Physiological Buffers
- All about maintaining equilibrium

1. **Protein buffer systems:**
   - help regulate pH in ECF and ICF
   - interact extensively with other buffer systems

2. **Carbonic acid–bicarbonate buffer system:**
   - most important in ECF

3. **Phosphate buffer system:**
   - buffers pH of ICF and urine

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**Carbonic acid** ↔ **Bicarbonate**

\[
\text{H}_2\text{CO}_3 \quad \leftrightarrow \quad \text{HCO}_3^- + \text{H}^+
\]

pKa = 6.4

Why this pair?

Carbonic Anhydrase (CA) catalyzes dissociation of carbonic acid into H\(^+\) and HCO\(_3^-\)

Removing CO\(_2\) from the blood helps **increase** the pH.

Removing HCO\(_3^-\) from the blood helps **lower** the pH.

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**OVERALL REACTION**

\[
\text{CO}_2 + \text{H}_2\text{O} \quad \leftrightarrow \quad \text{H}^+ + \text{HCO}_3^-
\]
Major buffer in blood (pH 7.4) and other extracellular fluids is the carbonic acid/bicarbonate pair (See Clinical Notes, p. 43)

\[
H_2CO_3 \xrightarrow{pK_a = 6.4} HCO_3^- + H^+
\]

A base added to blood would be neutralized by the following reaction:

\[
H_2CO_3 + OH^- \rightleftharpoons HCO_3^- + H_2O
\]

The addition of an acidic substance to blood also results in neutralization:

\[
HCO_3^- + H^+ \rightleftharpoons H_2CO_3
\]

The pH of blood (7.4) is at the upper limit of the buffering capability of this system. Seems ill-suited and inefficient as many metabolic processes generate acids.

But, we can regulate both CO_2 and HCO_3^-.

Depends on three equilibria:

- Gaseous CO_2 dissolves in blood to form carbonic acid
- Carbonic acid rapidly dissociates to H^+ and bicarbonate; sped up by presence of enzyme carbonic anhydrase in blood.
  - Concentration of carbonic acid in blood is very low
- Works in reverse as well, H^+ is removed from cells by blood plasma
  - Neutralized by the reaction with HCO_3^- and leads to release of CO_2 as gas from lungs; basically eliminates H^+
  - Can also adjust breathing if you need to keep more H^+ in the blood.
- Great system because it can be readily REGULATED!
  - CO_2 regulated by breathing
  - HCO_3^- regulated by kidneys
KIDNEYS:
- Short term adjustments made by changing breathing; longer term adjustments made by the kidneys
- Remove excess acid (hydrogen ion) or bases (bicarbonate) in the urine and keep the bicarbonate concentration in the blood normal
- $\text{HCO}_3^-$ in kidney filtrate is actively reclaimed before being lost in urine.
  1: $\text{H}^+$ leaves kidney in exchange for $\text{Na}^+$ via a $\text{Na}^+/\text{H}^+$ exchanger protein
  2: Expelled $\text{H}^+$ recombines with $\text{HCO}_3^-$ in the filtrate, forming $\text{CO}_2$.
  3: $\text{CO}_2$ is non-polar and can diffuse back into kidney cells, where it is converted to $\text{H}^+$ and $\text{HCO}_3^-$
- Kidneys also generate $\text{HCO}_3^-$ to offset losses (CO$_2$ loss, buffering metabolic acids)
  Metabolic activity in kidney generates $\text{CO}_2$, which is converted and $\text{HCO}_3^-$
  1: Cells excrete the $\text{H}^+$ via a $\text{H}^+$ pump, which goes to urine (why urine is acidic)
  2: Bicarbonate left in cell returned to blood in exchange for $\text{Cl}^-$

MEDICAL CONDITIONS ASSOCIATED WITH BLOOD pH:

