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Buffers

A guide for the preparation and use of buffers in biological systems

$$pH = pK_a + \log \frac{[CH_3COO^-]}{[CH_3COOH]}$$

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A guide for the preparation and use of buffers in biological systems

By
Chandra Mohan, Ph.D.

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A Word to Our Customers

We are pleased to present to you the newest edition of Buffers: A Guide for the Preparation and Use of Buffers in Biological Systems. This practical resource has been especially revamped for use by researchers in the biological sciences. This publication is a part of our continuing commitment to provide useful product information and exceptional service to you, our customers. You will find this booklet a highly useful resource, whether you are just beginning your research work or training the newest researchers in your laboratory.

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Marie Bergstrom
Marketing Manager
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Table of Contents:

Why Does Calbiochem® Biochemicals Publish a Booklet on Buffers?

We are frequently asked questions on the use of buffers that we offer to research laboratories. This booklet is designed to help answer several basic questions about the use of buffers in biological systems. The discussion presented here is by no means complete, but we hope it will help in the understanding of general principles involved in the use of buffers.

Almost all biological processes are pH dependent. Even a slight change in pH can result in metabolic acidosis or alkalosis, resulting in severe metabolic complications. The purpose of a buffer in biological system is to maintain intracellular and extracellular pH within a very narrow range and resist changes in pH in the presence of internal and external influences. Before we begin a discussion of buffers and how they control hydrogen ion concentrations, a brief explanation of the role of water and equilibrium constants of weak acids and bases is necessary.

Water: The Fluid of Life

Water constitutes about 70% of the mass of most living creatures. All biologic reactions occur in an aqueous medium. All aspects of cell structure and functi are adapted to the physical and chemical properties of water. Hence, it is essential to understand some basic properties of water and its ionization products, i.e., H^+ and OH^-. Both H^+ and OH^- influence the structure, assembly, and properties of all macromolecules in the cell.

Water is a polar solvent that dissolves most charged molecules. Water dissolve most salts by hydrating and stabilizing the cations and anions by weakening their electrostatic interactions (Figure 1). Compounds that readily dissolve in water are known as **HYDROPHILIC** compounds. Nonpolar compounds such as chloroform and ether do not interact with water in any favorable manner and known as **HYDROPHOBIC** compounds. These compounds interfere with hydrogen bonding among water molecules.

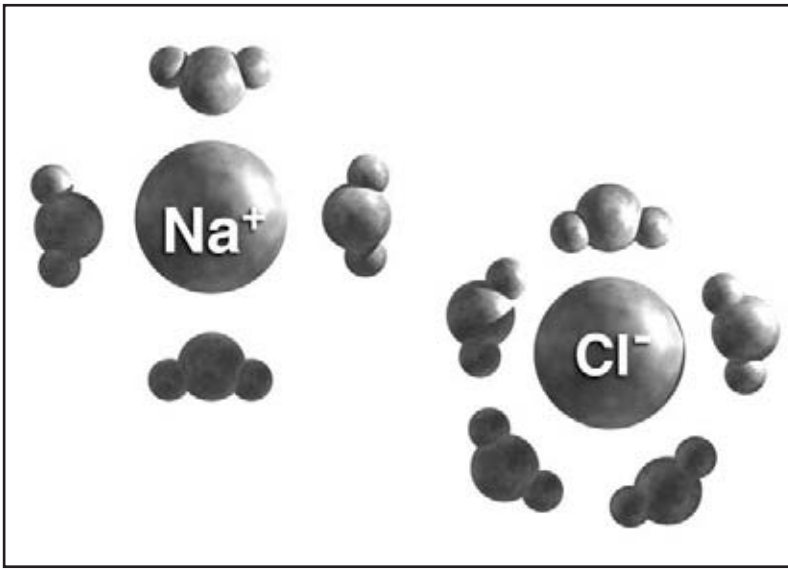

Figure 1: *Electrostatic interaction of Na^+ and Cl^- ions and water molecules.*

Several biological molecules, such as protein, certain vitamins, steroids, and phospholipids contain both polar and nonpolar regions. They are known as **AMPHIPATHIC** molecules. The hydrophilic region of these molecules are arranged in a manner that permits maximum interaction with water molecule However, the hydrophobic regions assemble together exposing only the small area to water.

Ionization of Water

Water molecules undergo reversible ionization to yield H^+ and OH^- as per the following equation.

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

The degree of ionization of water at equilibrium is fairly small and is given by the following equation where K_{eq} is the equilibrium constant.

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

At 25°C, the concentration of pure water is 55.5 M (1000 ÷ 18; M.W. 18.0).

Hence, we can rewrite the above equation as follows:

$$K_{eq} = \frac{[H^+][OH^-]}{55.5\ M}$$

or

$$(55.5)(K_{eq}) = [H+][OH^-]$$

For pure water electrical conductivity experiments give a K_{eq} value of 1.8×10^{-16} M at 25°C.

Hence,
$$(55.5\ M)(1.8 \times 10^{-16}\ M) = [H^+][OH^-]$$
or
$$99.9 \times 10^{-16}\ M^2 = [H^+][OH^-]$$
or
$$1.0 \times 10^{-14}\ M^2 = [H^+][OH^-]$$

$[H^+][OH^-]$, ion product of water, is always equal to $1.0 \times 10^{-14}\ M^2$ at 25°C. Whe $[H^+]$ and $[OH^-]$ are present in equal amounts then the solution gives a neutral

Here
$$[H^+][OH^-] = [H^+]^2$$
or
$$[H^+] = 1 \times 10^{-14}\ M^2$$
and
$$[H^+] = [OH^-] = 10^{-7}\ M$$

As the total concentration of H^+ and OH^- is constant, an increase in one ion is compensated by a decrease in the concentration of other ion. This forms the basis for the pH scale.

Dissociation Constants of Weak Acids and Bases

Strong acids (hydrochloric acid, sulfuric acid, etc.) and bases (sodium hydroxide, potassium hydroxide, etc.) are those that are completely ionized in dilute aqueous solutions.

In biological systems one generally encounters only weak acids and bases. Weak acids and bases do not completely dissociate in solution. They exist instead as an equilibrium mixture of undissociated and dissociated species. For example, in aqueous solution, acetic acid is an equilibrium mixture of acetate ion, hydrogen ion, and undissociated acetic acid. The equilibrium between these species can be expressed as:

$$CH_3COOH \underset{k_2}{\overset{k_1}{\rightleftarrows}} H^+ + CH_3COO^-$$

where k_1 represents the rate constant of dissociation of acetic acid to acetate and hydrogen ions, and k_2 represents the rate constant for the association of acetate and hydrogen ions to form acetic acid. The rate of dissociation of acetic acid, $-d[CH_3COOH]/dt$, is dependent on the rate constant of dissociation (k_1) and the concentration of acetic acid $[CH_3COOH]$ and can be expressed as:

$$\frac{d[CH_3COOH]}{dt} = k_1 [CH_3COOH]$$

Similarly, the rate of association to form acetic acid, $d[HAc]/dt$, is dependent on the rate constant of association (k_2) and the concentration of acetate and hydrogen ions and can be expressed as:

$$\frac{d[CH_3COOH]}{dt} = k_2 [H^+] [CH_3COO^-]$$

Since the rates of dissociation and reassociation are equal under equilibrium conditions:

$$k_1 [CH_3COOH] = k_2 [H^+] [CH_3COO^-]$$

or

$$\frac{k_1}{k_2} = \frac{[H^+] [CH_3COO^-]}{[CH_3COOH]}$$

and

$$K_a = \frac{[H^+] [CH_3COO^-]}{[CH_3COOH]}$$

where

$$\frac{k_1}{k_2} = K_a \textbf{ (Equilibrium constant)}$$

This equilibrium expression can now be rearranged to

$$[H^+] = K_a \frac{[CH_3COOH]}{[CH_3COO^-]}$$

where the hydrogen ion concentration is expressed in terms of the equilibrium constant and the concentrations of undissociated acetic acid and acetate ion. The equilibrium constant for ionization reactions is called the ionization constant or dissociation constant.

