Model Carbonate Blood Buffer

Introduction

The pH level in blood must be maintained within extremely narrow limits. What is the composition of the major buffer in blood?

Concepts

- Buffer
- Conjugate base
- Weak acid

Background

All buffers contain a mixture of a weak acid (HA) and its conjugate base (A^-). The buffer components HA and A^- are related to each other by means of the following chemical reaction (Equation 1).

$$\underset{\text{weak acid}}{HA} + H_2O \rightleftarrows \underset{\text{conjugate base}}{A^-} + H_3O^+ \qquad \textit{Equation 1}$$

Buffers control pH because the two buffer components react with and neutralize either strong acid or strong base that might be added. The weak acid HA reacts with any strong base, such as sodium hydroxide (NaOH), to give water and the conjugate base A^- (Equation 2). The conjugate base A^- reacts with any strong acid, such as hydrochloric acid (HCl), to give its acid partner HA (Equation 3).

$$HA + NaOH \rightleftarrows Na^+ + A^- + H_2O \qquad \textit{Equation 2}$$

$$A^- + HCl \rightleftarrows HA + Cl^- \qquad \textit{Equation 3}$$

These neutralization reactions can be visualized as a cyclic process (Figure 1). Buffer activity will continue as long as both components are present. The pH range in which a buffer system will be effective is called its *buffer range*. The buffer range is 2 pH units centered around the pK_a value of the weak acid.

The major buffer in blood is composed of carbonic acid (H_2CO_3) and its conjugate base, bicarbonate ion (HCO_3^-) (Equation 4). A carbonic acid–bicarbonate buffer has a buffer range of pH 5.4–7.4. The normal pH of blood (7.2) is at the upper limit of the effective range for the carbonic acid–bicarbonate buffer system. The buffer activity is kept in balance by a reserve supply of gaseous CO_2 in the lungs, which can replenish H_2CO_3 in the blood (Equation 5).

$$H_2CO_3 + H_2O \rightleftarrows HCO_3^- + H_3O^+ \qquad \textit{Equation 4}$$

$$\underset{\text{lungs}}{CO_2(g)} \rightleftarrows \underset{\text{blood}}{CO_2(aq)} + H_2O \rightleftarrows H_2CO_3 \qquad \textit{Eauation 5}$$

In this activity, seltzer will be used as a source of carbonic acid to prepare a model biological buffer. The effects of added acid and base on the pH and the buffer capacity of the model buffer will be examined. The pH of the buffer will be estimated using bromthymol blue, an acid–base indicator that is yellow when the pH is less than 6.0, blue when the pH is greater than 7.6, and green (an intermediate color) in the transition range 6.0–7.6.

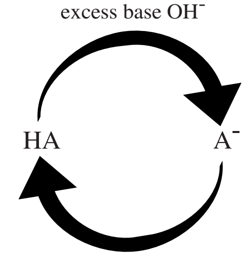

Figure 1.

Materials (for one student group)

- Bromthymol blue indicator solution, 0.04%, 3 mL
- Hydrochloric acid, HCl, 0.1 M, 3 mL
- Seltzer water, H_2CO_3, 8 mL
- Sodium bicarbonate solution, $NaHCO_3$, 0.1 M, 8 mL
- Sodium hydroxide solution, NaOH, 0.1 M, 3 mL
- Water, distilled or deionized
- Beral-type pipets, graduated, 3
- Graduated cylinders, 10-mL, 2
- pH paper, narrow range, 6.0–8.0
- Test tubes (medium), 6
- Test-tube rack

Safety Precautions

Dilute solutions of sodium bicarbonate, hydrochloric acid and sodium hydroxide are body tissue irritants. Avoid exposure to eyes and skin. Wear chemical splash goggles and chemical-resistant gloves and apron. Consult current Safety Data Sheets for additional safety, handling, and disposal information.

Procedure

1. Set up six medium-size test tubes in a rack. Label them 1–6.
2. Use a graduated cylinder to measure and add the indicated volumes of the following solutions to each test tube.