1. Acidosis:
   - Blood pH goes DOWN (Becomes ACIDIC)
   - $[\text{H}^+]$ goes UP

2. Alkalosis:
   - Blood pH goes UP (Becomes BASIC)
   - $[\text{H}^+]$ goes DOWN
ALKALOSIS:
- Characterized by increase in blood pH (pH 7.74)
- Becomes more basic/alkaline

- RESPIRATORY ALKALOSIS
  - Hyperventilation: Breathing rate more rapid than necessary for normal CO₂ elimination
    - Central nervous system disorders such as meningitis, encephalitis, cerebral hemorrhage
    - Drug or hormone induced physiological changes
    - Anxiety
  - Excessive intake of O₂: Abnormally low CO₂ due to excessive exhalation
  - pH goes so high that weakness and fainting result

  - Hyperventilation causes [CO₂] ↓
  - Equilibrium shifts to the LEFT
  - [H⁺] goes down and pH goes UP

  \[ \text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \]

  Treatment:
  - Correct underlying physiological problem
  - In short term, respiratory alkalosis can be helped by breathing a CO₂ rich atmosphere (breathing into a paper bag)
  - NH₄Cl infusion (for alkalosis). NH₄Cl dissociates into NH₄⁺ and Cl⁻. The NH₄⁺ (ammonium) is in equilibrium with NH₃ (ammonia) and H⁺. Because ammonia is volatile, it is respired through the lungs, leaving behind H⁺ and Cl⁻ or hydrochloric acid, which lowers the pH.

  - METABOLIC ALKALOSIS:
    - Caused by elevated HCO₃⁻ concentrations
      - Gain of HCO₃⁻ or loss of H⁺
    - Bicarbonate ions interact with H⁺ in solution
    - Reduced H⁺ causes alkalosis
    - Causes:
      - Addition of bicarbonate
      - Loss of fluids that have low conc. of bicarbonate (vomiting, diuretics)
      - Loss of hydrogen: For every hydrogen ion lost, one bicarbonate is added to the plasma. Vomiting; low potassium shifts K⁺ into plasma and H⁺ into cells
    - Compensation:
      - Respiratory: hypoventilation (more CO₂)
      - Increased HCO₃⁻ loss at kidney

  \[ \text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \]
ACIDOSIS:
- Blood pH goes **DOWN**
- \([H^+]\) goes **UP**

- **TWO TYPES:**
  - **Metabolic Acidosis**
    - **Causes:**
      - Uncontrolled diabetes (ketoacidosis)
      - Starvation diets
      - High-protein/low fat diets
      - Overproduce acidic compounds called ketone bodies that lower blood pH
      - Sudden surges in **LACTIC ACID** during exercise
  - **Respiratory Acidosis**
    - **Causes**
      - Obstructive lung disease
      - Hypoventilation (too much \(CO_2\))
      - Disease states that prevent efficient expiration of \(CO_2\)

- \([CO_2]\) ↑ (can’t be expelled)
- Equilibrium shifts to the **RIGHT**
- \([H^+]\) ↑; pH ↓

**Treatments:**
- **Treat the cause** - Stop alimentary loss of base; correct hypoxia; reduce renal acid load by diet; give insulin; treat shock with intra-venous fluids and stop hemorrhage etc
- In short term, can **ventilate**
- Commonly, **bicarbonate** is infused intravenously (\(NaHCO_3^−\))

### The Danger of Antifreeze

- Each year, thousands of pets die from consuming antifreeze
- Most brands of antifreeze contain **ethylene glycol**; taste sweet taste & give initial effect of drunkenness

- Metabolized in the liver to **glycolic acid**
  - \(HOCH_2COOH\) (weak acid) (p\(Ka\) 3.83)

- Can overwhelm the buffering ability of \(HCO_3^−\), causing the blood pH to drop: **ACIDOSIS**
- the treatment is to give the patient **ethanol**, which has a higher affinity for the enzyme that catalyzes the metabolism of ethylene glycol
Other Physiological Buffering Systems:

A. HEMOGLOBIN BUFFERING SYSTEM
   - Transports oxygen from lungs to peripheral tissues
   - Transports carbon dioxide from tissues to lungs for exhalation
   - Hemoglobin is a buffer for both CO₂ and H⁺.
   - CO₂ diffuses across RBC membrane from tissues.
   - The CO₂ can bind directly with hemoglobin and be released in the lungs. (20%)
   - The CO₂ that reacts with water forms carbonic acid that then dissociates into bicarbonate (70%) in RBC
     - Bicarbonate ions diffuse into plasma in exchange for chloride ions
     - H⁺ binds to hemoglobin and released in RBCs in lungs to combine with bicarbonate & reform CO₂ for exhalation

B. PHOSPHORIC ACID SPECIES
   - H₂PO₄⁻ and HPO₄²⁻ conjugate pair (pKa = 7.2)
     (dihydrogen phosphate/monohydrogen phosphate)
   - Used a lot in labs to mimic cellular conditions
   - Use the H-H equation to prepare these buffers

C. IONIZABLE GROUPS ON AMINO ACIDS IN PROTEINS ASSIST IN BUFFERING
CHM333 LECTURE 5: 1/21/13  SPRING 2013  Professor Christine Hrycyna

D. BUFFERS IN URINE:

- The ability to eliminate large amounts of H\(^+\) in a normal volume of urine depends on the presence of buffers
  - without buffers, would need to dilute the H\(^+\) in urine with 1000x more water!

1. **Carbonic acid–bicarbonate buffer system**
2. **Phosphate buffer system:** \(H_2PO_4^-\) is the weak acid, and \(HPO_4^{2-}\) is the conjugate base.
   (these two provided by filtration)
3. **Ammonia buffer system:**
   - Tubular deamination of glutamine generates NH\(_3\), that is transported into the tubule and buffers H\(^+\) by becoming NH\(_4^+\), that is transported into urinary space
   - Bicarbonate is reabsorbed along with Na\(^+\)

Control of bicarbonate reabsorption and H\(^+\) excretion

**pH Control in Kidneys**

- Blood
- Cells in walls of tubules
- Developing Urine

\[\text{Na}_2\text{HPO}_4 \rightarrow \text{Na}^+ \rightarrow \text{HPO}_4^{2-} \rightarrow \text{H}^+ + \text{HCO}_3^- \rightarrow \text{NaH}_2\text{PO}_4\]
AMINO ACIDS

- All 20 amino acids in pure form are white, crystalline, high-melting solids

- Amino acids act as: enzymes (catalysts), metabolic intermediates, carriers of energy and waste products and hormones.

- Amino acids are the building blocks of proteins

- Proteins are the most abundant macromolecules in living cells. May be 0.1 million different proteins in humans. Play pivotal role in almost every biological process.

- Generally, proteins composed of the 20 naturally occurring amino acids

- Only one way to link amino acids together – peptide bond

- Protein structure and function defined by sequence and type of the amino acids

- Proteins display great diversity in function and structure

- DEFINITION:
  Any organic molecule with at least one CARBOXYL group (organic acid) and at least one AMINO group (organic base)

GENERAL STRUCTURE OF THE 20 AMINO ACIDS:

At physiological pH (~7.4), amino acids exist as zwitterions – positive and negative charge on the same molecule. Dependent upon the pKa of the group. For example:

![Diagram of amino acid structure](image-url)

R = biochemical shorthand for “side chain”
Protonation of COOH and H$_2$N depends on pH of the solution
General anatomy of an amino acid. Except for proline and its derivatives, all of the amino acids commonly found in proteins possess this type of structure.