Henderson-Hasselbach Equation: pH and pK_a

The relationship between pH, pK_a, and the buffering action of any weak acid and its conjugate base is best explained by the Henderson-Hasselbach equation. In biological experiments, $[H^+]$ varies from 10^{-1} M to about 10^{-10} M. S.P.L. Sorenson, a Danish chemist, coined the "p" value of any quantity as the negative logarithm of the hydrogen ion concentration. Hence, for $[H^+]$ one can write the following equation:

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

Similarly pK_a can be defined as $-\log K_a$. If the equilibrium expression is converted to – log then

$$-\log [H^+] = -\log K_a - \log \frac{[CH_3COOH]}{[CH_3COO^-]}$$

and pH and pK_a substituted:

$$pH = pK_a - \log \frac{[CH_3COOH]}{[CH_3COO^-]}$$

or

$$pH = pK_a + \log \frac{[CH_3COO^-]}{[CH_3COOH]}$$

When the concentration of acetate ions equals the concentration of acetic acid, $\log [CH_3COO^-]/[CH_3COOH]$ approaches zero (the log of 1) and pH equals pK_a (the pK_a of acetic acid is 4.745). Acetic acid and acetate ion form an effective buffering system centered around pH 4.75. Generally, the pK_a of a weak acid or base indicates the pH of the center of the buffering region.

The terms pK and pK_a are frequently used interchangeably in the literature. The term pK_a ("a" refers to acid) is used in circumstances where the system is being considered as an acid and in which hydrogen ion concentration or pH is of

interest. Sometimes the term pK_b is used. pK_b ("b" refers to base) is used when the system is being considered as a base and the hydroxide ion concentration or pOH is of greater interest.

Determination of pK_a

pKa values are generally determined by titration. A carefully calibrated, automated, recording titrator is used, the free acid of the material to be measured is titrated with a suitable base, and the titration curve is recorded. The pH of the solution is monitored as increasing quantities of base are added to the solution. Figure 2 shows the titration curve for acetic acid. The point of inflection indicates the pK_a value. Frequently, automatic titrators record the first derivative of the titration curve, giving more accurate pK_a values.

Polybasic buffer systems can have more than one useful pK_a value. Figure 3 shows the titration curve for phosphoric acid, a tribasic acid. Note that the curve has five points of inflection. Three indicate pK_{a1}, pK_{a2} and pK_{a3}, and two additional points indicate where $H_2PO_4^-$ and HPO_4^- exist as the sole species.

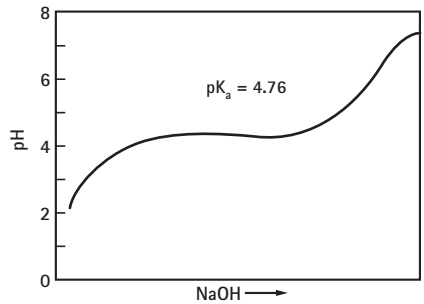

Figure 2: *Titration Curve for Acetic Acid*

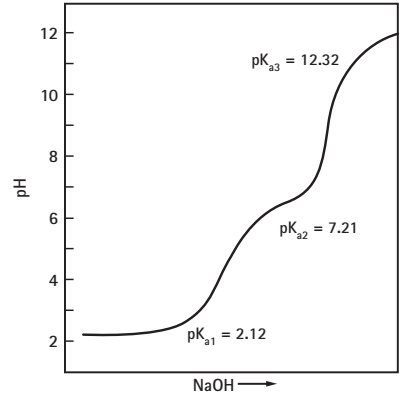

Figure 3: *Titration Curve for Phosphoric Acid*

Table 1: pK_a Values for Commonly Used Biological Buffers and Buffer Constituents

Product	Cat. No.	M.W.	pK_a at 20°C
ADA, Sodium Salt	114801	212.2	6.60
2-Amino-2-methyl-1,3-propanediol	164548	105.1	8.83
BES, ULTROL® Grade	391334	213.2	7.15
Bicine, ULTROL® Grade	391336	163.2	8.35
BIS-Tris, ULTROL® Grade	391335	209.2	6.50
BIS-Tris Propane, ULTROL® Grade	394111	282.4	6.80
Boric Acid, Molecular Biology Grade	203667	61.8	9.24
Cacodylic Acid	205541	214.0	6.27
CAPS, ULTROL® Grade	239782	221.3	10.40
CHES, ULTROL® Grade	239779	207.3	9.50
Citric Acid, Monohydrate, Molecular Biology Grade	231211	210.1	4.76
Glycine	3570	75.1	2.34[1]
Glycine, Molecular Biology Grade	357002	75.1	2.34[1]
Glycylglycine, Free Base	3630	132.1	8.40
HEPES, Free Acid, Molecular Biology Grade	391340	238.3	7.55
HEPES, Free Acid, ULTROL® Grade	391338	238.3	7.55
HEPES, Free Acid Solution	375368	238.3	7.55
HEPES, Sodium Salt, ULTROL® Grade	391333	260.3	7.55
HEPPS, ULTROL® Grade	391339	252.3	8.00
Imidazole, ULTROL® Grade	4015	68.1	7.00
MES, Free Acid, ULTROL® Grade	475893	195.2	6.15
MES, Sodium Salt, ULTROL® Grade	475894	217.2	6.15
MOPS, Free Acid, ULTROL® Grade	475898	209.3	7.20
MOPS, Sodium Salt, ULTROL® Grade	475899	231.2	7.20
PIPES, Free Acid, Molecular Biology Grade	528133	302.4	6.80
PIPES, Free Acid, ULTROL® Grade	528131	302.4	6.80
PIPES, Sodium Salt, ULTROL® Grade	528132	325.3	6.80
PIPPS	528315	330.4	3.73[2]
Potassium Phosphate, Dibasic, Trihydrate, Molecular Biology Grade	529567	228.2	7.21[3]
Potassium Phosphate, Monobasic	529565	136.1	7.21[3]
Potassium Phosphate, Monobasic, Molecular Biology Grade	529568	136.1	7.21[3]
Sodium Phosphate, Dibasic	567550	142.0	7.21[3]
Sodium Phosphate, Dibasic, Molecular Biology Grade	567547	142.0	7.21[3]
Sodium Phosphate, Monobasic	567545	120.0	7.21[3]
Sodium Phosphate, Monobasic, Monohydrate, Molecular Biology Grade	567549	138.0	7.21[3]
TAPS, ULTROL® Grade	394675	243.2	8.40
TES, Free Acid, ULTROL® Grade	39465	229.3	7.50
TES, Sodium Salt, ULTROL® Grade	394651	251.2	7.50
Tricine, ULTROL® Grade	39468	179.2	8.15
Triethanolamine, HCl	641752	185.7	7.66
Tris Base, Molecular Biology Grade	648310	121.1	8.30
Tris Base, ULTROL® Grade	648311	121.1	8.30
Tris, HCl, Molecular Biology Grade	648317	157.6	8.30
Tris, HCl, ULTROL® Grade	648313	157.6	8.30
Trisodium Citrate, Dihydrate	567444	294.1	—
Trisodium Citrate, Dihydrate, Molecular Biology Grade	567446	294.1	—

1. pK_{a1} = 2.34; pK_{a2} = 9.60
2. pK_{a1} = 3.73; pK_{a2} = 7.96 (100 mM aqueous solution, 25°C).
3. Phosphate buffers are normally prepared from a combination of the monobasic and dibasic salts, titrated against each other to the correct pH. Phosphoric acid has three pK_a values: pK_{a1} = 2.12; pK_{a2} = 7.21; pK_{a3} = 12.32

Buffers, Buffer Capacity and Range

Buffers are aqueous systems that resist changes in pH when small amounts of acid or base are added. Buffer solutions are composed of a weak acid (the proton donor) and its conjugate base (the proton acceptor). Buffering results from two reversible reaction equilibria in a solution wherein the concentration of proton donor and its conjugate proton acceptor are equal. For example, in a buffer system when the concentration of acetic acid and acetate ions are equal, addition of small amounts of acid or base do not have any detectable influence on the pH. This point is commonly known as the **isoelectric point**. At this point there is no net charge and pH at this point is equal to pK_a.

$$pH = pK_a + \log \frac{[CH3COO^-]}{[CH3COOH]}$$

At isoelectric point $[CH_3COO^-] = [CH_3COOH]$ hence, $pH = pK_a$

Buffer capacity is a term used to describe the ability of a given buffer to resist changes in pH on addition of acid or base. A buffer capacity of 1 is when 1 mol of acid or alkali is added to 1 liter of buffer and pH changes by 1 unit. The buffer capacity of a mixed weak acid-base buffer is much greater when the individual pK_a values are in close proximity with each other. It is important to note that the buffer capacity of a mixture of buffers is additive.

