

Calbiochem®

# Buffers

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



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

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## **A word to our valued 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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The Spotted Cucumber Beetle (*Diabrotica undecimpunctata*) is a native of North America and ranges from Mexico to Canada. The Cucumber Beetle can cause serious crop damage by feeding and by transmission of bacterial wilt disease. More information can be found at <http://bugguide.net/node/view/456>. *Photo credit: Scot Mitchell*

## **Why does Calbiochem® Biochemicals Publish a Booklet on Buffers?**

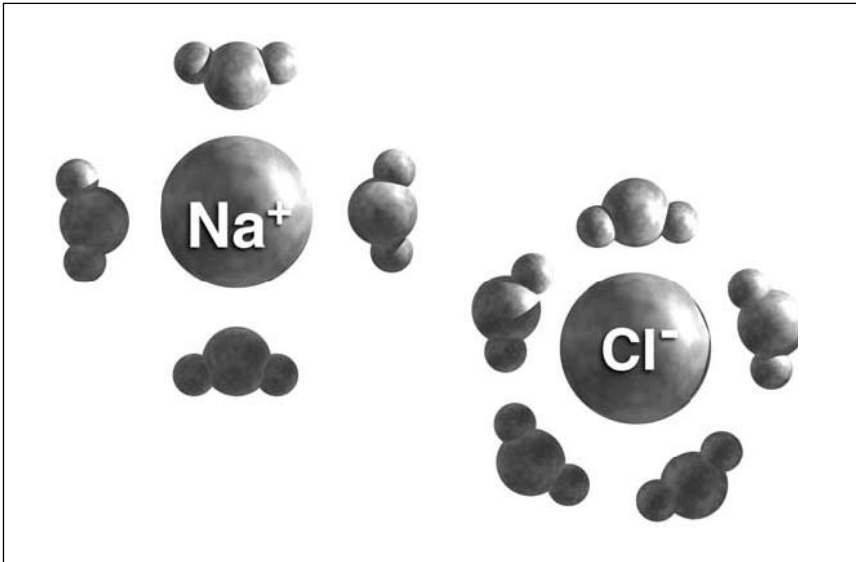
We are frequently asked questions on the use of buffers that we offer to research laboratories. This booklet is designed to help answer basic questions about the use of buffers in biological systems. The discussion presented here is by no means complete, but we hope it will help in the understanding 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 a 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 biological reactions occur in an aqueous medium. All aspects of cell structure and function 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 dissolves 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 are known as HYDROPHOBIC compounds. These compounds interfere with hydrogen bonding among water molecules.

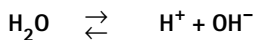


*Figure 1: Electrostatic interaction of  $Na^+$  and  $Cl^-$  ions and water molecules.*

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

## Ionization of Water

Water molecules undergo reversible ionization to yield  $\text{H}^+$  and  $\text{OH}^-$  as per the following equation.



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

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

At  $25^\circ\text{C}$ , the concentration of pure water is  $55.5 \text{ M}$  ( $1000 \div 18$ ; M.W. 18.0). Hence, we can rewrite the above equation as follows:

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

or

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

For pure water, electrical conductivity experiments give a  $K_{\text{eq}}$  value of  $1.8 \times 10^{-16} \text{ M}$  at  $25^\circ\text{C}$ .

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

or

$$99.9 \times 10^{-16} \text{ M}^2 = [\text{H}^+][\text{OH}^-]$$

or

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

$[\text{H}^+][\text{OH}^-]$ , the ion product of water, is always equal to  $1.0 \times 10^{-14} \text{ M}^2$  at  $25^\circ\text{C}$ .

When  $[\text{H}^+]$  and  $[\text{OH}^-]$  are present in equal amounts, the solution gives a neutral pH.

