Properties of Buffers

Chapter Overview

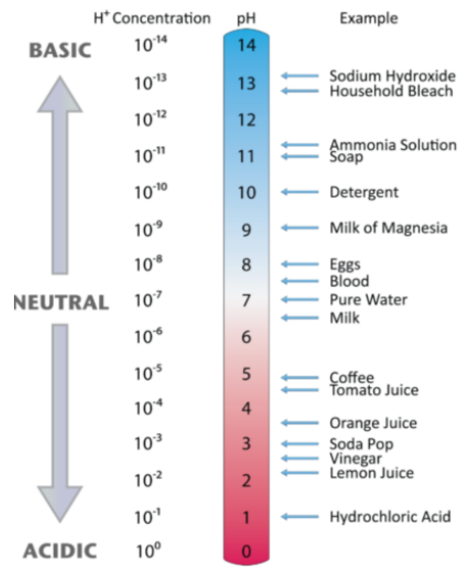
This chapter focuses on buffers—solutions that are able to maintain a stable pH even after the addition of acids or bases. A stable pH is essential both for the proper function of enzymes and cells in the human body, and for the reliability of laboratory methods.

In this chapter, we will look at the principle of how buffers work, buffering capacity and the Henderson–Hasselbalch equation, natural buffering systems in the human body, laboratory buffer solutions, and factors that affect their stability.

Table of Contents

1 Theoretical Introduction – pH

pH is a number that expresses the acidity or alkalinity of a solution. It is defined as the negative logarithm of the concentration of hydronium ions [H_3O^+]. The higher the proton concentration, the lower the pH (the solution is acidic); conversely, with fewer protons, the pH is higher (the solution is alkaline).

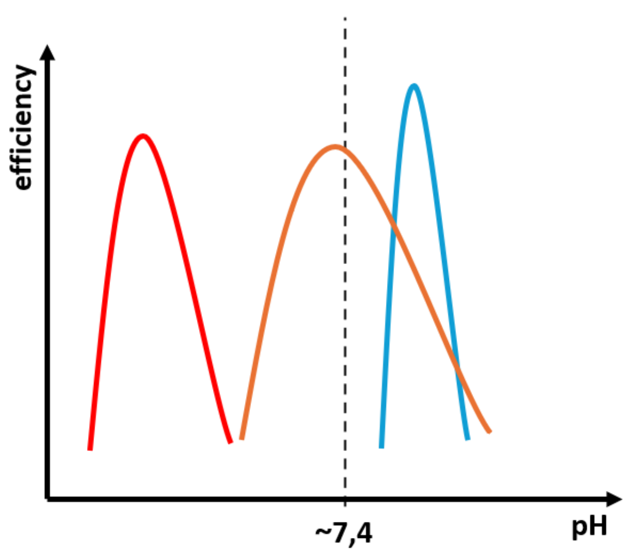


(Source: https://courses.lumenlearning.com/umes-cheminter/chapter/the-ph-scale/)

In water, autoprotolysis occurs—water molecules partially dissociate into [H_3O^+] and [OH^-]. The product of their concentrations is constant ($K_w = 10^{-14}$ at 25 °C), and therefore the relationship pH + pOH = 14 holds true.

The value of pH strongly influences the course of chemical reactions, particularly in aqueous solutions. **Enzymatic reactions** are especially sensitive to pH—a shift outside their optimal range can reduce catalytic activity or even lead to complete inactivation. Each enzyme has its own characteristic optimum (e.g., pepsin in the stomach at pH 1–2, trypsin in the intestine at pH 8–9).

pH also has a fundamental impact on **protein conformation**, membrane stability, ion distribution, and non-covalent interactions in the body. For this reason, maintaining pH within a narrow physiological range is essential—for instance, in blood, around 7.35–7.45. This balance is achieved by **buffer systems**, which dampen pH changes after the addition of acids or bases.



Pepsin – most active in an acidic environment

Trypsin – most active in an alkaline environment
(at physiological pH both are inactive)

Amylase – active at physiological pH

2 What is a buffer?

Buffers are solutions that can maintain a relatively stable pH even after the addition of small amounts of acid or base. This property is crucial both in biological systems and in laboratory practice. Chemically, buffers are solutions containing a weak acid and its conjugate base (or a weak base and its conjugate acid). Their effectiveness relies on the equilibrium of weak acid dissociation: added protons or hydroxide ions are captured by the buffer components, preventing major changes in pH.