Buffers have both intensive and extensive properties. The intensive property is a function of the pK_a value of the buffer acid or base. Most simple buffers work effectively in the pH scale of $pK_a \pm 1.0$. The extensive property of the buffers is also known as the buffer capacity. It is a measure of the protection a buffer offers against changes in pH. Buffer capacity generally depends on the concentration of buffer solution. Buffers with higher concentrations offer higher buffering capacity. On the other hand, pH is dependent not on the absolute concentrations of buffer components but on their ratio.

Using the above equation we know that when $pH = pK_a$ the concentrations of acetic acid and acetate ion are equal. Using a hypothetical buffer system of HA ($pK_a = 7.0$) and $[A^-]$, we can demonstrate how the hydrogen ion concentration, $[H^+]$, is relatively insensitive to external influence because of the buffering action.

For example:
If 100 ml of 10 mM ($1x\ 10^{-2}$ M) HCl are added to 1.0 liter of 1.0 M NaCl at pH 7.0, the hydrogen ion concentration, $[H^+]$, of the resulting 1.1 liter of solution can be calculated by using the following equation:

$$[H^+] \times Vol = [H^+]_o \times Vol_o$$

where

Vol_o = initial volume of HCl solution (in liters)

$[H^+]_o$ = initial hydrogen ion concentration (M)

Vol = final volume of HCl + NaCl solutions (in liters)

$[H^+]$ = final hydrogen ion concentration of HCl + NaCl solution (M)

Solving for $[H^+]$:

$$[H^+] \times 1.1 \text{ liter} = 1.0 \times 10^{-2} \times 0.1 = 1 \times 10^{-3}$$
$$[H^+] = 9.09 \times 10^{-4}$$
$$\text{or } pH = 3.04$$

Thus, the addition of 1.0×10^{-3} mol of hydrogen ion resulted in a pH change of approximately 4 pH units (from 7.0 to 3.04).

If a buffer is used instead of sodium chloride, a 1.0 M solution of HA at pH 7.0 will initially have:

$$[HA] = [A] = 0.5 \text{ M}$$

$$pH = pK + \log \frac{[A]}{[HA]}$$

$$pH = 7.0 + \log \frac{0.5}{0.5} \quad \text{or} \quad pH = 7.0$$

When 100 ml of 1.0×10^{-2} M (10 mM) HCl is added to this system, 1.0×10^{-3} mol of A^- is converted to 1.0×10^{-3} mol of HA, with the following result:

$$pH = 7.0 + \log \frac{0.499/1.1}{0.501/1.1}$$

$$pH = 7.0 - 0.002 \text{ or } pH = 6.998$$

Hence, it is clear that in the absence of a suitable buffer system there was a pH change of 4 pH units, whereas in a buffer system only a trivial change in pH was observed indicating that the buffer system had successfully resisted a change in pH. Generally, in the range from [A]/[HA] = 0.1 to [A]/[HA] = 10.0, effective buffering exists. However, beyond this range, the buffering capacity may be significantly reduced.

Biological Buffers

Biological buffers should meet the following general criteria:

- Their pK_a should reside between 6.0 to 8.0.
- They should exhibit high water solubility and minimal solubility in organic solvents.
- They should not permeate cell membranes.
- They should not exhibit any toxicity towards cells.
- The salt effect should be minimum, however, salts can be added as required.
- Ionic composition of the medium and temperature should have minimal effect of buffering capacity.
- Buffers should be stable and resistant to enzymatic degradation.
- Buffer should not absorb either in the visible or in the UV region.

Most of the buffers used in cell cultures, isolation of cells, enzyme assays, and other biological applications must possess these distinctive characteristics. Good's zwitterionic buffers meet these criteria. They exhibit pK_a values at or near physiological pH. They exhibit low interference with biological processes due to the fact that their anionic and cationic sites are present as non-interacting carboxylate or sulfonate and cationic ammonium groups respectively.

Buffering in Cells and Tissues

A brief discussion of hydrogen ion regulation in biological systems highlights the importance of buffering systems. Amino acids present in proteins in cells and tissues contain functional groups that act as weak acid and bases. Nucleotides and several other low molecular weight metabolites that undergo ionization also contribute effectively to buffering in the cell. However, phosphate and bicarbonate buffer systems are most predominant in biological systems.

The phosphate buffer system has a pK_a of 6.86. Hence, it provides effective buffering in the pH range of 6.4 to 7.4. The bicarbonate buffer system plays an important role in buffering the blood system where in carbonic acid acts as a weak acid (proton donor) and bicarbonate acts as the conjugate base (proton acceptor). Their relationship can be expressed as follows:

$$K_1 = \frac{[H^+][HCO_3^-]}{[H_2CO_3]}$$

In this system carbonic acid (H_2CO_3) is formed from dissolved carbon dioxide and water in a reversible manner. The pH of the bicarbonate system is dependent on the concentration of carbonic acid and bicarbonate ion. Since carbonic acid

concentration is dependent upon the amount of dissolved carbon dioxide the ultimate buffering capacity is dependent upon the amount of bicarbonate and the partial pressure of carbon dioxide.

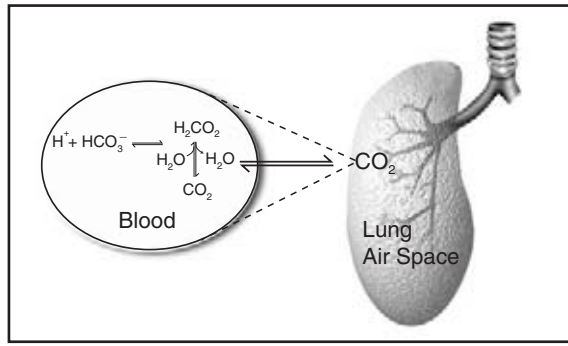

Figure 4: *Relationship between bicarbonate buffer system and carbon dioxide.*

In air breathing animals, the bicarbonate buffer system maintains pH near 7.4. This is possible due to the fact that carbonic acid in the blood is in equilibrium with the carbon dioxide present in the air. Figure 4 highlights the mechanism involved in blood pH regulation by the bicarbonate buffer system. Any increase in partial pressure of carbon dioxide (as in case of impaired ventilation) lowers the ratio of bicarbonate to pCO_2 resulting in a decrease in pH (**acidosis**). The acidosis is reversed gradually when kidneys increase the absorption of bicarbonate at the expense of chloride. Metabolic acidosis resulting from the loss of bicarbonate ions (such as in severe diarrhea or due to increased keto acid formation) leads to severe metabolic complications warranting intravenous bicarbonate therapy.

During hyperventilation, when excessive amounts of carbon dioxide are eliminated from the system (thereby lowering the pCO_2), pH of the blood increases resulting in **alkalosis**. This is commonly seen in conditions such as pulmonary embolism and hepatic failure. Metabolic alkalosis generally results when bicarbonate levels are higher in the blood. This is commonly observed after vomiting of acidic gastric secretions. Kidneys compensate for alkalosis by increasing the excretion of bicarbonate ions. However, an obligatory loss of sodium occurs under these circumstances.

In case of severe alkalosis the body is depleted of water, H^+, Cl^- and to some extent Na^+. A detailed account of metabolic acidosis and alkalosis is beyond the scope of this booklet. Readers are advised to consult a suitable text book of physiology for more detailed information on the mechanisms involved.

Effect of Temperature on pH

Generally when we consider the use of buffers we make following two assumptions.

(a) **The activity coefficients of the buffer ions is approximately equal to 1 over the useful range of buffer concentrations**
(b) **The value of K_a is constant over the working range of temperature.**

However, in real practice one observes that pH changes slightly with change in temperature. This might be very critical in biological systems where a precise hydrogen ion concentration is required for reaction systems to operate with maximum efficiency. Figure 5 presents the effect of temperature on the pH of phosphate buffer. The difference might appear to be slight but it has significant biological importance. Although the mathematical relationship of activity and temperature may be complicated, the actual change of pK_a with temperature ($\Delta pK_a/°C$) is approximately linear. Table 2 presents the pK_a and $\Delta pK_a/°C$ for several selected zwitterionic buffers commonly used in biological experimentation.