Here 
$$[\text{H}^+][\text{OH}^-] = [\text{H}^+]^2$$

or

$$[\text{H}^+] = 1 \times 10^{-14} \text{ M}^2$$

and

$$[\text{H}^+] = [\text{OH}^-] = 10^{-7} \text{ M}$$

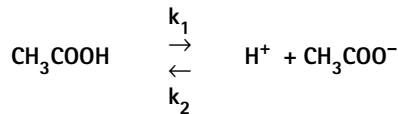
Because the total concentration of  $\text{H}^+$  and  $\text{OH}^-$  is constant, an increase in one ion is compensated by a decrease in the concentration of other ion. This forms the basis for the pH scale.



## 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:



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,  $d[\text{CH}_3\text{COOH}]/dt$ , is dependent on the rate constant of dissociation ( $k_1$ ) and the concentration of acetic acid  $[\text{CH}_3\text{COOH}]$  and can be expressed as:

$$\frac{d[\text{CH}_3\text{COOH}]}{dt} = k_1[\text{CH}_3\text{COOH}]$$

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

$$\frac{d[\text{CH}_3\text{COOH}]}{dt} = k_2[\text{H}^+][\text{CH}_3\text{COO}^-]$$

Since the rates of dissociation and re-association are equal under equilibrium conditions:

$$k_1[\text{CH}_3\text{COOH}] = k_2[\text{H}^+][\text{CH}_3\text{COO}^-]$$

or

$$\frac{k_1}{k_2} = \frac{[\text{H}^+][\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}]}$$

and

$$K_a = \frac{[\text{H}^+][\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}]}$$

where

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

This equilibrium expression can now be rearranged to

$$[\text{H}^+] = K_a \frac{[\text{CH}_3\text{COOH}]}{[\text{CH}_3\text{COO}^-]}$$

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 $\text{pK}_a$

The relationship between pH,  $\text{pK}_a$ , and the buffering action of any weak acid and its conjugate base is best explained by the Henderson-Hasselbach equation. In biological experiments,  $[\text{H}^+]$  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  $[\text{H}^+]$  one can write the following equation:

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

Similarly  $\text{pK}_a$  can be defined as  $-\log K_a$ . If the equilibrium expression is converted to  $-\log$ , then

$$-\log [\text{H}^+] = -\log K_a - \log \frac{[\text{CH}_3\text{COOH}]}{[\text{CH}_3\text{COO}^-]}$$

and pH and  $\text{pK}_a$  substituted:

$$\text{pH} = \text{pK}_a - \log \frac{[\text{CH}_3\text{COOH}]}{[\text{CH}_3\text{COO}^-]}$$

or

$$\text{pH} = \text{pK}_a + \log \frac{[\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}]}$$

When the concentration of acetate ions equals the concentration of acetic acid,  $\log [\text{CH}_3\text{COO}^-]/[\text{CH}_3\text{COOH}]$  approaches zero (the log of 1) and pH equals  $\text{pK}_a$  (the  $\text{pK}_a$  of acetic acid is 4.745). Acetic acid and acetate ion form an effective buffering system centered around pH 4.75. Generally, the  $\text{pK}_a$  of a weak acid or base indicates the pH of the center of the buffering region.

The terms pK and  $\text{pK}_a$  are frequently used interchangeably in the literature. The term  $\text{pK}_a$  (“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 $pK_a$

$pK_a$  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  $HPO_4^{2-}$  exist as the sole species.

