water ionization:

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

- Take  $-\log$  of both sides for convenience:

$$-\log (1 \times 10^{-14} M^2) = -\log [H^+] + -\log [OH^-]$$

$$14 = pH + pOH$$

- The pH scale ranges from 0 to 14
- pH scale is LOG BASED! Used to keep track of large changes important to acids and bases
- **Defined pH as the negative logarithm of the hydrogen ion concentration:**

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

- Water ionization:

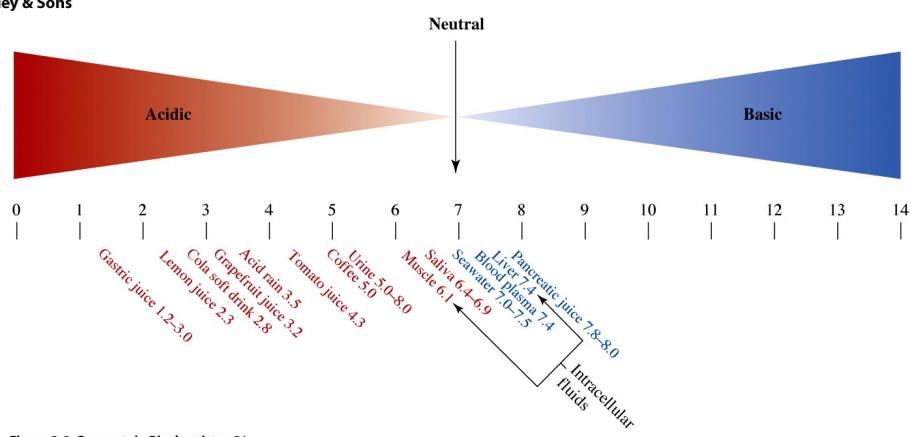
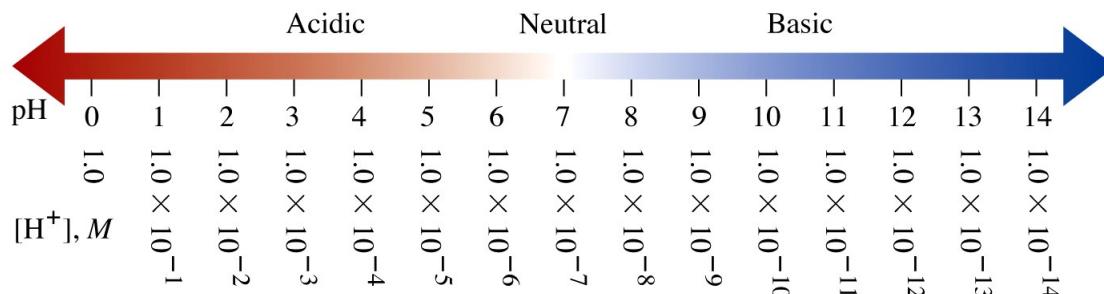
$$[H^+][OH^-] = 1.0 \times 10^{-14} M$$

- Take  $-\log$  of both sides for convenience:

$$-\log (1 \times 10^{-14} M^2) = -\log [H^+] + -\log [OH^-]$$

$$14 = pH + pOH$$

- The pH scale ranges from 0 to 14
- pH scale is LOG BASED! Used to keep track of large changes important to acids and bases
- Important to remember that the scale is **exponential**
  - o One pH unit = 10 times more acidic or basic

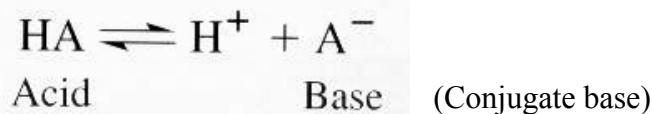


**Remember:**

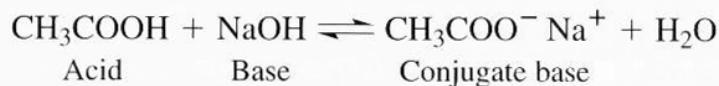
Add acid, pH gets smaller  
Add base, pH gets larger

**Acids and Bases**

- Applies to other acids and bases, not just water
  - o Acid = Releases proton in water (proton donor)
  - o Base = Accepts proton in water (proton acceptor)
  - o Water can act as an acid and a base = amphiprotic
  - o Strength of an acid is defined as its tendency to *release a proton (dissociate)*
- Define dissociation for an acid:



- Conjugate Base: base formed by the removal of a proton from an acid
- The acid and conjugate base are complementary species – every acid has a conjugate base
- Should be able to identify acids and their conjugate bases:

**Acetic acid/acetate pair:**

- Just as for water, we can write an equilibrium constant for the dissociation of the acid ( $K_a$ )

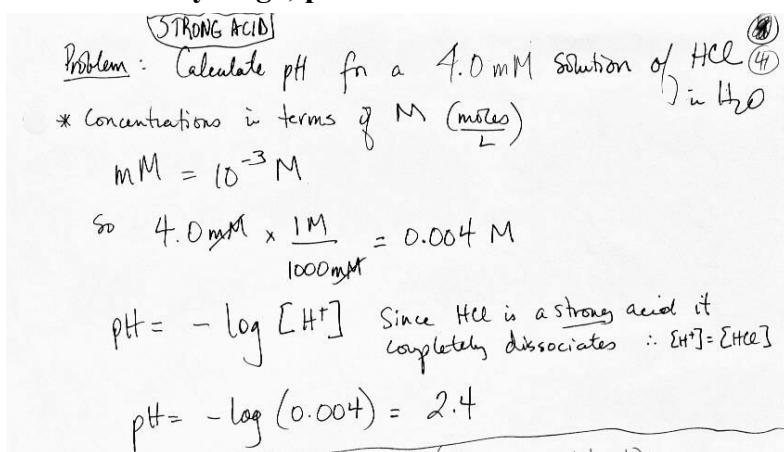
$$K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]}$$

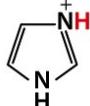
- We want  $K_a$  in convenient terms. Apply “p” rule and take the negative log of  $K_a$  to get  $\text{p}K_a$ .

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

- $\text{p}K_a$  is a QUANTITATIVE measure of acid strength.
  - o **Smaller  $\text{p}K_a \rightarrow$  Stronger acid**
  - o **Larger  $\text{p}K_a \rightarrow$  Weaker acid (stronger base)**
  - o Opposite of  $K_a$  where a LARGE number indicates strong acid
  - o Large  $K_a$  means mostly dissociated into  $\text{H}^+$  and  $\text{A}^-$ , not much HA left.
    - Numerator large, denominator small  $\rightarrow$  large number
    - **$K_a$  large =  $\text{p}K_a$  small**
- Examples of acids:
  - o **Strong acids**
    - $\text{HCl}$  and  $\text{HNO}_3$
    - Dissociate completely in water

- $[H^+]$  concentration is approx. equal to the [acid] in solution
- **K<sub>a</sub> is very large; pK<sub>a</sub> is small**

**TABLE 2-4 | pK Values of Some Acids**

Name	Formula <sup>a</sup>	pK
Trifluoroacetic acid	CF <sub>3</sub> COOH	0.18
Phosphoric acid	H <sub>3</sub> PO <sub>4</sub>	2.15 <sup>b</sup>
Formic acid	HCOOH	3.75
Succinic acid	HOOCCH <sub>2</sub> CH <sub>2</sub> COOH	4.21 <sup>b</sup>
Acetic acid	CH <sub>3</sub> COOH	4.76
Succinate	HOOCCH <sub>2</sub> CH <sub>2</sub> COO <sup>-</sup>	5.64 <sup>c</sup>
Thiophenol	C <sub>6</sub> H <sub>5</sub> SH	6.60
Phosphate	H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	6.82 <sup>c</sup>
N-(2-acetamido)-2-aminoethanesulfonic acid (ACES)	H <sub>2</sub> NCOCH <sub>2</sub> <sup>+</sup> NH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> SO <sub>3</sub> <sup>-</sup>	6.90
Imidazole		7.00
p-Nitrophenol		7.24
N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)	HOCH <sub>2</sub> CH <sub>2</sub> <sup>+</sup> NH <sub>2</sub> CH <sub>2</sub> SO <sub>3</sub> <sup>-</sup>	7.55
Glycinamide	<sup>+</sup> H <sub>3</sub> NCH <sub>2</sub> CONH <sub>2</sub>	8.20
Tris(hydroxymethyl)-aminomethane (Tris)	(HOCH <sub>2</sub> ) <sub>3</sub> CNH <sub>3</sub> <sup>+</sup>	8.30
Boric acid	H <sub>3</sub> BO <sub>3</sub>	9.24
Ammonium ion	NH <sub>4</sub> <sup>+</sup>	9.25
Phenol	C <sub>6</sub> H <sub>5</sub> OH	9.90
Methylammonium ion	CH <sub>3</sub> NH <sub>3</sub> <sup>+</sup>	10.60
Phosphate	HPO <sub>4</sub> <sup>2-</sup>	12.38 <sup>d</sup>

<sup>a</sup>The acidic hydrogen is highlighted in red; <sup>b</sup>pK<sub>1</sub>; <sup>c</sup>pK<sub>2</sub>; <sup>d</sup>pK<sub>3</sub>.