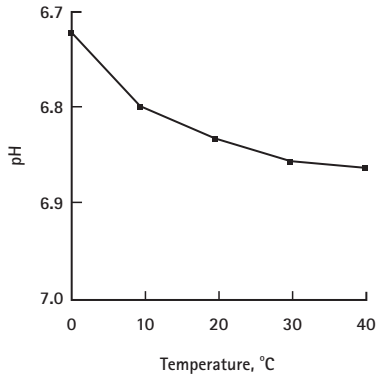

Figure 5: *Effect of Temperature on pH of Phosphate Buffer*

Table 2: pK_a and ΔpK_a/°C of Selected Buffers

Buffer	M.W.	pK_a (20°C)	pK_a (37°C)	ΔpK_a/°C	Binding to Metal Ions
MES	195.2	6.15	5.97	-0.011	Negligible metal ion binding
ADA	212.2	6.60	6.43	-0.011	Cu^{2+}, Ca^{2+}, Mn^{2+}. Weaker binding with Mg^{2+}.
BIS-Tris Propane*	282.4	6.80	–	-0.016	–
PIPES	302.4	6.80	6.66	-0.009	Negligible metal ion binding
ACES	182.2	6.90	6.56	-0.020	Cu^{2+}. Does not bind Mg^{2+}, Ca^{2+}, or Mn^{2+}.
BES	213.3	7.15	6.88	-0.016	Cu^{2+}. Does not bind Mg^{2+}, Ca^{2+}, or Mn^{2+}.
MOPS	209.3	7.20	6.98	-0.006	Negligible metal ion binding
TES	229.3	7.50	7.16	-0.020	Slightly to Cu^{2+}. Does not bind Mg^{2+}, Ca^{2+}, or Mn^{2+}.
HEPES	238.3	7.55	7.30	-0.014	None
HEPPS	252.3	8.00	7.80	-0.007	None
Tricine	179.2	8.15	7.79	-0.021	Cu^{2+}. Weaker binding with Ca^{2+}, Mg^{2+}, and Mn^{2+}.
Tris*	121.1	8.30	7.82	-0.031	Negligible metal ion binding
Bicine	163.2	8.35	8.04	-0.018	Cu^{2+}. Weaker binding with Ca^{2+}, Mg^{2+}, and Mn^{2+}.
Glycylglycine	132.1	8.40	7.95	-0.028	Cu^{2+}. Weaker binding with Mn^{2+}.
CHES	207.3	9.50	9.36	-0.009	–
CAPS	221.32	10.40	10.08	-0.009	–

* Not a zwitterionic buffer

Effects of Buffers on Factors Other than pH

It is of utmost importance that researchers establish the criteria and determine the suitability of a particular buffer system. Some weak acids and bases may interfere with the reaction system. For example, citrate and phosphate buffers are not recommended for systems that are highly calcium-dependent. Citric acid and its salts are powerful calcium chelators. Phosphates react with calcium producing insoluble calcium phosphate that precipitates out of the system. Phosphate ions in buffers can inhibit the activity of some enzymes, such as carboxypeptidase, fumarease, carboxylase, and phosphoglucomutase.

Tris(hydroxy-methyl)aminomethane can chelate copper and also acts as a competitive inhibitor of some enzymes. Other buffers such as ACES, BES, and TES, have a tendency to bind copper. Tris-based buffers are not recommended when studying the metabolic effects of insulin. Buffers such as HEPES and HEPPS are not suitable when a protein assay is performed by using Folin reagent. Buffers with primary amine groups, such as Tris, may interfere with the Bradford dye-binding method of protein assay. Borate buffers are not suitable for gel electrophoresis of protein, they can cause spreading of the zones if polyols are present in the medium.

Use of Water-Miscible Organic Solvents

Most pH measurements in biological systems are performed in the aqueous phase. However, sometimes mixed aqueous-water-miscible solvents, such as methanol or ethanol, are used for dissolving compounds of biological importance. These organic solvents have dissociation constants that are very low compared to that of pure water or of aqueous buffers (for example, the dissociation constant of methanol at 25°C is 1.45×10^{-17}, compared to 1.0×10^{-14} for water). Small amounts of methanol or ethanol added to the aqueous medium will not affect the pH of the buffer. However, even small traces of water in methanol or DMSO can significantly change the pH of these organic solvents.

Solubility Equilibrium: Effect of pH on Solubility

A brief discussion of the effect of pH on solubility is of significant importance when dissolution of compounds into solvents is under consideration. Changes in pH can affect the solubility of partially soluble ionic compounds.

Example:

$$Mg(OH)_2 \rightleftarrows Mg^{2+} + 2OH^-$$

Here

$$K = \frac{[Mg^{2+}]\,[OH^-]^2}{[Mg(OH)_2]}$$

As a result of the common ion effect, the solubility of $Mg(OH)_2$ can be increased or decreased. When a base is added the concentration of OH^- increases and shifts the solubility equilibrium to the left causing a diminution in the solubility of $Mg(OH)_2$. When an acid is added to the solution, it neutralizes the OH^- and shifts the solubility equilibrium to the right. This results in increased dissolution of $Mg(OH)_2$.

pH Measurements: Some Useful Tips

1. A pH meter may require a warm up time of several minutes. When a pH meter is routinely used in the laboratory, it is better to leave it "ON" with the function switch at "standby."

2. Set the temperature control knob to the temperature of your buffer solution. Always warm or cool your buffer to the desired temperature before checking final pH.

3. Before you begin make sure the electrode is well rinsed with deionized water and wiped off with a clean absorbent paper.

4. Always rinse and wipe the electrode when switching from one solution to another.

5. Calibrate your pH meter by using at least two standard buffer solutions.

6. Do not allow the electrode to touch the sides or bottom of your container. When using a magnetic bar to stir the solution make sure the electrode tip is high enough to prevent any damage.

7. Do not stir the solution while taking the reading.

8. Inspect your electrode periodically. The liquid level should be maintained as per the specification provided with the instrument .

9. Glass electrodes should not be left immersed in solution any longer than necessary. This is important especially when using a solution containing proteins. After several pH measurements of solutions containing proteins, rinse the electrode in a mild alkali solution and then wash several times with deionized water.

10. Water used for preparation of buffers should be of the highest possible purity. Water obtained by a method combining deionzation and distillation is highly recommended.

11. To avoid any contamination do not store water for longer than necessary. Store water in tightly sealed containers to minimize the amount of dissolved gases.

12. One may sterile-filter the buffer solution to prevent any bacterial or fungal growth. This is important when large quantities of buffers are prepared and stored over a long period of time.

CHOOSING A BUFFER

1. **Recognize the importance of the pK_a.** Select a buffer that has a pK_a value close to the middle of the range required. If you expect the pH to drop during the experiment, choose a buffer with a pK_a slightly lower than the working pH. This will permit the buffering action to become more resistant to changes in hydrogen ion concentration as hydrogen ions are liberated. Conversely, if you expect the pH to rise during the experiment, choose a buffer with a pK_a slightly higher than the working pH. For best results, the pK_a of the buffer should not be affected significantly by buffer concentration, temperature, and the ionic constitution of the medium.

2. **Adjust pH at desired temperature.** The pK_a of a buffer, and hence the pH, changes slightly with temperature. It is best to adjust the final pH at the desired temperature.

3. **Prepare buffers at working conditions.** Always try to prepare your buffer solution at the temperature and concentration you plan to use during the experiment. If you prepare stock solutions make dilutions just prior to use.

4. **Purity and cost.** Compounds used should be stable and be available in high purity and at moderate cost.

5. **Spectral properties:** Buffer materials should have no significant absorbance between 240 to 700 nm range.

6. **Some weak acids (or bases) are unsuitable for use as buffers in certain cases.** Citrate and phosphate buffers are not suitable for systems that are highly calcium-dependent. Citric acid and its salts are chelators of calcium and calcium phosphates are insoluble and will precipitate out. Use of these buffers may lower the calcium levels required for optimum reaction. Tris (hydroxymethyl) aminomethane is known to chelate calcium and other essential metals.

