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### The Control of pH and Total Alkalinity or Total Carbonate in Aquatic Bioassays

The concentrations of carbonate, bicarbonate, and carbon dioxide in an aquatic system are of importance because of their roles in pH control, complexation of metals, and control of metal solubility, and because of the toxicity of carbon dioxide to fishes. Few investigators have attempted to independently control the carbonate system and pH in bioassays of toxic metal ions even though these two variables regulate the chemical species of the metal used in the bioassay. This report presents a simple method for the control of total carbonate or total alkalinity and pH in synthetic or natural water systems.

The formation of significant quantities of metal carbanato or hydroxy complexes will affect the chemical relationships presented. The amount of base and the partial pressure of carbon dioxide required to establish a desired set of conditions can be predicted if the appropriate equilibrium constants in the equations presented are included.

#### EQUILIBRIUM RELATIONSHIPS OF TOTAL ALKALINITY AND pH

The total alkalinity of a sample is defined as its acid neutralizing capacity (Stumm and Morgan, 1970). This can be expressed as

$$\text{Total Alkalinity} = 2[\text{CO}_3^{2-}] + [\text{HCO}_3^-] + [\text{OH}^-] - [\text{H}^+] \quad (1)$$

where the brackets indicate concentrations and the alkalinity is expressed in equivalents per liter. For a sample exposed to the atmosphere the concentrations of carbonate and bicarbonate ions are related to the partial pressure of carbon dioxide ( $P_{\text{CO}_2}$ ) by the following relationships:

$$[\text{CO}_3^{2-}] = \frac{10^{-18.15} P_{\text{CO}_2}}{[\text{H}^+]^2} \quad \dots (2)$$

and

$$[\text{HCO}_3^-] = \frac{10^{-7.82} P_{\text{CO}_2}}{[\text{H}^+]} \quad \dots (3)$$

By substituting these relationships in equation 1, one obtains the following equation:

$$[\text{Total Alkalinity}] = \frac{10^{-17.85} P_{\text{CO}_2}}{[\text{H}^+]^2} + \frac{10^{-7.82} P_{\text{CO}_2}}{[\text{H}^+]} + \frac{10^{-14.00}}{[\text{H}^+]} - [\text{H}^+] \quad (4)$$

In this expression for total alkalinity the pH and partial pressure are the variables, but this expression will always equal zero unless a base is added to the solution. This can best be shown by an example.

In a solution of sodium bicarbonate, sodium carbonate, or sodium hydroxide, the following anion-cation balance will hold:

$$[\text{Na}^+] + [\text{H}^+] = 2[\text{CO}_3^{2-}] + [\text{HCO}_3^-] + [\text{OH}^-] \quad \dots (5)$$

Rearrangement gives:

$$[\text{Total Alkalinity}] = [\text{Na}^+] = 2[\text{CO}_3^{2-}] + [\text{HCO}_3^-] + [\text{OH}^-] - [\text{H}^+] \quad (6)$$

The total alkalinity is equal to the amount of base in solution and will equal zero if no base is present.

We can now substitute the sodium ion concentration for total alkalinity in equation 4 which on rearrangement yields an expression for the partial pressure of carbon dioxide as a function of the total alkalinity (or sodium ion concentration) and the pH:

$$P_{\text{CO}_2} = \frac{([\text{Na}^+][\text{H}^+] + [\text{H}^+]^2 - 10^{-14.00})[\text{H}^+]}{10^{-17.85} + 10^{-7.82}[\text{H}^+]} \quad (7)$$

This is an important equation because it shows that the  $[H^+]$  can be controlled by the partial pressure of carbon dioxide and the amount of base added to the solution. Both of these variables can be controlled by the investigator.

EQUILIBRIUM RELATIONSHIPS OF TOTAL CARBONATE AND pH

The total carbonate,  $[CO_3^{2-}] + [HCO_3^-] + [H_2CO_3]$ , is also controlled by the partial pressure of carbon dioxide and the pH according to the following relationship:

$$[\text{Total Carbonate}] = \frac{10^{-18.15} P_{CO_2}}{[H^+]^2} + \frac{10^{-7.82} P_{CO_2}}{[H^+]} + 10^{-1.47} P_{CO_2} \dots (8)$$

where  $[H_2CO_3] = 10^{-1.47} P_{CO_2} \dots (9)$

Solving equation 8 for the partial pressure of carbon dioxide

$$P_{CO_2} = \frac{[\text{Total Carbonate}][H^+]^2}{10^{-18.15} + 10^{-7.82}[H^+] + 10^{-1.47}[H^+]^2} (10)$$

one obtains an expression which can be used to calculate the necessary partial pressure of carbon dioxide for any desired pH and total carbonate concentration. The concentration of sodium ion necessary to establish the desired conditions is given by equation 7 which can be rewritten

$$[Na^+] = \frac{(10^{-17.85} + 10^{-7.82}[H^+])P_{CO_2}}{[H^+]^2} - \frac{[H^+]^2 - 10^{-14.00}}{[H^+]} \dots (11)$$

Addition of  $NaHCO_3$ ,  $Na_2CO_3$ , or  $NaOH$  is necessary so that the total carbonate and pH may be independently varied.