© John Wiley &amp; Sons, Inc. All rights reserved.

**Weak Acids:**

Common in biological systems – will focus on these common acids: Acetic acid, phosphoric acid, carbonic acid, and lactic acid

Not completely dissociated in water, so  $[H^+]$  will be much lower than  $[HA]$

Need to be concerned with equilibrium in solution

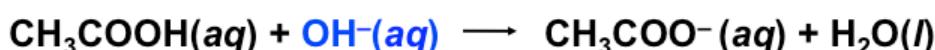
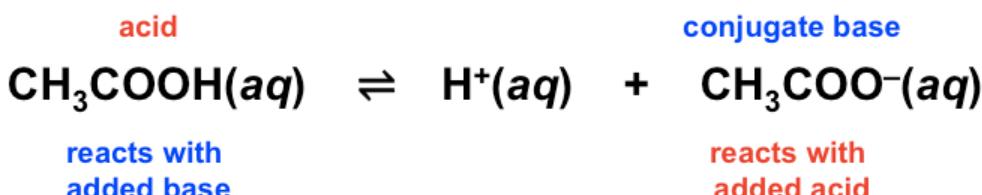
Amino acids are also weak acids  $pK_a > 1$

## BUFFERS

- Buffers are extremely important in biological systems
  - Buffers are solutions that resist changes in pH upon addition of acid or base
  - Examples:
    - o Maintaining blood pH
    - o Maintaining physiological pH inside cells

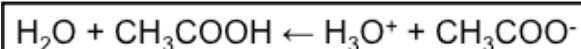
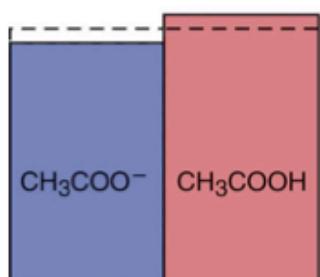
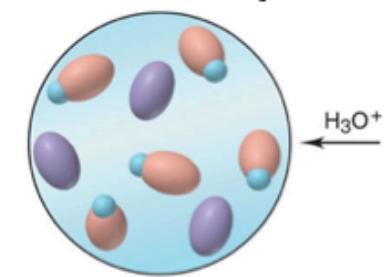
A solution that contains a weak acid and its conjugate base (or a weak base and its conjugate acid) is a ***buffer***.

A buffer is LeChatelier's Principle in action



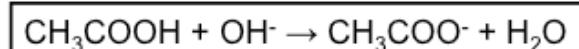
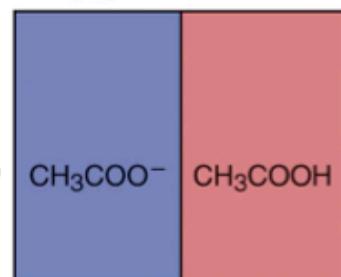
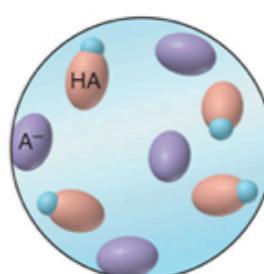
How buffer solutions resist changes in pH.

*Buffer has more HA after addition of  $H_3O^+$ .*



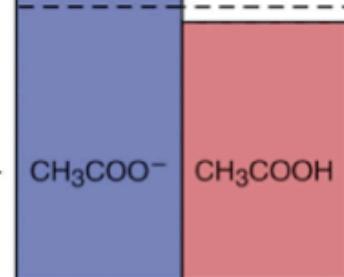
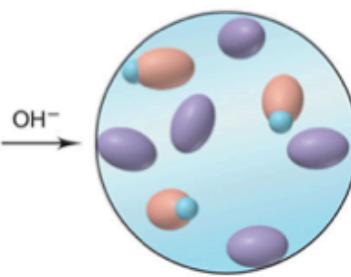
Note: no net increase in  $[H^+]$

