

## Characterizing a TRIS Buffer

When microorganisms reproduce they release waste products that may change the pH of their environment in a manner that prevents their further reproduction. This can present a problem in biology labs where microorganisms are grown in the closed environment of a culture dish. Culture media, therefore, are usually buffered. One common buffer is made using the weak base tris(hydroxymethyl)aminomethane, which is more commonly called TRIS, and its conjugate weak acid, TRISH<sup>+</sup>. Relevant information about this buffering system is provided here:

TRIS is (HOCH<sub>2</sub>)<sub>3</sub>CNH<sub>2</sub> and has a  $K_b$  of  $1.19 \times 10^{-6}$

TRISH<sup>+</sup> is (HOCH<sub>2</sub>)<sub>3</sub>CNH<sub>3</sub><sup>+</sup> and is available as the salt (HOCH<sub>2</sub>)<sub>3</sub>CNH<sub>3</sub>Cl

1. What range of pH values are possible for buffers made using TRIS and TRISH<sup>+</sup>?

A buffer's optimal pH range is defined as  $\text{pH} = \text{p}K_a \pm 1$ . To define the range, therefore, we need to know the  $\text{p}K_a$  for TRISH<sup>+</sup>. We know that for a conjugate weak acid/weak base pair, the following relationships apply

$$K_a \times K_b = 1.00 \times 10^{-14} \text{ or } \text{p}K_a + \text{p}K_b = 14$$

The  $\text{p}K_b$  for TRIS is  $-\log(1.19 \times 10^{-6})$  or 5.92. The  $\text{p}K_a$  for TRISH<sup>+</sup>, therefore, is 8.08 and the optimal pH range is 7.1 – 9.1.

2. At what pH will a TRIS/TRISH<sup>+</sup> buffer have its greatest capacity for neutralizing against the addition of strong acid?

The greatest buffering capacity against the addition of strong acid is when the buffer is at its most basic limit; that is, at a pH of 9.1. The buffer under these conditions has  $10 \times$  as much TRIS as TRISH<sup>+</sup> and has, therefore, as much weak base as possible to neutralize the addition of a strong acid. This buffer, of course, has essentially no capacity to neutralize any added strong base.

3. Suppose you want to make a TRIS/TRISH<sup>+</sup> buffer with a pH of 9.00 and that the equilibrium concentration of TRIS needs to be 0.100 M. What concentration of TRISH<sup>+</sup> will you need?

We can solve for this using the Henderson-Hasselbalch equation; thus

$$\text{pH} = \text{p}K_a + \log \frac{[\text{TRIS}]}{[\text{TRISH}^+]}$$

$$9.00 = 8.08 + \log \frac{0.100}{[\text{TRISH}^+]}$$

$$0.92 = \log \frac{0.100}{[\text{TRISH}^+]}$$

$$\frac{0.100}{[\text{TRISH}^+]} = 8.32$$

which leaves us with  $[\text{TRISH}^+] = 0.0120 \text{ M}$

4. A TRIS/TRISH<sup>+</sup> buffer is prepared by dissolving 50.0 g of TRIS and 65.0 g of TRIS•HCl in deionized water and diluting to 2.00 L. What is the pH of this buffer?

The moles of TRIS and moles of TRISH<sup>+</sup> are, respectively

$$50.0 \text{ g TRIS} \times \frac{1 \text{ mol TRIS}}{121.138 \text{ g}} = 0.4128 \text{ mol TRIS}$$

$$65.0 \text{ g TRIS} \cdot \text{HCl} \times \frac{1 \text{ mol TRIS} \cdot \text{HCl}}{157.559 \text{ g}} \times \frac{1 \text{ mol TRISH}^+}{\text{mol TRIS} \cdot \text{HCl}} = 0.4125 \text{ mol TRISH}^+$$

The pH of the buffer, therefore, is

$$\text{pH} = \text{pK}_a + \log \frac{(\text{moles TRIS})_0}{(\text{moles TRISH}^+)_0} = 8.08 + \log \frac{0.4128}{0.4125} = 8.08$$

5. What is the pH after adding 0.5 mL of 12.0 M HCl to a 200.0-mL portion of the buffer from Problem 4?

Adding 0.5 mL of 12.0 M HCl is the same as adding 0.006 mol of H<sub>3</sub>O<sup>+</sup>. Since this is being added to only 10% of the solution from Problem 4, the original 200.0-mL portion of the buffer contains 0.04128 mol TRIS and 0.04125 mol TRISH<sup>+</sup>. The pH after adding the HCl is

$$\text{pH} = \text{pK}_a + \log \frac{\text{mol TRIS} - \text{mol H}_3\text{O}^+}{\text{mol TRISH}^+ + \text{mol H}_3\text{O}^+} = 8.08 + \log \frac{0.04128 - 0.006}{0.04125 + 0.006} = 7.95$$

As expected, adding a strong acid makes the buffer slightly more acidic.

6. What is the buffer capacity against the addition of strong base for a 200.0-mL portion of the buffer from Problem 4? Express your answer in terms of the maximum volume, in mL, of 6.0 M NaOH that can be added?

To find the buffer capacity against the addition of strong base, we look for the amount of OH<sup>-</sup> needed to reach a 10:1 ratio for TRIS:TRISH<sup>+</sup>. As in the previous problem, the initial moles of TRIS and TRISH<sup>+</sup> are, respectively, 0.04128 and 0.0412; thus

$$\frac{\text{mol TRIS} + \text{mol OH}^-}{\text{mol TRISH}^+ - \text{mol OH}^-} = 10 = \frac{0.04128 + \text{mol OH}^-}{0.0412 - \text{mol OH}^-}$$

Solving, gives

$$0.04128 + \text{mol OH}^- = 0.412 - 10 \times \text{mol OH}^-$$

$$11 \times \text{mol OH}^- = 0.37072$$

$$\text{mol OH}^- = 0.0337 \text{ mol OH}^-$$

This amount of NaOH is equivalent to

$$0.0337 \text{ mol NaOH} \times \frac{1 \text{ L}}{6.0 \text{ M NaOH}} \times \frac{1000 \text{ mL}}{\text{L}} = 5.6 \text{ mL}$$