

**ANSC 619**  
**PHYSIOLOGICAL CHEMISTRY OF LIVESTOCK SPECIES**  
**SOLUTIONS AND BUFFERS**

A solution can be defined as a homogeneous mixture of two or more substances. By homogeneous, we ordinarily mean uniform to visual observation by eye or microscope. Sea water, filtered if necessary to remove seaweed, surf boards and sharks, is a solution of various salts in water.

In a gas-liquid or solid-liquid solution, we ordinarily refer to the liquid as the "solvent" and the other component (gas or solid) as the "solute". If both components are liquids, the designations solute and solvent are more ambiguous. Frequently, the component present in the greater amount is called the solvent. In a solution of 1 ml of ethanol in 100 ml of water, we would probably speak of the water as the solvent and the ethanol as the solute. If the amounts are more nearly equal, the choice becomes less clear-cut and must be specified.

Concentration units. The properties of solutions depend upon the relative amounts of solute and solvent present. Thus, it is important to specify concentrations. This can be done by stating the relative amount of solute and solvent, or stating the amount of one component relative to the mass or volume of solution.

**Molality** (*m*) is defined as the number of mol of solute per kilogram of solvent:

$$m = \frac{\text{no. of mol solute}}{\text{no. kg solvent}}$$

**Molarity** (*M*) Concentrations of reagents in biochemistry are most often specified in terms of **molarity**, which is defined as the number of mol of solute per liter of solution:

$$M = \frac{\text{no. mol solute}}{\text{no. of liters solution}}$$

**Normality** (in the biochemical, not sociological, sense) (*N*) is defined as the number of gram equivalent weights (GEW) of solute per liter of solution:

$$N = \frac{\text{no. of GEW solute}}{\text{no. of liters solution}}$$

For acids and bases, the GEW is defined as follows:

1 GEW acid = weight of acid which reacts with one mol of OH<sup>-</sup>

1 GEW base = weight of base which reacts with one mol of H<sup>+</sup>

By this definition:

1 GEW HCl = 36.5 g HCl = 1 mol HCl  
 1 GEW NaOH = 40 g NaOH = 1 mol NaOH  
 1 GEW H<sub>2</sub>SO<sub>4</sub> = 49 g H<sub>2</sub>SO<sub>4</sub> = 1/2 mol H<sub>2</sub>SO<sub>4</sub>

Thus, normality and molarity are related. For example,

1.0 N HCl = 1.0 M HCl  
 1.0 N NaOH = 1.0 M NaOH  
 1.0 N H<sub>2</sub>SO<sub>4</sub> = 0.5 M H<sub>2</sub>SO<sub>4</sub>

Metabolites and solutes within cells rarely are present in molar concentrations. Nor is it likely that you will observe cell volumes approaching one liter. Thus, it usually is more convenient to express concentrations as millimolar (mM) and micromol/mL (μmol/mL). To make this conversion, remember:

1 mol = 1,000 mmol  
 1 mmol = 1,000 μmol  
 1 mM = 1 mmol/liter = 1,000 μmol/1,000 mL = 1 μmol/mL

## pH AND BUFFERS

pH. For simplicity, and because of the use of relatively weak solutions in most biochemical settings, we will accept the following definition of pH:

pH is the logarithm of the reciprocal of the hydrogen ion concentration, i.e.,

$$\text{pH} = \log 1/[\text{H}^+]$$

What this tells us in an operational sense is that we may ignore all other ions (such as OH<sup>-</sup>) for considerations of pH in dilute solutions. It also implies that the only species in solution that contributes to pH is the hydrogen ion (H<sup>+</sup>, i.e., a proton).

The importance of this is apparent when we consider the contribution of a weak acid to the pH of a solution. By definition a strong acid is one that dissociates completely:

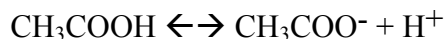


so that the concentration of hydrogen ions in a solution of HCl is equal to the concentration of HCl added to the solution (i.e., no HCl will be present, only H<sup>+</sup> and Cl<sup>-</sup>).