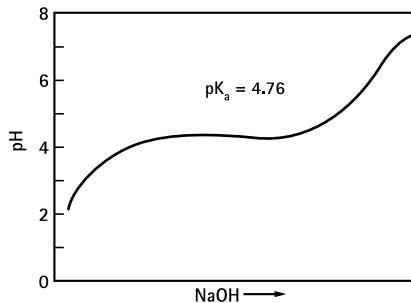


Figure 2: Titration Curve for Acetic Acid

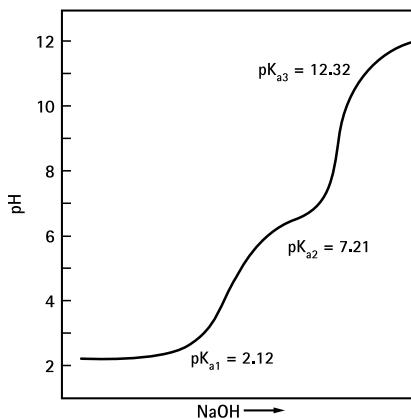


Figure 3: Titration Curve for Phosphoric Acid

**Table 1: pK<sub>a</sub> Values for Commonly Used Biological Buffers and Buffer Constituents**

Product	Cat. No.	M.W.	pK <sub>a</sub> at 20°C
BES, ULTROL <sup>®</sup> Grade	391334	213.2	7.15
Bicine, ULTROL <sup>®</sup> Grade	391336	163.2	8.35
BIS-Tris, ULTROL <sup>®</sup> Grade	391335	209.2	6.50
BIS-Tris Propane, ULTROL <sup>®</sup> 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 <sup>®</sup> Grade	239782	221.3	10.40
CHES, ULTROL <sup>®</sup> Grade	239779	207.3	9.50
Citric Acid, Monohydrate, Molecular Biology Grade	231211	210.1	4.76
Glycine	3570	75.1	2.341
Glycine, Molecular Biology Grade	357002	75.1	2.341
Glycylglycine, Free Base	3630	132.1	8.40
HEPES, Free Acid, Molecular Biology Grade	391340	238.3	7.55
HEPES, Free Acid, ULTROL <sup>®</sup> Grade	391338	238.3	7.55
HEPES, Free Acid Solution	375368	238.3	7.55
HEPES, Sodium Salt, ULTROL <sup>®</sup> Grade	391333	260.3	7.55
Imidazole, ULTROL <sup>®</sup> Grade	4015	68.1	7.00
MES, Free Acid, ULTROL <sup>®</sup> Grade	475893	195.2	6.15
MES, Sodium Salt, ULTROL <sup>®</sup> Grade	475894	217.2	6.15
MOPS, Free Acid, ULTROL <sup>®</sup> Grade	475898	209.3	7.20
MOPS, Sodium Salt, ULTROL <sup>®</sup> Grade	475899	231.2	7.20
PIPES, Free Acid, ULTROL <sup>®</sup> Grade	528131	302.4	6.80
PIPES, Sodium Salt, ULTROL <sup>®</sup> Grade	528132	325.3	6.80
PIPPS	528315	330.4	3.732
Potassium Phosphate, Dibasic, Trihydrate, Molecular Biology Grade	529567	228.2	7.213
Potassium Phosphate, Monobasic, Molecular Biology Grade	529568	136.1	7.213
Sodium Phosphate, Dibasic	567550	142.0	7.213
Sodium Phosphate, Dibasic, Molecular Biology Grade	567547	142.0	7.213
Sodium Phosphate, Monobasic	567545	120.0	7.213
Sodium Phosphate, Monobasic, Monohydrate, Molecular Biology Grade	567549	138.0	7.213
TAPS, ULTROL <sup>®</sup> Grade	394675	243.2	8.40
TES, Free Acid, ULTROL <sup>®</sup> Grade	39465	229.3	7.50
Tricine, ULTROL <sup>®</sup> 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 <sup>®</sup> Grade	648311	121.1	8.30
Tris, HCl, Molecular Biology Grade	648317	157.6	8.30
Tris, HCl, ULTROL <sup>®</sup> Grade	648313	157.6	8.30
Trisodium Citrate, Dihydrate, Molecular Biology Grade	567446	294.1	–

1. pK<sub>a1</sub> = 2.34; pK<sub>a2</sub> = 9.6

2. pK<sub>a1</sub> = 3.73; pK<sub>a2</sub> = 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 pKa values: pK<sub>a1</sub> = 2.12; pK<sub>a2</sub> = 7.21; pK<sub>a3</sub> = 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  $pK_a$ .

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

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

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.

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.

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 ( $1 \times 10^{-2}$  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  $[H^+]$ :

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

Thus, the addition of  $1.0 \times 10^{-3}$  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:

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

$$\text{pH} = \text{p}K_a + \log \frac{[A]}{[HA]}$$

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

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

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

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

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.
- They should not interfere with any biological process.
- The salt effect should be minimal, 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 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.