CALCULATION PROCEDURES FOR SYNTHETIC WATERS

Case I: Total Alkalinity and pH Control.

The procedure for controlling total alkalinity and pH in solutions made from distilled or deionized water is very simple:

- 1) Decide what total alkalinity is desired and add that molar

concentration of  $NaHCO_3$ ,  $NaOH$ , or half as much  $Na_2CO_3$  to the solution.

- 2) Mix carbon dioxide with air or a nitrogen-oxygen mixture, and bubble the final mixture through the solution, varying the amount of carbon dioxide until the desired pH is obtained and maintained in equilibrium.

The pH may be varied simply by adjustment of the amount of carbon dioxide in the mixture, and the alkalinity will remain constant at any pH.

Case II: Total Carbonate and pH Control.

The control of total carbonate and pH involves the following calculation:

- 1) Calculate the amount of  $NaHCO_3$ ,  $NaOH$ , or  $Na_2CO_3$  necessary for the desired total carbonate and pH from the following equation which can be derived from equations 10 and 11.

$$[Na^+] = \frac{[\text{Total Carbonate}] \times (10^{-17.85} + 10^{-7.82}[H^+])}{10^{-18.15} + 10^{-7.82}[H^+] + 10^{-1.47}[H^+]^2} - \frac{[H^+]^2 - 10^{-14.00}}{[H^+]} \dots (12)$$

- 2) After the addition of the sodium ion, the pH is adjusted with a mixture of carbon dioxide and air or a nitrogen-oxygen mixture as described in the total alkalinity case. Once the desired pH has been obtained, the total carbonate concentration should also be at the desired level. These calculations and additions of base must be done for each pH and total carbonate concentration.

CALCULATION PROCEDURES FOR NATURAL  
WATERS

Case III: Total Alkalinity and pH Control.

Natural waters have an inherent total alkalinity and just as in a solution of  $\text{NaHCO}_3$ , the total alkalinity will be equal to the concentration of basic material dissolved in it.

Taking the same example as before, a sodium bicarbonate solution, we find

$$[\text{Total Alkalinity}] = [\text{Na}^+] \quad . \quad . \quad (6)$$

if we add a monoprotic acid (HA) to this solution, rearrangement of the charge balance equation would give

$$\begin{aligned} [\text{Total Alkalinity}] &= [\text{Na}^+] - [\text{A}^-] \\ &= 2[\text{CO}_3^{2-}] + [\text{HCO}_3^-] \\ &\quad + [\text{OH}^-] - [\text{H}^+] \quad . \quad . \quad (13) \end{aligned}$$

This example shows that we can adjust the total alkalinity of a natural water to any value desired by measuring its native total alkalinity and adding a base to increase it or an acid to decrease it.

The procedure would be:

- 1) Determine the total alkalinity of the natural water being used (APHA, 1971).
- 2) If the desired total alkalinity is greater than the measured value, the amount of  $\text{NaHCO}_3$ ,  $\text{Na}_2\text{CO}_3$ , or  $\text{NaOH}$  that should be added to the solution would be:

$$[\text{Na}^+] = [\text{Desired Total Alkalinity}] - [\text{Measured Total Alkalinity}] \quad . \quad (14)$$

- 3) If the desired total alkalinity is less than the measured value, the amount of monoprotic acid to add would be:

$$\begin{aligned} [\text{HA}] &= \\ &[\text{Measured Total Alkalinity}] \\ &- [\text{Desired Total Alkalinity}] \quad . \quad . \quad (15) \end{aligned}$$

- 4) The investigator can control the pH by adjusting the partial pressure of carbon dioxide without altering the total alkalinity.

Case IV: Total Carbonate and pH Control.

- 1) Calculate the amount of  $\text{NaHCO}_3$ ,  $\text{NaOH}$ ,  $\text{Na}_2\text{CO}_3$  necessary for the desired total carbonate and pH from equation 12.
- 2) Determine the total alkalinity of the natural water (APHA, 1971).
- 3) If the calculated  $[\text{Na}^+]$  is less than the measured total alkalinity, the amount of monoprotic acid to add is:

$$[\text{HA}] = [\text{Measured Total Alkalinity}] - [\text{Calculated } [\text{Na}^+]] \quad . \quad . \quad . \quad (16)$$

- 4) If the calculated  $[\text{Na}^+]$  is greater than the measured total alkalinity, the amount of  $\text{NaHCO}_3$ ,  $\text{Na}_2\text{CO}_3$ , or  $\text{NaOH}$  to add is:

$$[\text{Na}^+] = [\text{Calculated } [\text{Na}^+]] - [\text{Measured Total Alkalinity}] \quad . \quad (17)$$

- 5) Adjust the pH to the designated value using the appropriate  $\text{CO}_2$ -gas mixture.