**Example:** What is the pH of a 1 M HCl solution?

$$\begin{aligned} \text{pH} &= \log 1/[\text{H}^+] \\ \text{pH} &= \log 1/1 \\ \text{pH} &= 0, \text{ because the log of } 1 = 0 \end{aligned}$$

A weak acid, conversely, is one that dissociates only sparingly. In fact, for the dissociation of acetic acid,



equilibrium lies far to the left, so that very little acetic acid exists in the acetate form. This is an important concept, because **only the  $\text{H}^+$  ion contributes to the pH, not  $\text{CH}_3\text{COOH}$** . Also, **we cannot predict  $[\text{H}^+]$  based solely on the concentration of acetic acid in solution**.

### The Henderson-Hasselbach equation

Acetate ( $\text{CH}_3\text{COO}^-$ ) is commonly referred to as a **salt**. Thus, the equilibrium solution indicated above consists of a weak acid plus its salt; **this is one definition of a buffer**. For practical purposes, we will use the Bronsted definition of acids and salts: an acid is something that can donate a proton, and a salt is something that can accept a proton.

**Buffers.** The degree to which a weak acid dissociates tells us a great deal about the characteristics of a buffer (a weak acid plus its salt). Because buffer solutions rapidly reach equilibrium, the dissociation (and reassociation) of the acid can be described by a **dissociation constant**. **This is equivalent to the equilibrium constant for all chemical reactions.**

For acetic acid, this constant is

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

Taking the reciprocal of both sides of the equation, we obtain

$$1/K_a = [\text{CH}_3\text{COOH}]/[\text{H}^+][\text{CH}_3\text{COO}^-]$$

or

$$1/K_a = 1/[\text{H}^+] * [\text{CH}_3\text{COOH}]/[\text{CH}_3\text{COO}^-]$$

Taking the logarithm of both sides, we have

$$1/K_a = \log 1/[\text{H}^+] + \log[\text{CH}_3\text{COOH}]/[\text{CH}_3\text{COO}^-]$$

From the definition of pH, you know that  $\log 1/[\text{H}^+] = \text{pH}$ . By analogy, we define

$$\log 1/K_a = \text{p}K_a = -\log K_a$$

Thus,

$$\text{p}K_a = \text{pH} + \log [\text{CH}_3\text{COOH}]/[\text{CH}_3\text{COO}^-]$$

or

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

or

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

or

$$\text{pH} = \text{p}K_a + \log \frac{[\text{salt}]}{[\text{acid}]}$$

This is the Henderson-Hasselbalch equation, and it leads us to a definition of  $\text{p}K_a$ : **the  $\text{p}K_a$  is that pH at which the concentration of salt equals the concentration of acid in a solution.** That is

$$\text{pH} = \text{p}K_a + \log \frac{1}{1}$$

Because  $\log 1 = 0$ ,  $\text{pH} = \text{p}K_a$  under these conditions.

## **BUFFER SYSTEMS IN ANIMALS: The bicarbonate buffer system**

### **I. Role of buffers in compensating for pH changes**

A.  $\text{pH} = \text{p}K_a + \log \frac{[\text{salt}]}{[\text{acid}]}$

B. Major blood buffers

1. Bicarbonate:  $\text{p}K_a = 6.1$
2. Phosphate:  $\text{p}K_a = 6.7$
3. Proteins:  $\text{p}K_a$  range from 2 to 12
  - a. Especially important is hemoglobin.
  - b. Histidine has variable  $\text{p}K_a$ ;  $6.2 \rightarrow 7.7$

C. Buffers function as a team.

1. Any change in pH is reflected in adjustments of  $[\text{S}]/[\text{A}]$  ratio for all buffers.
2. Change in ratio is determined by each buffer's  $\text{p}K_a$ .

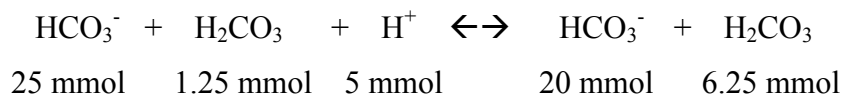
D. Adding 5 meq of  $\text{H}^+$  to the blood (e.g., from lactic acid, acetoacetic acid, acetic acid)

1. Blood contains 26.25 meq/L (i.e., 26.25 mmol) of total carbonate
2. This is distributed in the ratio of 20/1 between bicarbonate and carbonic acid.