Have both **ACID** and **BASE** groups in same molecule

**STEREOCHEMISTRY OF AMINO ACIDS:**

- Stereochemistry imparts certain characteristics to a compound.
  - Every compound has a mirror image
  - Sometimes mirror images of a molecule are superimposable in space with the original object and some are not
  - **NON-superimposable mirror images = CHIRAL**
    - Have no plane of symmetry
    - **General rule:** Carbon atom with 4 DIFFERENT atoms or groups bonded to it is chiral.
    - Other Chiral examples:
      - Hand or foot
      - Right hand is a mirror image of your left hand – note how they cannot be superimposed
  - **Superimposable mirror images = ACHIRAL**
    - Achiral examples:
      - Two plain coffee mugs
      - You can turn the mirror image of the plain mug in space to make it superimposable.
Each amino acid (like your hand) also has a mirror image. These images are NOT superimposable; No matter how you turn the mirror image in space you won’t regain the original. Note no plane of symmetry.

Chirality movie:
http://cwx.prenhall.com/petrucci/medialib/media_portfolio/text_images/083_Chirality.MOV

These are called ENANTIOMERS = non-superimposable mirror images
- Most amino acids (except glycine) have four different groups attached to the a-carbon and therefore are chiral or have a chiral center.
- For glycine, R is hydrogen. Therefore, the mirror images ARE superimposable and NOT chiral. A plane of symmetry exists.
- Each amino acid except glycine has 2 ENANTIOMERS
- The enantiomers are classified based on the ability to rotate polarized light – optically active.
  o Rotate light in either (+) or (−) direction
  o Called D or L enantiomers (again non-superimposable mirror images)
  o Both D and L amino acids exist in nature but only L amino acids are used as building blocks for proteins.
  o D-amino acids are found in a few rare bacteria in the cell walls.
EXAMPLE 1:
Different flavors tasted depending on chirality of one carbon in carvone.

(-) = spearmint  
(+ ) = caraway

Taste receptors can tell the difference in 3-dimensional structure of the small molecule.

EXAMPLE 2: Thalidomide
- A sedative given to women in late 1950s and early 1960s to help morning sickness.
- Mixture of R- and S- forms.
- R- form is effective
- S- form caused severe birth defects, including appendage.
- Can racemize in vivo - Both forms can interconvert.

EXAMPLE 3: Conversion of L $\rightarrow$ D amino acids in proteins with age
- Part of aging process may include the isomerization of the amino acid aspartic acid (aspartate) from L $\rightarrow$ D in proteins in teeth and eyes.
- Isomerized proteins are less biologically active
- Scientists are identifying the enzymes that change D back to L to try and slow the aging process.

EXAMPLE 4: Isomer Isolation Leads Researchers To More Effective Psychiatric Drugs
In an effort to increase effectiveness and reduce dosing, side effects, and potential drug interactions, pharmacologists are isolating and purifying specific forms of psychiatric drugs.

Mirror image (or racemic) isomers are labeled as "right handed" (termed "R-" or dex- ) or "left handed" (termed "S-" or "levo-"). Because the different isomers are difficult to separate, the FDA allows drug companies to include both in medicines, as long as the less-functional isomer is not harmful.
Successes:

- **Dexamethylphenidate** (Focalin) (Treatment of ADHD)
- **Citalopram** (Celexa) (SSRI; Treatment of depression)
  - S-citalopram under the generic name of escitalopram
  - Escitalopram (Lexapro) is nearly four times more potent as an SSRI than the mixture present in the parent compound citalopram
  - Escitalopram (Lexapro) has better side-effect and drug-interaction profiles.

Failure:

- **Prozac** = R is the more effective enantiomer. S is thought to be potentially dangerous. Isolating R-fluoxetine could yield a more effective drug with fewer side effects. However, late in 2000, Eli Lilly and Company halted development of the R-isomer of fluoxetine (Prozac) after Stage III clinical trials revealed unexpected cardiac side effects (*Psychiatric News*, December 1, 2000). The company had hoped that R-fluoxetine would replace fluoxetine, which was facing imminent expiration of its patent protection.