7. **Buffer materials and their salts can be used together for convenient buffer preparation.** Many buffer materials are supplied both as a free acid (or base) and its corresponding salt. This is convenient when making a series of buffers with different pH's. For example, solutions of 0.1 M HEPES and 0.1 M HEPES, sodium salt, can be mixed in an infinite number of ratios between 10:1 and 1:0 to provide 0.1 M HEPES buffer with pH values ranging from 6.55 to 8.55.

8. **Use stock solutions to prepare phosphate buffers.** Mixing precalculated amounts of monobasic and dibasic sodium phosphates has long been established as the method of choice for preparing phosphate buffer. By mixing the appropriate amounts of monobasic and dibasic sodium phosphate solutions buffers in the desired pH range can be prepared (see examples on page 17).

9. **Adjust buffer materials to the working pH.** Many buffers are supplied as crystalline acids or bases. The pH of these buffer materials in solution will not be near the pK_a, and the materials will not exhibit any buffering capacity until the pH is adjusted. In practice, a buffer material with a pK_a near the desired working pH is selected. If this buffer material is a free acid, pH is adjusted to desired working pH level by using a base such as sodium hydroxide, potassium hydroxide, or tetramethyl-ammonium hydroxide. Alternatively, pH for buffer materials obtained as free bases must be adjusted by adding a suitable acid.

10. **Use buffers without mineral cations when appropriate.** Frequently, buffers without mineral cations are appropriate. Tetramethylammonium hydroxide fits this criterion. The basicity of this organic quaternary amine is equivalent to that of sodium or potassium hydroxide. Buffers prepared with this base can be supplemented at will with various inorganic cations during the evaluation of mineral ion effects on enzymes or other bioparticulate activities.

11. **Use a graph to calculate buffer composition.** Figure 6 shows the theoretical plot of ΔpH versus $[A^-]/[HA]$ on two-cycle semilog paper. As most commonly used buffers exhibit only trivial deviations from theoretical value in the pH range, this plot can be of immense value in calculating the relative amounts of buffer components required for a particular pH.

    For example, suppose one needs 0.1 M MOPS buffer, pH 7.6 at 20°C. At 20°C, the pK_a for MOPS is 7.2. Thus, the working pH is about 0.4 pH units above the reported pK_a. According to the chart presented, this pH corresponds to a MOPS sodium/MOPS ratio of 2.5, and 0.1 M solutions of MOPS and MOPS sodium mixed in this ratio will give the required pH. If any significant deviations from theoretical values are observed one should check the proper working conditions and specifications of their pH meter. The graph can also be used to calculate the amount of acid (or base) required to adjust a free base buffer material (or free acid buffer material) to the desired working pH.

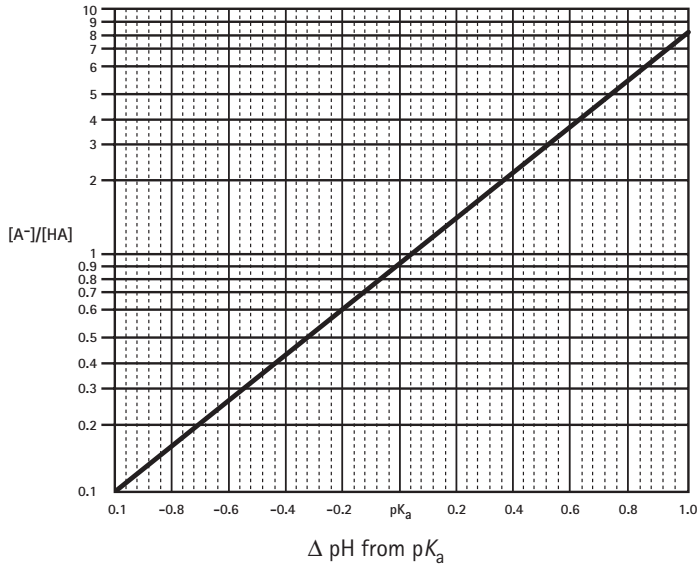

Figure 6: *Theoretical plot of ΔpH versus [A⁻]/[HA] on two-cycle semilog paper.*

Preparation of Some Common Buffers for Use in Biological Systems

The information provided below is intended only as a general guideline. We strongly recommend the use of a sensitive pH meter with appropriate temperature setting for final pH adjustment. Addition of other chemicals, after adjusting the pH, may change the final pH value to some extent. **The buffer concentrations in the tables below are used only as examples. You may select higher or lower concentrations depending upon your experimental needs.**

1. **Hydrochloric Acid-Potassium Chloride Buffer (HCl-KCl); pH Range 1.0 to 2.2**
   (a) 0.1 M Potassium chloride : 7.45 g/l (M.W.: 74.5)
   (b) 0.1 M Hydrochloric acid

Mix 50 ml of potassium chloride and indicated volume of hydrochloric acid. Mix and adjust the final volume to 100 ml with deionized water. Adjust the final pH using a sensitive pH meter.

ml of HCl	97	64.5	41.5	26.3	16.6	10.6	6.7
pH	1.0	1.2	1.4	1.6	1.8	2.0	2.2

2. Glycine-HCl Buffer; pH range 2.2 to 3.6

(a) 0.1 M Glycine: 7.5 g/l (M.W.: 75.0)

(b) 0.1 M Hydrochloric acid

Mix 50 ml of glycine and indicated volume of hydrochloric acid. Mix and adjust the final volume to 100 ml with deionized water. Adjust the final pH using a sensitive pH meter.

ml of HCl	44.0	32.4	24.2	16.8	11.4	8.2	6.4	5.0
pH	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6

3. Citrate Buffer; pH range 3.0 to 6.2

(a) 0.1 M Citric acid: 19.21 g/l (M.W.: 192.1)

(b) 0.1 M Sodium citrate dihydrate: 29.4 g/l (M.W.: 294.0)

Mix citric acid and sodium citrate solutions in the proportions indicated and adjust the final volume to 100 ml with deionized water. Adjust the final pH using a sensitive pH meter. The use of pentahydrate salt of sodium citrate is not recommended.

ml of Citric acid	46.5	40.0	35.0	31.5	25.5	20.5	16.0	11.8	7.2
ml of Sodium citrate	3.5	10.0	15.0	18.5	24.5	29.5	34.0	38.2	42.8
pH	3.0	3.4	3.8	4.2	4.6	5.0	5.4	5.8	6.2

4. Acetate Buffer; pH range 3.6 to 5.6

(a) 0.1 M Acetic acid (5.8 ml made to 1000 ml)

(b) 0.1 M Sodium acetate; 8.2 g/l (anhydrous; M.W. 82.0) or 13.6 g/l (trihydrate; M.W. 136.0)

Mix acetic acid and sodium acetate solutions in the proportions indicated and adjust the final volume to 100 ml with deionized water. Adjust the final pH using a sensitive pH meter.

ml of Acetic acid	46.3	41.0	30.5	20.0	14.8	10.5	4.8
ml of Sodium acetate	3.7	9.0	19.5	30.0	35.2	39.5	45.2
pH	3.6	4.0	4.4	4.8	5.0	5.2	5.6

5. Citrate-Phosphate Buffer; pH range 2.6 to 7.0

(a) 0.1 M Citric acid; 19.21 g/l (M.W. 192.1)

(b) 0.2 M Dibasic sodium phosphate; 35.6 g/l (dihydrate; M.W. 178.0) or 53.6 g/l (heptahydrate; M.W. 268.0)

Mix citric acid and sodium phosphate solutions in the proportions indicated and adjust the final volume to 100 ml with deionized water. Adjust the final pH using a sensitive pH meter.

ml of Citric acid	44.6	39.8	35.9	32.3	29.4	26.7	24.3	22.2	19.7	16.9	13.6	6.5
ml of Sodium phosphate	5.4	10.2	14.1	17.7	20.6	23.3	25.7	27.8	30.3	33.1	36.4	43.6
pH	2.6	3.0	3.4	3.8	4.2	4.6	5.0	5.4	5.8	6.2	6.6	7.0