*Buffer has equal concentrations of A<sup>-</sup> and HA.*



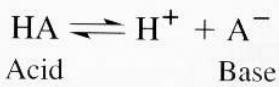
Note: no net increase in  $[OH^-]$

*Buffer has more A<sup>-</sup> after addition of OH<sup>-</sup>.*



- **Henderson-Hasselbalch Equation**

- o Equation that describes the behavior of weak acids in solution
- o Allows us to calculate the concentration of an acid and conjugate base at various pH



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

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

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

- We measure pH
- Since the pH of a solution is determined by the relative concentrations of acids and bases, let's express this in terms of pH.
- Isolate  $[\text{H}^+]$  factor
- Take log of both sides
- Rearrange to get  $\log[\text{H}^+]$  by itself
- We then apply the definitions of pH and  $pK_a$  to get:

## Henderson–Hasselbalch equation

$$\text{pH} = pK_a + \log \frac{[\text{A}^-]}{[\text{HA}]} \quad \frac{\text{[conjugate base]}}{\text{[acid]}}$$

USE THIS RATIO OF THE TWO SPECIES TO CALCULATE BUFFER COMPONENTS!

- Note that if  $[\text{A}^-] = [\text{HA}]$ , the following is true:

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

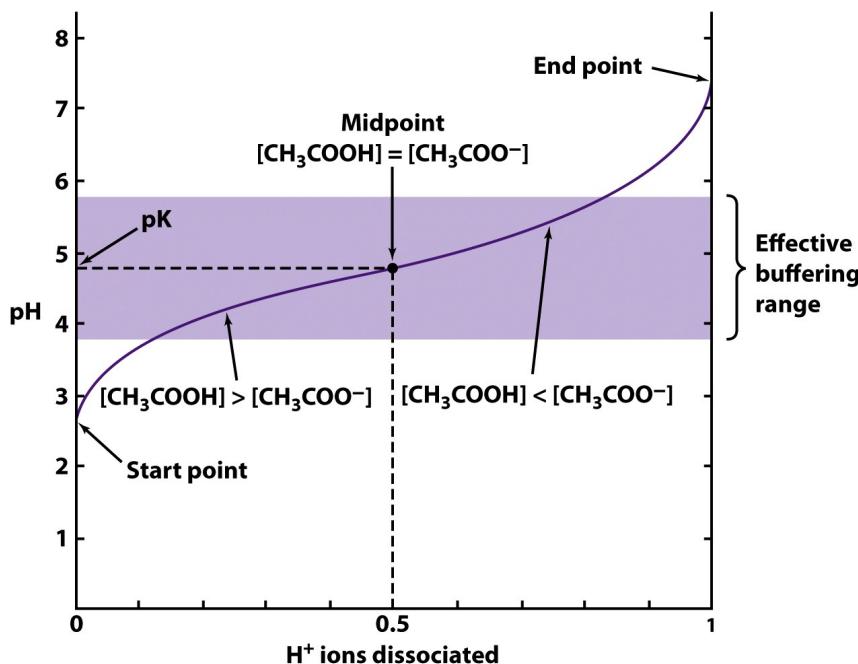
$$\text{pH} = pK_a + \log 1 \quad (\log 1 = 0)$$

$$\text{pH} = pK_a$$

## “MAXIMUM BUFFER CAPACITY”

### **\*\*A buffer is effective at pH +/- 1 of the pKa\*\***

This is mathematical proof of the behavior of buffers. At or near the point where the conjugate base and acid concentrations are equal is the best region of buffering.



© John Wiley & Sons, Inc. All rights reserved.

If we look at a titration curve for a weak acid such as acetic acid, you can see the behavior.

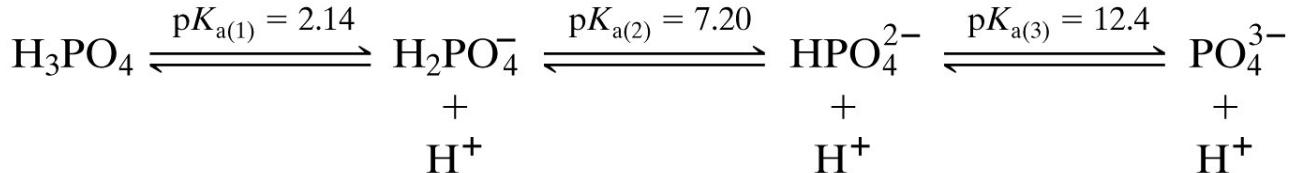
- Plot equivalents of  $\text{OH}^-$  vs. pH
- To start, acid form predominates – fully protonated
- As  $\text{NaOH}$  is added gradually,  $\text{OH}^-$  combines with free  $\text{H}^+$  in solution to form  $\text{H}_2\text{O}$ .
- As free  $\text{H}^+$  is removed,  $\text{CH}_3\text{COOH}$  dissociates further to stay in equilibrium.
- Net result  $\rightarrow$  More and more  $\text{CH}_3\text{COOH}$  ionizes, forming  $\text{CH}_3\text{COO}^-$
- **Equivalence point** is reached around pH 7 when all  $\text{CH}_3\text{COOH}$  has lost protons
- One titratable proton from  $\text{CH}_3\text{COOH}$  is titrated (removed)
- Midpoint of curve (inflection point) is where there are equal amounts of acid and conjugate base. The pH of this solution at the midpoint is the  $\text{pKa}$ !