2.1 Examples of Buffer Composition

As mentioned above, a buffer is an aqueous solution composed of a conjugate pair—a weak acid and its salt with a strong base, or a weak base and its salt with a strong acid.

- Acidic buffers: acetate buffer (CH_3COOH + CH_3COONa), bicarbonate buffer (H_2CO_3 + $NaHCO_3$).
- Basic buffers: ammonia buffer (NH_3 + NH_4Cl).

2.2 Mechanism of effect

Their effect relies on the presence of a weak acid and its conjugate base.

- When **acid (H^+)** is added, the conjugate base (e.g., acetate ion, CH_3COO^-) reacts with the proton to form the weak acid (CH_3COOH).
- When **base (OH^-)** is added, the weak acid neutralizes the hydroxide ions, producing water and its conjugate base.

As a result, the increase in free H^+ or OH^- concentration is limited, and the pH remains relatively stable. Buffers work most effectively around their **pKa value**, when the weak acid and conjugate base are present in comparable amounts.

- The **weak acid** captures added OH^- ions, preventing a rise in pH.
- The **conjugate base** captures added H^+ ions, preventing a drop in pH.

Let's break this down with a model example: the acetate buffer, composed of acetic acid (weak acid) and sodium acetate (salt of the weak acid with a strong base).

- When a strong base (e.g., NaOH) is added: Hydroxide ions react with acetic acid to form acetate. Some free acetic acid is consumed, but since acetic acid is only weakly dissociated (≈1%), the concentration of H^+ ions decreases only slightly. The pH change is therefore much smaller than it would be in pure water.

- When a strong acid (e.g., HCl) is added: Protons from the acid react with acetate ions to form acetic acid. Since acetic acid is again only weakly dissociated, it does not release large amounts of H^+ into the solution. The H^+ concentration therefore increases only partially compared to what would happen without sodium acetate present.

? ***Question**: What happens if you add a strong acid to pure water compared with adding it to a solution containing an acetate buffer?*

3 Henderson–Hasselbalch Equation and Buffer Capacity

3.1 Henderson–Hasselbalch Equation

The Henderson–Hasselbalch equation helps us understand how stable pH is maintained. It is based on the fact that in any solution containing a weak acid and its "basic" form (the conjugate base), the pH depends on their ratio. When the acidic and basic components are present in equal amounts, the pH equals the **pKa**, which is characteristic for each acid.

The Henderson–Hasselbalch equation allows calculation of the buffer pH based on the concentrations of its components:

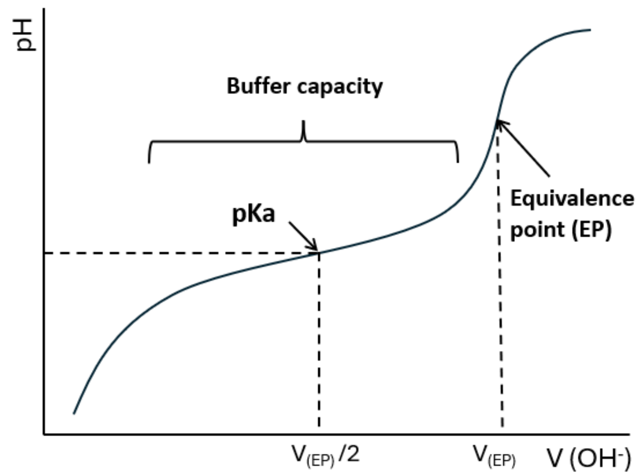
$$\mathrm{pH} = \mathrm{p}K_\mathrm{a} + \log \frac{[\mathrm{A}^-]}{[\mathrm{HA}]}$$

? ***Question**: What is the ratio of base to acid if pH = pKa + 1? What about if pH = pKa – 1?*

3.2 Buffer capacity

Buffer capacity represents the ability of a buffer to resist changes in pH when acid or base is added. It expresses the amount of strong acid or base that can be added without changing the pH by more than one unit. The higher the buffer capacity, the “stronger” the buffer in stabilizing its environment.

The figure below shows the pH change during titration of acetic acid with NaOH. The segment highlighted approximately marks the range of the acetate buffer (buffer capacity).



- The highest buffer efficiency occurs when the ratio of acid to conjugate base is **1:1**. At this point, the solution’s pH equals the pKa of the acid.
- For acetic acid (pKa ≈ 4.76), maximum buffer efficiency is achieved at a 1:1 ratio of acetic acid to sodium acetate, corresponding to half of the equivalence point of the titration.