- a.  $7.4 = 6.1 + \log [S]/[A]$
- b.  $1.3 = \log [S]/[A]$
- c.  $20/1 = [S]/[A]$
- d.  $[S] + [A] = 26.25$
- e.  $[S] = 20[A]$  (from c)
- f.  $20[A] + [A] = 26.25$  (i.e.,  $21[A] = 26.25$ )
- g.  $[A] = 1.25 \text{ mmol/L}$  ( $26.25/21 = 1.25$ )
- h.  $[S] = 20(1.25) = 25 \text{ mmol/L}$

E. After adding 5 meq  $H^+$ /L, the ratio would be predicted to change and the pH would decrease:

1. Reaction



- a.  $pH = 6.1 + \log 20/6.25$
  - b.  $pH = 6.1 + \log 3.2$
  - c.  $pH = 6.1 + 0.505$
  - d.  $pH = 6.6$
2. This is very acidic, but the actual pH change is minimal because:
- a. The lungs attenuate the pH change by removing  $CO_2$ .



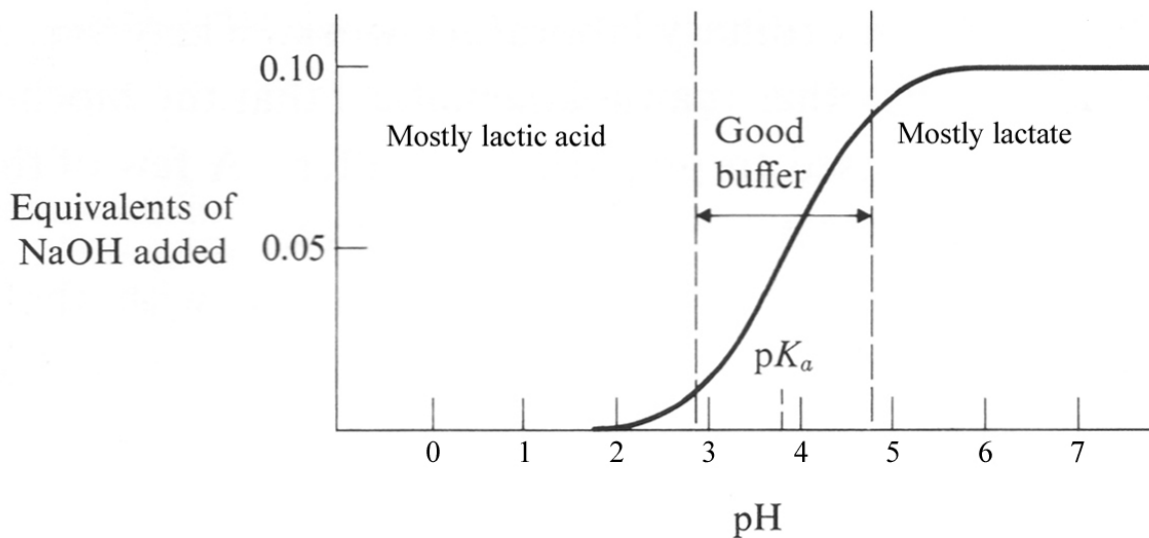
The  $H_2O$  is exhaled, effectively removing a source of acid from the circulatory system.

- b. Other buffers in the blood, especially phosphate and proteins, provide additional buffer capacity, maintaining pH within a narrow range.