6. Phosphate Buffer; pH range 5.8 to 8.0

(a) 0.1 M Sodium phosphate monobasic; 13.8 g/l (monohydrate, M.W. 138.0)

(b) 0.1 M Sodium phosphate dibasic; 26.8 g/l (heptahydrate, M.W. 268.0)

Mix Sodium phosphate monobasic and dibasic solutions in the proportions indicated and adjust the final volume to 200 ml with deionized water. Adjust the final pH using a sensitive pH meter.

ml of Sodium phosphate, Monobasic	92.0	81.5	73.5	62.5	51.0	39.0	28.0	19.0	13.0	8.5	5.3
ml of Sodium phosphate, Dibasic	8.0	18.5	26.5	37.5	49.0	61.0	72.0	81.0	87.0	91.5	94.7
pH	5.8	6.2	6.4	6.6	6.8	7.0	7.2	7.4	7.6	7.8	8.0

7. Tris-HCl Buffer, pH range 7.2 to 9.0

(a) 0.1 M Tris(hydroxymethyl)aminomethane; 12.1 g/l (M.W.: 121.0)

(b) 0.1 M Hydrochloric acid

Mix 50 ml of Tris(hydroxymethyl)aminomethane and indicated volume of hydrochloric acid and adjust the final volume to 200 ml with deionized water. Adjust the final pH using a sensitive pH meter.

ml of HCl	44.2	41.4	38.4	32.5	21.9	12.2	5.0
pH	7.2	7.4	7.6	7.8	8.2	8.6	9.0

8. Glycine-Sodium Hydroxide, pH 8.6 to 10.6

(a) 0.1 M Glycine; 7.5 g/l (M.W.: 75.0)

(b) 0.1 M Sodium hydroxide; 4.0 g/l (M.W.: 40.0)

Mix 50 ml of glycine and indicated volume of sodium hydroxide solutions and adjust the final volume to 200 ml with deionized water. Adjust the final pH using a sensitive pH meter.

ml Sodium hydroxide	4.0	8.8	16.8	27.2	32.0	38.6	45.5
pH	8.6	9.0	9.4	9.8	10.0	10.4	10.6

9. Carbonate-Bicarbonate Buffer, pH range 9.2 to 10.6

(a) 0.1 M Sodium carbonate (anhydrous), 10.6 g/l (M.W.: 106.0)

(b) 0.1 M Sodium bicarbonate, 8.4 g/l (M.W.: 84.0)

Mix sodium carbonate and sodium bicarbonate solutions in the proportions indicated and adjust the final volume to 200 ml with deionized water. Adjust the final pH using a sensitive pH meter.

ml of Sodium carbonate	4.0	9.5	16.0	22.0	27.5	33.0	38.5	42.5
ml of Sodium bicarbonate	46.0	40.5	34.0	28.0	22.5	17.0	11.5	7.5
pH	9.2	9.4	9.6	9.8	10.0	10.2	10.4	10.6

Commonly Used Buffer Media in Biological Research

Krebs-Henseleit bicarbonate buffer, pH 7.4

119 mM NaCl
4.7 mM KCl
2.5 mM $CaCl_2$
1.2 mM $MgSO_4$
1.2 mM KH_2PO_4
25 mM $NaHCO_3$

pH 7.4 (at 37°C) when equilibrated with 95% O_2 and 5% CO_2. Adjust the pH before use.

Hank's Biocarbonate Buffer, pH 7.4

137 mM NaCl
5.4 mM KCl
0.25 mM Na_2HPO_4
0.44 mM KH_2PO_4
1.3 mM $CaCl_2$
1.0 mM $MgSO_4$
4.2 mM $NaHCO_3$

pH 7.4 (at 37°C) when equilibrated with 95% O_2 and 5% CO_2. Adjust the pH before use.

Phosphate Buffered Saline (PBS), pH 7.4

150 mM NaCl
10 mM Potassium Phosphate buffer
(1 liter PBS can be prepared by dissolving 8.7 g NaCl, 1.82 g $K_2HPO_4.3H_2O$, and 0.23 g KH_2PO_4 in 1 liter of distilled water. Adjust the pH before use).

A variation of PBS can also be prepared as follows:

137 mM NaCl
2.7 mM KCl
10 mM Na_2HPO_4
1.76 mM KH_2PO_4

Tris Buffered Saline (TBS), pH 7.4

10 mM Tris
150 mM NaCl

(1 liter of TBS can be prepared by dissolving 1.21 g of Tris base and 8.7 g of NaCl in 1 liter of distilled water. Adjust the pH before use. Note: Tris has a pK_a of 8.3. Hence, the buffering capacity at pH 7.4 is minimal compared to phosphate buffer (pK_a = 7.21).

TBST (Tris Buffered saline and TWEEN®-20)

10 mM Tris-HCl, pH 8.0
150 mM NaCl
0.1% TWEEN®-20

Stripping Buffer for Western Blotting Applications

62.5 mM Tris buffer, pH 6.7 to 6.8
2% Sodium dodecyl sulfate (SDS)
100 mM β-Mercaptoethanol

Cell Lysis Buffer

20 mM Tris-HCl (pH 7.5)
150 mM NaCl
1 mM Sodium EDTA
1 mM EGTA
1% TRITON® X-100
2.5 mM Sodium pyrophosphate
1 mM β-Glycerophosphate
1 mM Sodium orthovanadate
1µg/ml Leupeptin

Isoelectric Point of Selected Proteins

Protein	Organism/Tissue	Isoelectric Point
Acetylcholinesterase	Electric eel, Electric organ	4.5
α1-Acid Glycoprotein	Human serum	1.8
Acid Protease	*Penicillium duponti*	3.9
Aconitase	Porcine heart	8.5
Adenosine deaminase	Human erythrocytes	4.7 - 5.1
Adenylate cyclase	Mouse brain	5.9 - 6.1
Adenylate kinase	Rat liver	7.5 - 8.0
Adenylate Kinase	Human erythrocytes	8.5 - 9.0
Albumin	Human serum	4.6 - 5.3
Alcohol dehydrogenase	Horse liver	8.7 - 9.3
Aldehyde dehydrogenase	Rat Liver (cytosol)	8.5
Aldolase	Rabbit muscle	8.2 - 8.6
Alkaline phosphatase	Bovine intestine	4.4
Alkaline phosphatase	Human liver	3.9
cAMP-phosphodiesterase	Rat brain	6.1
Amylase	Guinea Pig pancreas	8.4
Amylase	Human saliva	6.2 - 6.5
Arginase	Rat liver	9.4
Arginase	Human liver	9.2
ATPase (Na^+-K^+)	Dog heart	5.1
Carbonic Anhydrase	Porcine intestine	7.3
Carboxypeptidase B	Human pancreas	6.9
Carnitine acetyltransferase	Calf liver	6.0
Catalase	Mouse liver (particulate)	6.7
Cathepsin B	Human liver	5.1
Cathepsin D	Bovine spleen	6.7
Choline acetyltransferase	Human brain	7.8
α-Chymotrypsin	Bovine pancreas	8.8
Collagenase	*Clostridium*	5.5
C-Reactive protein	Human	7.4
DNA polymerase	Human lymphocytes	4.7
DNase I	Bovine	4.7
Dipeptidase	Porcine kidney	4.9
Enolase	Rat liver	5.9
Epidermal Growth Factor	Mouse submaxillary glands	4.6
Erythropoietin	Rabbit plasma	4.8 - 5.0
Ferritin	Human liver	5.0 - 5.6
α-Fetoprotein	Human serum	4.8
Follicle stimulating hormone	Sheep pituitary	4.6
Fructose 1,6-diphosphatase	Crab muscle	5.9
Galactokinase	Human placenta	5.8
β-Galactosidase	Rabbit brain	6.3
Glucose-6-phosphate dehydrogenase	Human erythrocytes	5.8 - 7.0
β-Glucuronidase	Rat liver microsomes	6.7

Isoelectric Point of Selected Proteins, cont.