Buffer capacity defines how much strong acid or base must be added to shift the pH by one unit. The higher this value, the more stable the solution is against pH fluctuations.

3.3 Factors Affecting Buffer Capacity

- **Concentration of buffer components**: higher concentrations of acid and base give higher buffer capacity.
- **Ratio between acid and base**: maximum effect at 1:1 (see above). Shifts in this ratio move the pH toward acidic or basic values.
- **Type of acid and base**: buffers are most effective when the acid’s pKa is close to the desired pH of the system.

Example: The acetate buffer (CH_3COOH/CH_3COONa) is most effective in the pH range 3.7–5.7. By adjusting the ratio of components, the pH can be fine-tuned for the needs of a given reaction or experiment.

4 Laboratory Buffers and Their Use

In laboratory biochemistry, buffers are essential for stabilizing pH during chemical and biological reactions. They create a defined environment that ensures reproducibility, reliability, and accuracy of results. A well-chosen buffer also minimizes the risk of biomolecule denaturation and preserves enzyme activity.

4.1 Properties of Laboratory Buffers

- **Stable pH** within the required range
- **Compatibility with biological systems** (non-toxic, no unwanted interactions)
- **Resistance to sterilization** (autoclaving, filtration) without losing effectiveness

? ***Question:*** *Why is it important to use a buffer when measuring enzyme activity under in vitro conditions?*

4.2 Commonly Used Laboratory Buffers

- **PBS (phosphate-buffered saline):** a physiological solution combining a phosphate buffer ($H_2PO_4^-$/HPO_4^{2-}) with Na^+ and Cl^- ions. The phosphate system stabilizes pH around 7.4, while salts ensure isotonicity with body fluids. PBS is widely used in cell and tissue work, immunology, and immunoassays such as ELISA.

- **Tris-HCl (tris(hydroxymethyl)aminomethane)**: an organic base combined with HCl, effective in the pH range 7.0–9.0. Protonation/deprotonation of the amine group maintains pH. Because of this range, Tris-HCl is highly versatile and used in molecular biology—from DNA electrophoresis to protein extraction and Western blotting.

- **HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid):** one of the so-called Good's buffers, characterized by minimal temperature dependence. Its sulfonated organic base maintains pH between 6.8–8.2. This is particularly important for sensitive enzymatic reactions and cell culture, where even small pH shifts can affect viability.

- **MOPS (3-(N-morpholino)propanesulfonic acid) and MES (2-(N-morpholino) ethanesulfonic acid):** also part of Good's buffers, acting via protonation of sulfonic groups. MOPS (pH ~7.2) and MES (pH ~6.1) are especially used in RNA work and biochemical experiments requiring stable mildly acidic to neutral conditions.

- **TAE and TBE:** specialized buffers for nucleic acid electrophoresis. Both are based on Tris to stabilize pH, with acetate (TAE) or borate (TBE) as conjugate bases. EDTA is added to chelate divalent cations (e.g., Mg^{2+}), protecting DNA from enzymatic degradation. These buffers thus not only stabilize pH but also safeguard nucleic acids during analysis.

Note: *Good's buffers were developed in the 1960s by biologist Norman Good to provide ideal environments for biological and biochemical experiments. They are effective near physiological pH (6–8), have low toxicity, are stable, show minimal temperature dependence, are not broken down by enzymes, and do not interfere with ions or biological processes—making them highly versatile tools for working with cells and biomolecules.*

5 Natural Buffer Systems in the Human Body

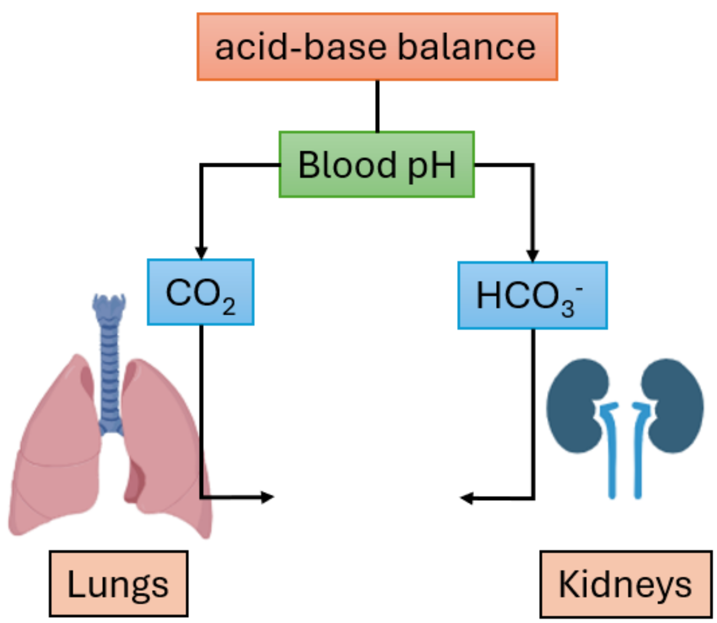
Maintaining a stable pH is essential for the proper function of cells, enzymes, and overall metabolism. In the human body, several natural buffer systems prevent drastic changes in pH during metabolic and respiratory processes.