Test Tube Number	**1**	**2**	**3**	**4**	**5**	**6**
Solution	Carbonic Acid (Reference)	Model Carbonate Blood Buffer	Water (Control)	Model Carbonate Blood Buffer	Water (Control)	Sodium Bicarbonate (Reference)
Seltzer water (mL)	4	2	0	2	0	0
Sodium bicarbonate (mL)	0	2	0	2	0	4
Distilled water (mL)	0	0	4	0	4	0

3. Thoroughly mix the contents of each test tube by gentle shaking or swirling.
4. Add 5 drops of bromthymol blue to each test tube and shake to mix. Record the initial color of each solution 1–6.
5. Measure the initial pH of each solution using a piece of narrow-range pH paper, pH 6.0–8.0. Record the results.
6. Using a Beral-type pipet, add 0.1 M hydrochloric acid solution *dropwise* to the model carbonate blood buffer in test tube 2. Swirl the contents after each drop to ensure thorough mixing.
7. Count and record the number of drops of HCl required to change the color to the same shade as the carbonic acid reference solution in test tube 1.
8. Add 0.1 M hydrochloric acid solution *dropwise* to the water (control) in test tube 3. Count and record the number of drops of HCl required to change the color to that of the carbonic acid reference solution in test tube 1.
9. Using a different Beral-type pipet, add 0.1 M sodium hydroxide solution *dropwise* to the model carbonate blood buffer in test tube 4. Swirl the contents after each drop to ensure thorough mixing.
10. Count and record the number of drops of NaOH required to change the color to the same shade as the sodium bicarbonate reference solution in test tube 6.
11. Add 0.1 M sodium hydroxide solution *dropwise* to the water (control) in test tube 5. Count and record the number of drops of NaOH required to change the color to that of the sodium bicarbonate reference solution in test tube 6.

Discussion Questions

1. Compare the measured pH value for the model carbonate blood buffer to (a) the expected pH of an ideal carbonic acid–bicarbonate buffer, and (b) the actual pH of blood.
2. Based on the pH comparisons in Question #1, which solution, the model carbonate blood buffer or an actual blood buffer, is more likely to contain a greater proportion of carbonic acid compared to bicarbonate? Explain.
3. What is the effect of adding even 1 drop of HCl or NaOH on the pH value of the control (water)? Compare this to the effect of adding HCl or NaOH to the model carbonate blood buffer.

Disposal

Consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. All solutions can be rinsed down the drain with excess water according to Flinn Scientific Disposal Method #26b.

Sample Data *(Student data will vary.)*

Test Tube Number	1	2	3	4	5	6
Solution	Carbonic Acid (Reference)	Model Carbonate Blood Buffer	Water (Control)	Model Carbonate Blood Buffer	Water (Control)	Sodium Bicarbonate (Reference)
Bromthymol blue indicator color	yellow	green	teal green	green	teal green	blue
Initial pH value	< 6.0	6.8–7.0	7.0–7.2	6.8–7.0	7.0–7.2	> 8.0
Number of drops of HCl to convert solutions 2 and 3 to acid reference color	NA	35–40 drops	1 drop	NA	NA	NA
Number of drops of NaOH to convert solutions 4 and 5 to bicarbonate reference color	NA	NA	NA	15–20 drops	1 drop	NA

Answers to Discussion Questions *(Student answers will vary.)*

1. *The model carbonate blood buffer has a pH value equal to 6.8–7.0. This is greater than the pH of an ideal carbonic acid–bicarbonate buffer (6.4). The pH of the model blood buffersimilar to the actual pH of blood, which is regulated at pH = 7.2 ±0.2.*

2. *The pH of the model carbonate blood buffer indicates that it is more acidic than the actual buffer present in blood. Therefore, it is more likely that the model buffer contains a greater amount of the weak acid component relative to the bicarbonate (conjugate base) component.*

3. *The pH of water was dramatically affected by the addition of even one drop of strong acid or strong base. Addition of 1 drop of HCl was sufficient to decrease the pH to the "acid" color (pH <6). In contrast, the buffer solution was approximately 35 times more resistant to pH change, since 35–40 drops of HCl were necessary to change the pH of the buffer solution to the acid color. The model carbonate blood buffer was not quite as resistant to the effect of NaOH as it was to the effect of HCl. The buffer capacity with respect to NaOH addition, however, was still 15–20 times greater than that of water.*

Materials for *Model Carbonate Blood Buffer* are available from Flinn Scientific, Inc.

Catalog No.	Description
B0173	Bromthymol Blue indicator Solution, 100 mL
AP335	Hydrion Narrow Range pH Paper, 6.0–8.0

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.