Protein	Organism/Tissue	Isoelectric Point
γ-Glutamyl transpeptidase	Rat hepatoma	3.0
Glutathione S-transferase	Rat liver	6.9, 8.1
D-Glyceraldehyde 3-phosphate dehydrogenase	Rabbit muscle	8.3
L-Glycerol-3-phosphate dehydrogenase	Rabbit kidney	6.4
Glycogen phosphorylase b	Human muscle	6.3
Growth hormone	Horse pituitary	7.3
Guanylate kinase	Human erythrocytes	5.1
Hemoglobin	Rabbit erythrocyte	7.0
Hemoglobin A	Human erythrocytes	7.0
Hexokinase	Yeast	5.3
Insulin	Bovine pancreas	5.7
Lactate dehydrogenase	Rabbit muscle	8.5
Leucine aminopeptidase	Porcine kidney	4.5
Lipase	Human pancreas	4.7
Malate dehydrogenase	Rabbit heart (cytosol)	5.1
Malic Enzyme	Rabbit heart mitochondria	5.4
Myoglobin	Horse muscle	6.8, 7.3
Ornithine decarboxylase	Rat liver	4.1
Phosphoenolpyruvate carboxykinase	Mouse liver	6.1
Phosphofructokinase	Porcine liver	5.0
3-Phosphoglycerate kinase	Bovine liver	6.4
Phospholipase A	Bee venom	10.5
Phospholipase C	*C. perfringens*	5.3
Phosphorylase kinase	Rabbit muscle	5.8
Pepsin	Porcine stomach	2.2
Plasmin	Human plasma	7.0 - 8.5
Plasminogen	Human plasma	6.4 - 8.5
Plasminogen proactivator	Human plasma	8.9
Prolactin	Human pituitary	6.5
Protein kinase A	Bovine brain catalytic subunit	7.8
Prothrombin	Human plasma	4.6 - 4.7
Pyruvate kinase	Rat liver	5.7
Pyruvate kinase	Rat muscle	7.5
Renin	Human kidney	5.3
Ribonuclease	Bovine pancreas	9.3
RNA polymerase II	Human HeLa, KB cells	4.8
Superoxide dismutase	*Pleurotus olearius*	7.0
Thrombin	Human plasma	7.1
Transferrin	Human plasma	5.9
Trypsin inhibitor	Soybean	4.5
Trypsinogen Guinea	Porcine pancreas	8.7
Tubulin	Porcine brain	5.5
Urease	Jack bean	4.9

Isoelectric Points of Selected Plasma/Serum Proteins

Protein	M.W.	Species	Isoelectric Point
α_1-Acid Glycoprotein	44,000	Human	2.7
Albumin	66,000	Human	5.2
α_1-Antitrypsin	51,000	Human	4.2 - 4.7
Ceruloplasmin	135,000	Human	4.4
Cholinesterase	320,000	Human	4.0
Conalbumin	—	Human	5.9
C-Reactive Protein	110,000	Human	4.8
Erythropoietin	—	Rabbit	4.8 - 5.0
α-Fetoprotein	70,000	Human	4.8
Fibrinogen	340,000	Human	5.5
IgG	150,000	Human	5.8 - 7.3
IgD	172,000	Human	4.7 - 6.1
β-Lactoglobulin	44,000	Bovine	5.2
α_2-Macroglobulin	725,000	Human	5.4
β_2-Macroglobulin	11,800	Human	5.8
Plasmin	—	Human	7.0 - 8.5
Prealbumin	50,000 - 60,000	Human	4.7
Prothrombin	—	Bovine	4.6 - 4.9
Thrombin	37,000	Human	7.1
Thyroxine Binding Protein	63,000	Human	4.2 - 5.2
Transferrin	79,600	Human	5.9

Approximate pH and Bicarbonate Concentration in Extracellular Fluids

Fluid	pH	meq $HCO3^-$/liter
Plasma	7.35 - 7.45	28
Cerebrospinal Fluid	7.4	25
Saliva	6.4 - 7.4	10 - 20
Gastric Secretions	1.0 - 2.0	0
Tears	7.0 - 7.4	5 - 25
Aqueous Humor	7.4	28
Pancreatic Juice	7.0 - 8.0	80
Sweat	4.5 - 7.5	0 - 10

Ionization Constants K and pK_a for Selected Acids and Bases in Water

Acids and Bases	Ionization Constant (K)	pK_a
Acetic Acid	1.75×10^{-5}	4.76
Citric Acid	7.4×10^{-4} 1.7×10^{-5} 4.0×10^{-7}	3.13 4.77 6.40
Formic Acid	1.76×10^{-4}	3.75
Glycine	4.5×10^{-3} 1.7×10^{-10}	2.35 9.77
Imidazole	1.01×10^{-7}	6.95
Phosphoric Acid	7.5×10^{-3} 6.2×10^{-8} 4.8×10^{-13}	2.12 7.21 12.32
Pyruvic Acid	3.23×10^{-3}	2.49
Tris(hydroxymethyl)aminomethane	8.32×10^{-9}	8.08

Physical Properties of Some Commonly Used Acids

Acid	Molecular Weight	Specific Gravity	% Weight/ Weight	Approx. Normality	ml required to make 1 liter of 1 N solution
Acetic Acid	60.05	1.06	99.50	17.6	57
Hydrochloric Acid	36.46	1.19	37	12.1	83
Nitric Acid	63.02	1.42	70	15.7	64
Perchloric Acid (72%)	100.46	1.68	72	11.9	84
Phosphoric Acid	98.00	1.70	85	44.1	23
Sulfuric Acid	98.08	1.84	96	36.0	28

Some Useful Tips for Calculation of Concentrations and Spectrophotometric Measurements

As per Beer's law

$A = abc$

Where A = absorbance
a = proportionality constant defined as absorptivity
b = light path in cm
c = concentration of the absorbing compound

When **b** is 1 cm and **c** is moles/liter, the symbol **a** is substituted by a symbol **ε** (epsilon).

ε is a constant for a given compound at a given wavelength under prescribed conditions of solvent, temperature, pH and is called as molar absorptivity. e is used to characterize compounds and establish their purity.

Example:

Bilirubin dissolved in chloroform at 25°C should have a molar absorptivity (e) of 60,700.
Molecular weight of bilirubin is 584.
Hence 5 mg/liter (0.005 g/l) read in 1 cm cuvette should have an absorbance of

$$A = (60{,}700)(1)(0.005/584) = 0.52 \quad \{A = abc\}$$

Conversely, a solution of this concentration showing absorbance of 0.49 should have a purity of 94% (0.49/0.52).

In most biochemical and toxicological work, it is customary to list constants based on concentrations in g/dl rather than mol/liter. This is also common when molecular weight of the substance is not precisely known.

Here for **b** = 1 cm; and **c** = 1 g/dl (1%), A can be written as $A^{1\%}_{1cm}$

This constant is known as absorption coefficient.

The direct proportionality between absorbance and concentration must be established experimentally for a given instrument under specified conditions.

Frequently there is a linear relationship up to a certain concentration. Within these limitations, a calibration constant (**K**) may be derived as follows:

$$A = abc.$$

Therefore,

$$c = A/ab = A \times 1/ab.$$

The absorptivity (**a**) and light path (**b**) remain constant in a given method of analysis. Hence, 1/ab can be replaced by a constant (**K**).

Then,

c = A x **K**; where **K** = **c**/A. The value of the constant **K** is obtained by measuring the absorbance (A) of a standard of known concentration (**c**).

CALBIOCHEM® Buffers

We offer an extensive line of buffer materials that meet the highest standards of quality. We are continuing to broaden our line of ULTROL® Grade Buffer materials, which are of superior quality and are manufactured to meet stringent specifications. In addition, whenever possible, they are screened for uniform particle size, giving uniform solubility characteristics.