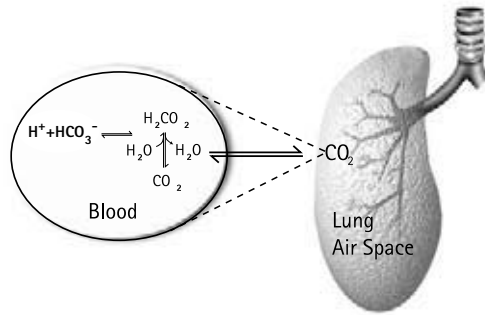


Figure 4: Relationship between bicarbonate buffer system and carbon dioxide.

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  $pCO_2$ ), the 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. Please consult a suitable textbook 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 the 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 ( $\Delta pK_a / ^\circ C$ ) is approximately linear. Table 2 presents the  $pK_a$  and  $\Delta pK_a / ^\circ C$  for several selected zwitterionic buffers commonly used in biological experimentation.

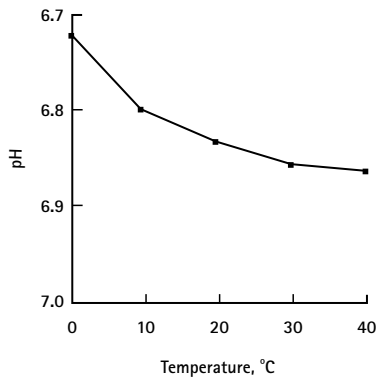


Figure 5: Effect of Temperature on pH of Phosphate Buffer

**Table 2:  $pK_a$  and  $\Delta pK_a/^\circ C$  of Selected Buffers**

Buffer	M.W.	$pK_a$ (20 $^\circ C$ )	$pK_a$ (37 $^\circ C$ )	$pK_a/^\circ C$	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.

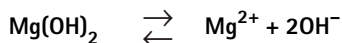
## Use of Water–Miscible Organic Solvents

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  $1.45 \times 10^{-17}$ , compared to  $1.0 \times 10^{-14}$  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:



Here

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

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

## 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 deionization 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 the 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 in the 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. 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 18).
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. 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  $\Delta pH$  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.

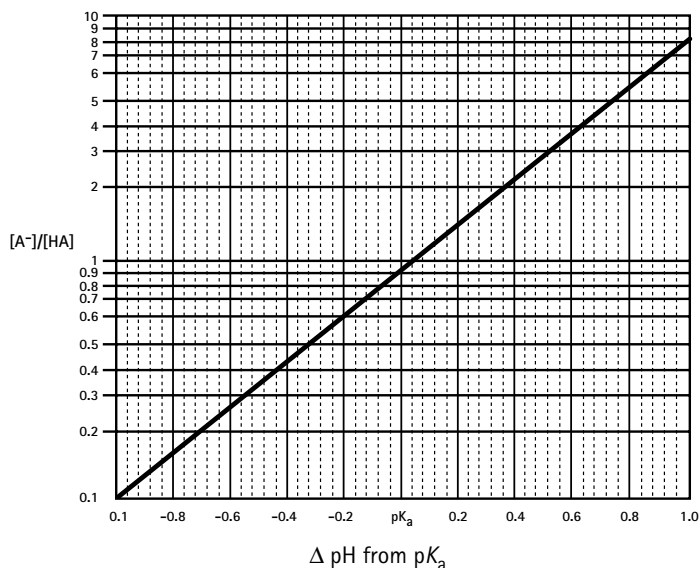


Figure 6: Theoretical plot of  $\Delta\text{pH}$  versus  $[A^-]/[HA]$  on two-cycle semilog paper.

## 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 of 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

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## 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<sub>2</sub>  
1.2 mM MgSO<sub>4</sub>  
1.2 mM KH<sub>2</sub>PO<sub>4</sub>  
25 mM NaHCO<sub>3</sub>

pH 7.4 (at 37°C) when equilibrated with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Adjust the pH before use.