#### REMEMBER:

- Titration curves for weak acids show that a weak acids and conjugate base can act as a buffer. Resists change in pH upon addition of acid or base.
- Buffers best around the  $\text{pKa}$  (just like equation demonstrated!)
- Established an equilibrium between buffer components (remember log of the ratio!) – large additions needed to make changes in pH
- The H-H equation represents removal of one proton from an acid – one equilibrium between acid and conjugate base.

- Some buffers are **POLYPROTIC** – that is they have more than ONE acidic proton
- All  $\text{H}^+$  do NOT dissociate at the same  $\text{pK}_a$  but are released SEQUENTIALLY at different  $\text{pK}_a$ 's – start at lowest pH and go to higher pH

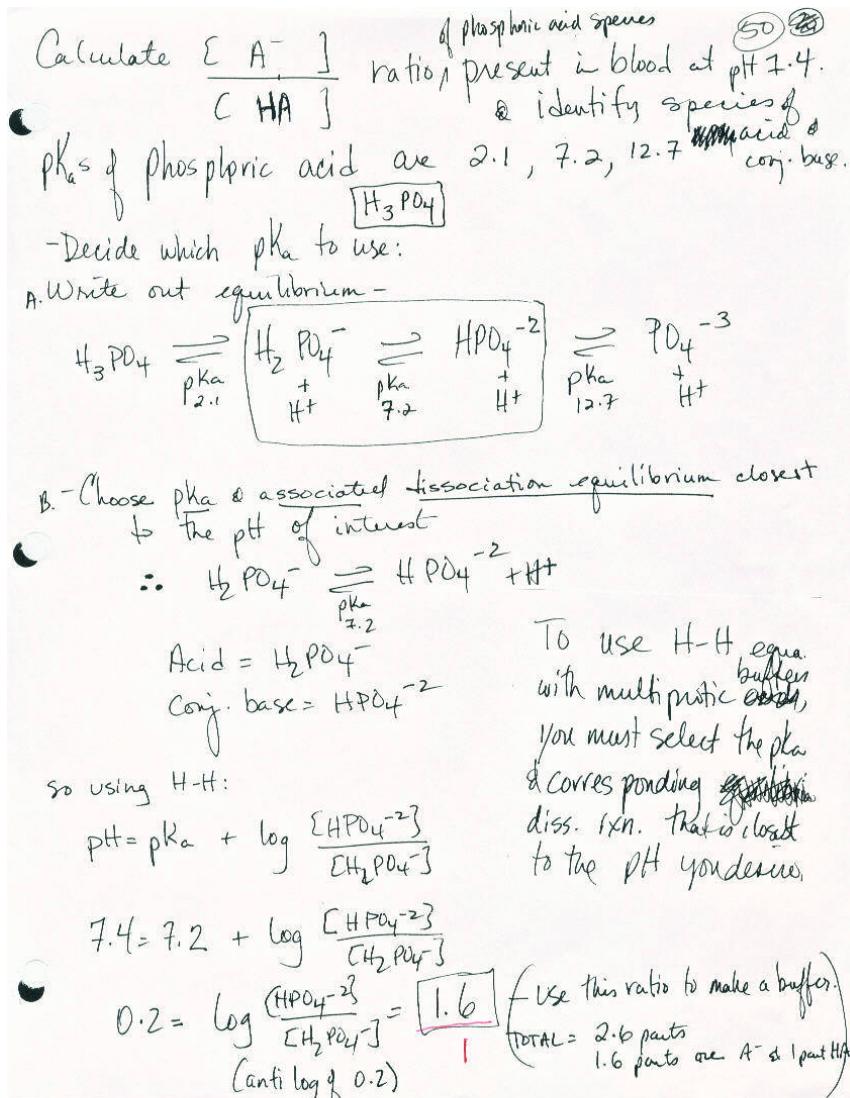
For example, phosphoric acid has 3 titratable protons:



Unnumbered figure pg 52 Concepts in Biochemistry, 3/e  
© 2006 John Wiley & Sons

- 3 Different equilibria exist
- Note the conjugate base from 1<sup>st</sup> ionization is the acid for the 2<sup>nd</sup> and so on
- Each can be represented by the H-H equation
- Write a separate equation for each step
- To use H-H: select the reaction and pK<sub>a</sub> closest to the pH of interest

### AN EXAMPLE:



Calculate the ratio inside a muscle at pH 6.6. (3)

Which pKa use? - Same - still closest to 7.2

Use  $\text{H}^+ - \text{H}^-$ :

$$6.6 = 7.2 + \log \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]}$$

$$-0.6 = \log \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]}$$

$$\frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} = \frac{0.25}{1}$$

At which pH is buffer capacity greater?

Ans: 7.4 (greatest at pKa) = pH  
7.2 is the next closest.

Good buffer within  $\pm 1$  pH unit of pKa

When  $\frac{[\text{A}^-]}{[\text{HA}]} = 1$  Greatest Buffer Capacity

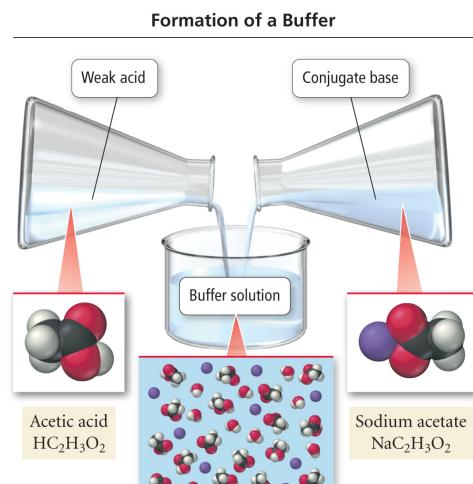
TOTAL BUFFER CONCENTRATION =

CONCENTRATION OF ACID (HA)

+

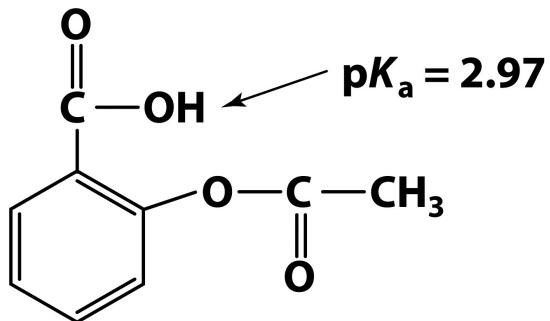
CONCENTRATION OF CONJUGATE BASE (A<sup>-</sup>)  
(salt of conjugate base)

Example: Buffer made of **0.1 M Acetic Acid** and **0.1 M Sodium Acetate** has a total buffer concentration of **0.2 M**.  
Also called a 0.2 M acetic acid buffer.



### In Class Problem #1:

Absorption of food in the stomach and intestine depends on the ability of molecules to penetrate the cell membranes and pass into the bloodstream. Because hydrophobic molecules are more likely to be absorbed than hydrophilic or charged molecules, the absorption of orally administered drugs may depend on their pK<sub>a</sub> values and the pH of the digestive organs.



**Acetylsalicylic acid (aspirin)**

© John Wiley & Sons, Inc. All rights reserved.

### Aspirin (acetylsalicylic acid) has a pK<sub>a</sub> of 2.97

- Draw the structure and give the name the conjugate base of aspirin.
- Calculate the **percentage** of aspirin (acetylsalicylic acid) available for absorption in the stomach (pH = 2.0) and in the duodenum at (pH = 4.5).

### In Class Problem #2:

For the following buffer system:

- Find the pH of a 2 L solution containing 80 g of lactic acid (MW = 90.8g/mol) and 120 g of sodium lactate (MW = 112.06g/mol) and the K<sub>a</sub> of lactic acid =  $1.38 \times 10^{-4}$
- What is the total buffer concentration of the solution in part a?
- What is the resulting pH if you add 10 mL of 3 M HCl to the buffer above? (Ignore the volume change upon addition of the 10 mL)