Bicarbonate Buffer (H_2CO_3 / HCO_3^-)

The most important **extracellular buffer system**, regulating blood pH. It is based on the equilibrium between carbon dioxide, carbonic acid, and bicarbonate:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

This equilibrium is influenced by the **respiratory system** (removal of CO_2) and the **kidneys** (reabsorption and secretion of HCO_3^-).



Interesting fact*: Human saliva has a pH of about 6.5–7.4 and contains a bicarbonate buffer, which protects teeth from acids in food and drinks.*

Hemoglobin Buffer

In red blood cells, **hemoglobin** contributes to acid–base balance by binding protons formed during the conversion of CO_2 to bicarbonate. Importantly, **deoxygenated hemoglobin** binds protons better than oxygenated hemoglobin. This phenomenon, known as the **Bohr effect**, explains why oxygen is released more easily in tissues where CO_2 production (and thus proton concentration) is higher.

Phosphate Buffer ($H_2PO_4^-$ / HPO_4^{2-})

Significant mainly in the **intracellular space** and in **urine**. It is most effective around pH 6.8, playing a key role in pH regulation in the kidneys.

***Interesting fact**: The phosphate buffer in malt is crucial for proper enzyme activity during beer brewing—showing that "buffer chemistry" matters even in brewing.*

Protein Buffer

Plasma proteins, especially **albumin**, contain ionizable groups that can accept or release protons. This system significantly contributes to buffering in blood plasma.

6 Role of Buffer Systems in Diagnostics

Physiological buffers (bicarbonate, phosphate, hemoglobin, and protein) together maintain stable pH in blood and tissues, which is essential for cellular function. Their imbalance leads to **acid–base disorders**, assessed using blood gas analyzers measuring pH, pCO_2, and HCO_3^-. These parameters reveal whether the disorder is **metabolic** or **respiratory**, and whether compensation is occurring.

Clinically, four major types of acid–base disturbances are recognized:

- **Respiratory acidosis** (hypoventilation, e.g., COPD)
- **Respiratory alkalosis** (hyperventilation)
- **Metabolic acidosis** (e.g., ketoacidosis, renal failure, intoxications)
- **Metabolic alkalosis** (e.g., recurrent vomiting, excessive diuretic use, bicarbonate administration)

Therapy depends on diagnosis—common approaches include administration of **$NaHCO_3$** in severe metabolic acidosis or targeted **urine alkalinization** in intoxications and in the treatment of urolithiasis and infections. Monitoring pH, pCO_2, and HCO_3^- is especially critical in **critically ill patients**, where it enables early detection of life-threatening imbalances.

(*Further details on acid–base balance will be covered in the summer semester in the topic "Homeostasis: Acid base balance, ions, water; Metabolism in kidneys, urine."*)

Summary

Buffers are solutions that maintain stable pH through the equilibrium between a weak acid and its conjugate base (or vice versa). Their effectiveness is described by the **Henderson–Hasselbalch equation** and depends on **buffer capacity**. In the human body, the most important buffer systems are the **bicarbonate, hemoglobin, phosphate, and protein buffers**. In the laboratory, buffers are essential for stabilizing conditions during enzymatic reactions, immunological assays, or cell cultures. Incorrect preparation, storage, or CO_2 influence can lead to measurement errors. Buffers are therefore indispensable tools in **biology, medicine, and everyday laboratory practice**.

Control Questions

1. What is the principle of buffer action when an acid or a base is added?
2. What is the Henderson–Hasselbalch equation and when is it used?
3. At what acid-to-base ratio does a buffer have the highest efficiency?
4. Which buffering system is the most important for maintaining blood pH?
5. What is the difference between the bicarbonate buffer and the phosphate buffer?