ADA, Sodium Salt
(N-2-Acetamido-2-iminodiacetic Acid, Na)
M.W. 212.2 100 g
Cat. No. 114801

2-Amino-2-methyl-1,3-propanediol
M.W. 105.1 50 g
Cat. No. 164548 500 g

BES, Free Acid, ULTROL® Grade
[N,N-*bis*-(2-Hydroxyethyl)-2-aminoethane-sulfonic Acid]
M.W. 213.3 25 g
Cat. No. 391334

Bicine, ULTROL® Grade
[N,N-bis-(2-Hydroxyethyl)glycine]
M.W. 163.2 100 g
Cat. No. 391336 1 kg

BIS-Tris, ULTROL® Grade
{*bis*(2-Hydroxyethyl)imino]-*tris*(hydroxymethyl)methane}
M.W. 209.2 100 g
Cat. No. 391335 1 kg

BIS-Tris Propane, ULTROL® Grade
{1,3-*bis*[tris(Hydroxymethyl)methylamino]-propane}
M.W. 282.4 100 g
Cat. No. 394111 1 kg

Boric Acid, Molecular Biology Grade
M.W. 61.8 500 g
Cat. No. 203667 1 kg
5 kg

Cacodylic Acid, Sodium Salt
(Sodium Dimethyl Arsenate)
M.W. 160.0 100 g
Cat. No. 205541

CAPS, ULTROL® Grade
[3-(Cyclohexylamino)propanesulfonic Acid]
M.W. 221.3 100 g
Cat. No. 239782 1 kg

CHES, ULTROL® Grade
[2-(N-Cyclohexylamino)ethanesulfonic Acid]
M.W. 207.3 100 g
Cat. No. 239779

Citric Acid, Monohydrate, Molecular Biology Grade
M.W. 210.1 100 g
Cat. No. 231211 1 kg

Glycine, Free Base
M.W. 75.1 500 g
Cat. No. 3570

Glycine, Molecular Biology Grade
M.W. 75.1 100 g
Cat. No. 357002 1 kg

Glycylglycine, Free Base
M.W. 132.1 25 g
Cat. No. 3630 100 g

HEPES, Free Acid, Molecular Biology Grade
(N-2-Hydroxyethylpiperazine-N′-2-ethanesulfonic Acid)
M.W. 238.3 25 g
Cat. No. 391340 250 g

HEPES, Free Acid, ULTROL® Grade
(N-2-Hydroxyethylpiperazine-N′-2-ethanesulfonic Acid)
M.W. 238.3 25 g
Cat. No. 391338 100 g
500 g
1 kg
5 kg

HEPES, Free Acid, ULTROL® Grade, 1 M Solution
M.W. 238.3 100 ml
Cat. No. 375368 500 ml

HEPES, Sodium Salt, ULTROL® Grade
(N-2-Hydroxyethylpiperazine-N′-2-ethanesulfonic Acid, Na)
M.W. 260.3 100 g
Cat. No. 391333 500 g
1 kg

HEPPS, ULTROL® Grade
(EPPS; N-2-Hydroxyethylpiperazine-N′-3-propane sulfonic acid)
M.W. 252.3 100 g
Cat. No. 391339

Imidazole, ULTROL® Grade
(1,3-Diaza-2,4-cyclopentadiene)
M.W. 68.1 25 g
Cat. No. 4015 100 g

MES, Free Acid, ULTROL® Grade
[2-(N-Morpholino)ethanesulfonic Acid]
M.W. 195.2 100 g
Cat. No. 475893 500 g
1 kg

MES, Sodium Salt, ULTROL® Grade
[2-(N-Morpholino)ethanesulfonic Acid, Na]
M.W. 217.2 100 g
Cat. No. 475894 1 kg

MOPS, Free Acid, ULTROL® Grade
[3-(N-Morpholino)propanesulfonic Acid]
M.W. 209.3 100 g
Cat. No. 475898 500 g
1 kg

MOPS, Sodium, ULTROL® Grade
[3-(N-Morpholino)propanesulfonic Acid, Na]
M.W. 231.2 100 g
Cat. No. 475899 1 kg

MOPS/EDTA Buffer, 10X Liquid Concentrate, Molecular Biology Grade
M.W. 209.3 100 ml
Cat. No. 475916

PBS Tablets
(Phosphate Buffered Saline Tablets)
Cat. No. 524650 1 each

PBS-TWEEN® Tablets
(Phosphate Buffered Saline-TWEEN® 20 Tablets)
Cat. No. 524653 1 each

PIPES, Free Acid, Molecular Biology Grade
[Piperazine-N,N′-*bis*(2-ethanesulfonic Acid)]
M.W. 302.4 25 g
Cat. No. 528133 250 g

PIPES, Free Acid, ULTROL® Grade
[Piperazine-N,N′-*bis*(2-ethanesulfonic Acid)]
M.W. 302.4 100 g
Cat. No. 528131 1 kg

PIPES, Sesquisodium Salt, ULTROL® Grade
[Piperazine-N,N′-*bis*(2-ethanesulfonic Acid), 1.5Na]
M.W. 335.3 100 g
Cat. No. 528132 1 kg

PIPPS
[Piperazine-N,N′-*bis*(3-propanesulfonic Acid)]
M.W. 330.4 10 g
Cat. No. 528315

Potassium Phosphate, Dibasic, Trihydrate, Molecular Biology Grade
M.W. 228.2 250 g
Cat. No. 529567 1 kg

Potassium Phosphate, Monobasic
M.W. 136.1 100 g
Cat. No. 529565 500 g

Potassium Phosphate, Monobasic, Molecular Biology Grade
M.W. 136.1 250 g
Cat. No. 529568 1 kg

Sodium Citrate, Dihydrate
(Citric Acid, 3Na)
M.W. 294.1 1 kg
Cat. No. 567444

Sodium Citrate, Dihydrate, Molecular Biology Grade
M.W. 294.1 100 g
Cat. No. 567446 1 kg
5 kg

Sodium Phosphate, Dibasic
M.W. 142.0 500 g
Cat. No. 567550 1 kg

Sodium Phosphate, Dibasic, Molecular Biology Grade
M.W. 142.0 250 g
Cat. No. 567547 1 kg

Sodium Phosphate, Monobasic
M.W. 120.0 250 g
Cat. No. 567545 500 g
1 kg

Sodium Phosphate, Monobasic, Monohydrate, Molecular Biology Grade
M.W. 138.0 250 g
Cat. No. 567549 1 kg

SSC Buffer, 20X Powder Pack, ULTROL® Grade
Cat. No. 567780 2 pack

SSPE Buffer, 20X Powder Pack, ULTROL® Grade
Cat. No. 567784 2 pack

TAPS, ULTROL® Grade
(3-{[*tris*(Hydroxymethyl)methyl]amino}-propanesulfonic Acid)
M.W. 243.2 100 g
Cat. No. 394675 1 kg

TBE Buffer, 10X Powder Pack, ULTROL® Grade
(10X Tris-Borate-EDTA Buffer)
Cat. No. 574796 2 pack

TES, Free Acid, ULTROL® Grade
(2-{[*tris*(Hydroxymethyl)methyl]amino}-ethanesulfonic Acid)
M.W. 229.3 100 g
Cat. No. 39465 1 kg

TES, Sodium Salt, ULTROL® Grade
M.W. 251.2 100 g
Cat. No. 394651

Tricine, ULTROL® Grade
{N-[*tris*(Hydroxymethyl)methyl]glycine}
M.W. 179.2 100 g
Cat. No. 39468 1 kg

Triethanolamine, Hydrochloride*
M.W. 185.7 1 kg
Cat. No. 641752

Triethylammonium Acetate, 1 M Solution
M.W. 161.2 1 liter
Cat. No. 625718

Tris Base, Molecular Biology Grade
[*tris*(Hydroxymethyl)aminomethane]
M.W. 121.1 100 g
Cat. No. 648310 500 g
1 kg
2.5 kg

Tris Base, ULTROL® Grade
[*tris*(Hydroxymethyl)aminomethane]
M.W. 121.1 100 g
Cat. No. 648311 500 g
1 kg
5 kg
10 kg

Tris Buffer, 1.0 M, pH 8.0, Molecular Biology Grade
M.W. 121.1 100 ml
Cat. No. 648314

Tris Buffer, 100 mM, pH 7.4, Molecular Biology Grade
M.W. 121.1 100 ml
Cat. No. 648315

Tris, Hydrochloride, Molecular Biology Grade
[*tris*(Hydroxymethyl)aminomethane, HCl]
M.W. 157.6 100 ml
Cat. No. 648317 1 kg

Tris, Hydrochloride, ULTROL® Grade
[*tris*(Hydroxymethyl)aminomethane, HCl]
M.W. 157.6 250 g
Cat. No. 648313 500 g
1 kg

* Not for international sales outside the US.