## II. Relationship of $pK_a$ to buffering capacity

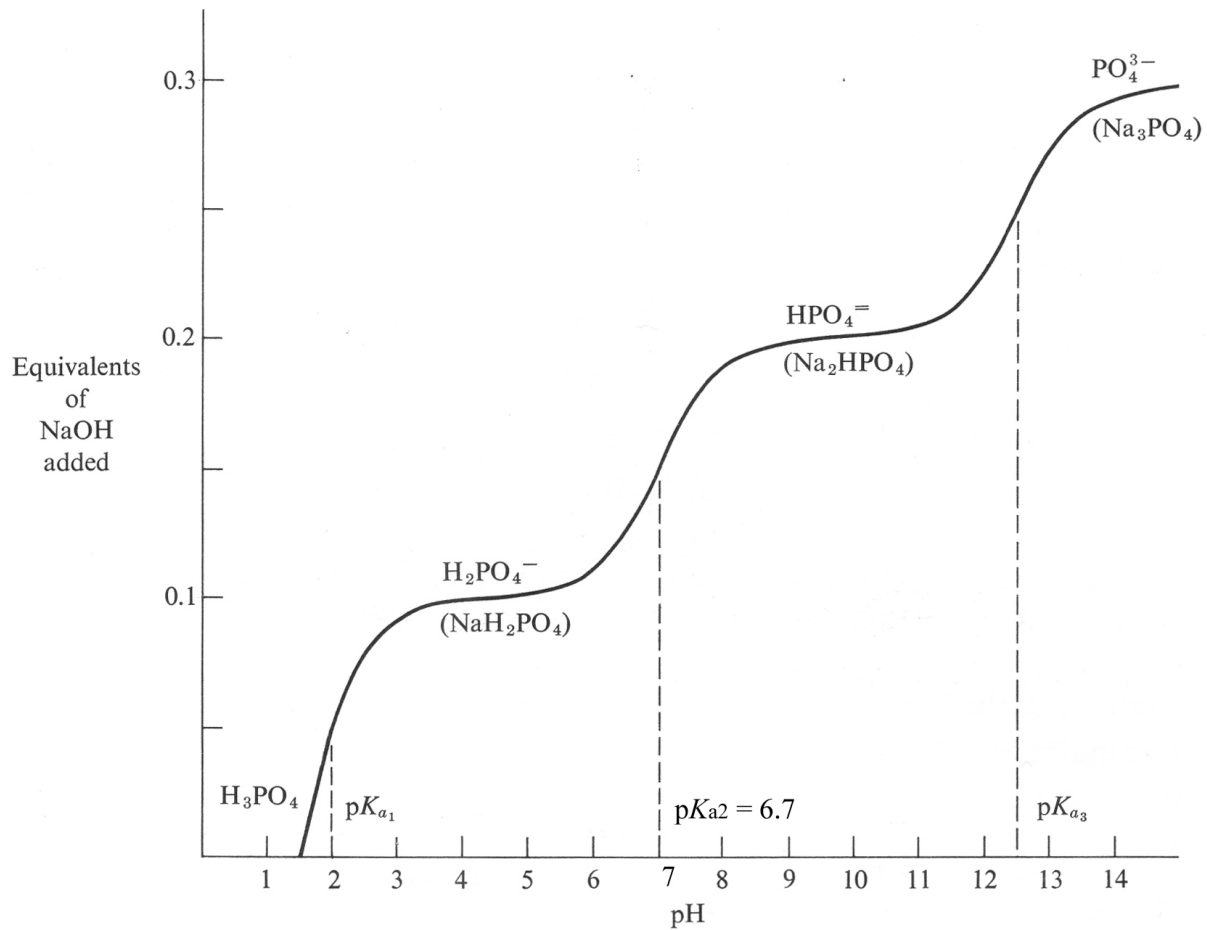
### A. Is lactic acid a good buffer?

1. The concentration of lactate in blood ranges from 0.5 to 2.2 mM (2.2 mmol/L), so there is not sufficient lactate to serve as a buffer.
2. Also, the  $pK_a$  of lactic acid is too low (3.86), so it would only be a good buffer around pH 4.
3. **The best buffers have  $pK_a$ s near the desired final pH.**



Titration of lactic acid with NaOH

B. Is phosphoric acid a good buffer?



**6-3** Titration of 1 liter of 0.1 M  $\text{H}_3\text{PO}_4$  with  $\text{NaOH}$ .

1. The  $\text{pK}_{a2}$  for  $\text{H}_2\text{PO}_4^- \rightarrow \text{HPO}_4^{=}$  = 6.7, so it would have strong buffering capacity at blood pH (7.4).
2. However, the concentration of phosphates in plasma is only 1.12 to 1.45 mmol/L, so it does not have sufficient **buffering capacity** by itself.

Take home message: **Buffers do not prevent pH change; they only minimize pH change.**