These calculations must be repeated for any change in desired pH or total carbonate concentration.

COMPARISON OF TOTAL ALKALINITY AND  
TOTAL CARBONATE METHODS

The concentration of sodium ion and the partial pressure of carbon dioxide necessary for establishing a  $10^{-3}$  molar total carbonate solution and  $10^{-3}$  equivalents/liter total alkalinity solution are shown in Figure 1. While the required partial pressure of carbon dioxide is essentially the same for control of either total alkalinity or total carbonate at high pH, the divergence at lower pH is due to the formation of a significant concentration of free carbon dioxide which is included in the ex-

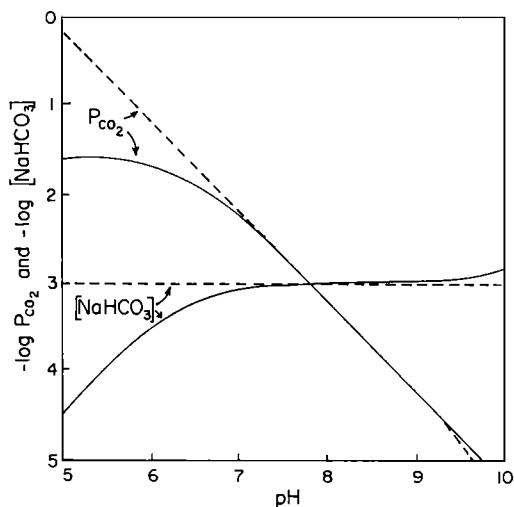


FIGURE 1.—Partial pressure of carbon dioxide and mole/liter sodium bicarbonate required to maintain total carbonate =  $10^{-3}$  molar (solid line) or total alkalinity =  $10^{-3}$  equivalents/liter (dashed lines).

pression for total carbonate but not for total alkalinity. Similarly, while the required sodium ion in the total alkalinity case is constant over the entire pH range and is nearly the same as required for the control of total carbonate at high pH, the values diverge at low pH.

#### EXPERIMENTAL APPARATUS

In the authors' laboratory a system is presently being used to simultaneously control the pH and total carbonate in six 250-gallon tanks used as reservoirs for continuous flow aquatic bioassays. The system has been operated over the pH range of 5.5–8.0 with a mixture of carbon dioxide and air. The required concentration of sodium ion is added to the water in the form of sodium bicarbonate and the ratio of gases adjusted manually until the desired equilibrium pH is achieved. Gas flows are monitored by flowmeters and the gases are mixed in a 1 ft  $\times$  1 ft  $\times$  3 ft baffled airtight mixing chamber. Aeration of the tanks is achieved by bubbling the gas mixture through large aquarium stones; the desired equilibrium pH can be adjusted and obtained in less than 3 hours. Once the controls for the required gas mixture have been set, daily checking and minor adjustments are

sometimes required. The system maintains pH to less than  $\pm 0.1$  units. At pH 5.5 and a total carbonate of  $10^{-3}$  M, less than one cylinder of carbon dioxide is consumed in a week for the 1500 gallons of water.

#### LIMITATIONS

While it is theoretically possible to achieve any pH and total alkalinity or total carbonate by this procedure, practical restraints limit its usable range. At low pH's and high total alkalinity or total carbonate, high concentrations of free carbon dioxide may be lethal to fish. At high pH's the high ionic strength present in the control of total carbonate may prove deleterious; also it may be difficult in practice to control the low carbon dioxide pressure required to maintain a high pH. These problems should not develop in the control of bioassay chemical conditions because extreme values of pH and total alkalinity or total carbonate would not be desired.

Generally, the predominant factor in controlling pH and alkalinity is the carbonate system. However, certain bioassay conditions may significantly alter the total alkalinity, total carbonate, and pH levels. For example, high hardness, alkalinity, and pH may cause carbonate precipitation and reduce the total alkalinity. High concentrations of metal toxicants such as lead may also result in the formation of metal hydroxides which would lower the alkalinity. These conditions can be recognized if the pH obtained is significantly lower than the predicted value.

#### CONCLUSIONS

We have employed basic chemical equilibrium concepts to develop a method for precise control of pH and total alkalinity or total carbonate for use in bioassay studies. By precision control of these variables along with control of other variables, e.g., temperature, hardness, dissolved oxygen, and dissolved organics, determination of the quantitative effects of these variables on the toxicity of heavy metals is possible.

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