### Hank's Bicarbonate Buffer, pH 7.4

137 mM NaCl  
5.4 mM KCl  
0.25 mM Na<sub>2</sub>HPO<sub>4</sub>  
0.44 mM KH<sub>2</sub>PO<sub>4</sub>  
1.3 mM CaCl<sub>2</sub>  
1.0 mM MgSO<sub>4</sub>  
4.2 mM NaHCO<sub>3</sub>

pH 7.4 (at 37°C) when equilibrated with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. 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<sub>2</sub>HPO<sub>4</sub> · 3H<sub>2</sub>O, and 0.23 g KH<sub>2</sub>PO<sub>4</sub> 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<sub>2</sub>HPO<sub>4</sub>  
1.76 mM KH<sub>2</sub>PO<sub>4</sub>

### **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  $\beta$ -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  $\beta$ -Glycerophosphate

1 mM Sodium orthovanadate

1  $\mu$ g/ml Leupeptin

## Isoelectric Point

The isoelectric point (pI) of a protein is the pH at which any given protein has an equal number of positive and negative charges. At a pH below this point, proteins carry a net positive charge, and above this point they have a net negative charge. This property has important biochemical implications in protein purification and electrophoresis. If the pH of the buffer is higher than the isoelectric point of the protein, it will migrate towards the positive terminal. On the other hand, if the pH of the buffer is lower than the isoelectric point of the protein, it will migrate towards the negative terminal. When the buffer pH is equal to the pI of a protein, it will not migrate at all.

A protein can exhibit different charges depending on the pH of the medium. At their pI, proteins exhibit the least electrostatic repulsion, hence they have the lowest solubility at this point and can easily precipitate. This property is useful in crystallization of proteins.

In general, positive and negative charges on the surface of protein are balanced around neutral pH. The electrostatic attraction provides the compact shape and greater stability to the protein. However, at extremely low pH, the carboxyl groups are protonated and negative charges decrease. Here proteins gain more electrostatic repulsion and become denatured.

When acidic proteins are denatured in an acidic condition, they aggregate easily. On the other hand, when basic proteins are denatured in acidic conditions, they do not aggregate much because the proteins have greater number of positive charges in the acidic condition and the electrostatic repulsion is high. When the pH is brought back to neutral, non-precipitated basic proteins may return to their native structure. This is not generally true for precipitated acidic proteins, which often require treatment with strong denaturants, such as urea or guanidine hydrochloride.

## Isoelectric Point of Selected Proteins

Protein	Organism/Tissue	Isoelectric Point
Acetylcholinesterase	Electric eel, Electric organ	4.5
$\alpha$ 1-Acid glycoprotein	Human serum	1.8
Acid protease	<i>Penicillium duponti</i>	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

## Isoelectric Point of Selected Proteins, *continued*

Protein	Organism/Tissue	Isoelectric Point
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 <sup>+</sup> -K <sup>+</sup> )	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	<i>Clostridium</i>	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
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

Protein	Organism/Tissue	Isoelectric Point
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
Malate dehydrogenase	Rabbit heart (mitochondria)	9.2
Malic enzyme	Rabbit heart mitochondria	5.4
Myoglobin	Horse muscle	6.8, 7.3
Nerve Growth Factor	Mouse salivary gland	9.3
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
Protein kinase A	Porcine brain	4.6
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
$\alpha_1$ -Acid glycoprotein	44,000	Human	2.7
Albumin	66,000	Human	5.2
$\alpha_1$ -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	21,000 (unglycosylated)	Human	4.8–5.0
$\alpha$ -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
$\beta$ -Lactoglobulin	44,000	Bovine	5.2
$\alpha_2$ -Macroglobulin	725,000	Human	5.4
$\beta_2$ -Macroglobulin	11,800	Human	5.8
Plasmin	83,000	Human	7.0–8.5
Prealbumin	50,000–60,000	Human	4.7
Prothrombin	72,000	Human	4.7–4.9
$\alpha$ -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 $\text{HCO}_3^-$ /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



## Ionic Composition of Body Fluids

Ion	Plasma	Intracellular Fluid
	[mEq/l]	
Sodium	142	10
Potassium	4	160
Calcium	5	2
Magnesium	2	26
Chloride	101	3
Bicarbonate	27	10
Phosphate	2	100
Sulfate	1	20

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

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

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

Example:

Bilirubin dissolved in chloroform at 25°C should have a molar absorptivity  $\epsilon$  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

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

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  $A_{\text{cm}}^{1\%}$

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,

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

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Bicine, ULTROL <sup>®</sup> Grade [N,N-bis-(2-Hydroxyethyl)glycine] M.W. 163.2 Cat. No. 391336	100 g 1 kg	Cacodylic Acid, Sodium Salt (Sodium Dimethyl Arsenate) M.W. 160.0 Cat. No. 205541	100 g
BIS-Tris, ULTROL <sup>®</sup> Grade {bis(2-Hydroxyethyl)imino}- tris(hydroxymethyl)methane} M.W. 209.2 Cat. No. 391335	100 g 1 kg	CAPS, ULTROL <sup>®</sup> Grade [3-(Cyclohexylamino)propanesulfonic Acid] M.W. 221.3 Cat. No. 239782	100 g 1 kg
BIS-Tris Propane, ULTROL <sup>®</sup> Grade {1,3-bis[tris(Hydroxymethyl)methylamino]- propane} M.W. 282.4 Cat. No. 394111	100 g 1 kg	CHES, ULTROL <sup>®</sup> Grade [2-(N-Cyclohexylamino)ethanesulfonic Acid] M.W. 207.3 Cat. No. 239779	100 g
		Citric Acid, Monohydrate, Molecular Biology Grade M.W. 210.1 Cat. No. 231211	100 g 1 kg

Glycine, Free Base		MOPS, Sodium, ULTROL® Grade	
M.W. 75.1	500 g	[3-(N-Morpholino)propanesulfonic Acid, Na]	
Cat. No. 3570		M.W. 231.2	100 g
		Cat. No. 475899	1 kg
Glycine, Molecular Biology Grade		MOPS/EDTA Buffer, 10X Liquid Concentrate,	
M.W. 75.1	100 g	Molecular Biology Grade	
Cat. No. 357002	1 kg	M.W. 209.3	100 ml
		Cat. No. 475916	
Glycylglycine, Free Base		PBS Tablets	
M.W. 132.1	25 g	(Phosphate Buffered Saline Tablets)	
Cat. No. 3630	100 g	Cat. No. 524650	1 each (10 tablets)
HEPES, Free Acid, Molecular Biology Grade		PBS-TWEEN® Tablets	
(N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic Acid)		(Phosphate Buffered Saline-TWEEN® 20 Tablets)	
M.W. 238.3	25 g	Cat. No. 524653	1 each (10 tablets)
Cat. No. 391340	50 g		
HEPES, Free Acid, ULTROL® Grade		PIPES, Free Acid, ULTROL® Grade	
(N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic Acid)		[Piperazine-N,N -bis(2-ethanesulfonic Acid)]	
M.W. 238.3	25 g	M.W. 302.4	100 g
Cat. No. 391338	100 g	Cat. No. 528131	1 kg
	500 g		
	1 kg	PIPES, Sesquisodium Salt, ULTROL® Grade	
	5 kg	[Piperazine-N,N -bis(2-ethanesulfonic Acid),1.5Na]	
HEPES, Free Acid, ULTROL® Grade, 1 M Solution		M.W. 335.3	100 g
M.W. 238.3	100 ml	Cat. No. 528132	1 kg
Cat. No. 375368	500 ml		
HEPES, Sodium Salt, ULTROL® Grade		PIPPS	
(N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic Acid, Na)		[Piperazine-N,N -bis(3-propanesulfonic Acid)]	
M.W. 260.3	100 g	M.W. 330.4	10 g
Cat. No. 391333	500 g	Cat. No. 528315	
	1 kg	Potassium Phosphate, Dibasic, Trihydrate,	
Imidazole, ULTROL® Grade		Molecular Biology Grade	
(1,3-Diaza-2,4-cyclopentadiene )		M.W. 228.2	250 g
M.W. 68.1	25 g	Cat. No. 529567	1 kg
Cat. No. 4015	100 g	Potassium Phosphate, Monobasic, Molecular	
		Biology Grade	
MES, Free Acid, ULTROL® Grade		M.W. 136.1	250 g
[2-(N-Morpholino)ethanesulfonic Acid]		Cat. No. 529568	1 kg
M.W. 195.2	100 g	Sodium Citrate, Dihydrate, Molecular Biology	
Cat. No. 475893	500 g	Grade	
	1 kg	M.W. 294.1	100 g
MES, Sodium Salt, ULTROL® Grade		Cat. No. 567446	1 kg
[2-(N-Morpholino)ethanesulfonic Acid, Na]			5 kg
M.W. 217.2	100 g	Sodium Phosphate, Dibasic	
Cat. No. 475894	1 kg	M.W. 142.0	500 g
		Cat. No. 567550	1 kg
MOPS, Free Acid, ULTROL® Grade		Sodium Phosphate, Dibasic, Molecular	
[3-(N-Morpholino)propanesulfonic Acid]		Biology Grade	
M.W. 209.3	100 g	M.W. 142.0	250 g
Cat. No. 475898	500 g	Cat. No. 567547	1 kg
	1 kg		

<b>Sodium Phosphate, Monobasic</b> M.W. 120.0 Cat. No. 567545	250 g 500 g 1 kg	<b>Triethylammonium Acetate, 1 M Solution</b> M.W. 161.2 Cat. No. 625718	1 liter
<b>Sodium Phosphate, Monobasic, Monohydrate, Molecular Biology Grade</b> M.W. 138.0 Cat. No. 567549	250 g 1 kg	<b>Tris Base, Molecular Biology Grade</b> [tris(Hydroxymethyl)aminomethane] M.W. 121.1 Cat. No. 648310	100 g 500 g 1 kg 2.5 kg
<b>SSC Buffer, 20X Powder Pack, ULTROL® Grade</b> Cat. No. 567780	2 pack	<b>Tris Base, ULTROL® Grade</b> [tris(Hydroxymethyl)aminomethane] M.W. 121.1 Cat. No. 648311	100 g 500 g 1 kg 5 kg 10 kg
<b>TAPS, ULTROL® Grade</b> (3-{{tris(Hydroxymethyl)methyl}amino}-propanesulfonic Acid) M.W. 243.2 Cat. No. 394675	100 g 1 kg		
<b>TBE Buffer, 10X Powder Pack, ULTROL® Grade</b> (10X Tris-Borate-EDTA Buffer) Cat. No. 574796	2 pack	<b>Tris Buffer, 1.0 M, pH 8.0, Molecular Biology Grade</b> M.W. 121.1 Cat. No. 648314	100 ml
<b>TES, Free Acid, ULTROL® Grade</b> (2-{{tris(Hydroxymethyl)methyl}amino}-ethanesulfonic Acid) M.W. 229.3 Cat. No. 39465	100 g 1 kg	<b>Tris Buffer, 100 mM, pH 7.4, Molecular Biology Grade</b> M.W. 121.1 Cat. No. 648315	100 ml
<b>Tricine, ULTROL® Grade</b> {N-{{tris(Hydroxymethyl)methyl}glycine} M.W. 179.2 Cat. No. 39468	100 g 1 kg	<b>Tris, Hydrochloride, Molecular Biology Grade</b> [tris(Hydroxymethyl)aminomethane, HCl] M.W. 157.6 Cat. No. 648317	100 ml 1 kg
<b>Triethanolamine, Hydrochloride*</b> M.W. 185.7 Cat. No. 641752	1 kg	<b>Tris, Hydrochloride, ULTROL® Grade</b> [tris(Hydroxymethyl)aminomethane, HCl] M.W. 157.6 Cat. No. 648313	250 g 500 g 1 kg

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