

20.3: BIOLOGICAL ACIDS AND THE HENDERSON-HASSELBALCH EQUATION

OBJECTIVES

After completing this section, you should be able to

1. identify the form that carboxylic acids take within living cells.
2. use the Henderson-Hasselbalch equation to calculate the percentages of dissociated and undissociated acids $[A^-]$ and $[HA]$ in a solution, given the pK_a value of the acid and the pH of the solution.
3. explain why cellular carboxylic acids are always referred to by the name of their anion.

KEY TERMS

Make certain that you can define, and use in context, the key terms below.

- Henderson-Hasselbalch equation
- physiological pH

CARBOXYLIC ACIDS IN BUFFERED SOLUTIONS

Due to the acidic nature of carboxylic acids, their level of dissociation is directly related to the pH of the solution. Under acidic conditions (low pH), carboxylic acids are almost completely undissociated and exist primarily in their protonated form ($RCOOH$). Under, basic conditions (high pH), carboxylic acids become nearly completely dissociated into their deprotonated carboxylate form ($RCOO^-$). Because the environment inside a living cell is an aqueous buffer with a nearly neutral pH = 7.3 (sometimes referred to as the **physiological pH**), then in what form do carboxylic acids exist? An important part of answering this question lies in the **Henderson-Hasselbalch equation**:

$$pH = pK_a + \log \left(\frac{[\text{concentration of conjugate base}]}{[\text{concentration of weak acid}]} \right) \quad (20.3.1)$$

This equation shows that the amount of dissociation in a carboxylic acid is related to the pH of the solution and the pK_a of the acid. In particular, the Henderson-Hasselbalch equation shows that when the concentration of a carboxylic acid is equal to the concentration of carboxylate then the pH of the solution equals the pK_a of the acid. Also, the Henderson-Hasselbalch allows for the pH of the solution to be calculated when the pK_a of the acid is known along with the concentrations of the carboxylic acid and carboxylate are known.

✓ EXAMPLE 20.3.1

What is the pH of an aqueous buffer solution that is 30 mM in acetic acid and 40 mM in sodium acetate? The pK_a of acetic acid is 4.8.

Solution

This is a direct application of the Henderson-Hasselbalch equation (Equation 20.3.1).

$$pH = pK_a + \log \left(\frac{[\text{concentration of conjugate base}]}{[\text{concentration of weak acid}]} \right)$$

The ratio of base to acid is 40/30, or 1.33. Therefore, substituting these values and the pK_a results in

$$\begin{aligned} pH &= 4.8 + \log \left(\frac{40}{30} \right) \\ &= 4.8 + \log 1.33 \\ &= 4.8 + 0.125 \\ &= 4.9 \end{aligned}$$

The Henderson-Hasselbalch equation is particularly useful when determining the protonation state of different biomolecule functional groups under physiological pH conditions. This is done under the assumption that the concentration of the biomolecule is small compared to the concentration of the buffer components. (The actual composition of a physiological buffer is complex, but it is primarily based on phosphoric and carbonic acids.).

Aspartic acid is an amino acid with a carboxylic acid functional group as part of its side chain. When aspartic acid comprises an amino acid residue of a protein in a human cell, it's side chain is in full contact with the physiological buffer. In what state is the side chain functional group: the protonated state (a carboxylic acid) or the deprotonated state (a carboxylate ion)?

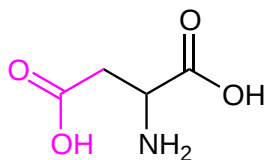


Figure 20.3.1: Aspartic acid with the carboxylic acid side-chain highlighted. (CC BY-SA 4.0; Layne Morsch via LibreTexts)

Using the buffer pH of 7.3 and a rough approximation of $pK_a = 5$ for the carboxylic acid side chain, we find that the ratio of carboxylate to carboxylic acid is about 200 to 1. The carboxylic acid is almost completely ionized to the carboxylate inside the cell. This result extends to all other carboxylic acid groups you might find on natural biomolecules or drug molecules. In the physiological environment, carboxylic acids are almost completely deprotonated. Indeed, cellular carboxylic acids are often referred to by the name of their anion. So rather than pyruvic acid, or acetic acid or lactic acid, they are discussed as pyruvate, acetate or lactate.

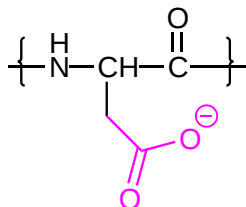


Figure 20.3.2: Aspartate (as part of a protein or polypeptide). (CC BY-SA 4.0; Layne Morsch via LibreTexts)

Using the same process, the Henderson-Hasselbalch equation (Equation 20.3.1) can also be used to determine the protonation state of carboxylic acids in solutions buffered to other pH levels.

$$\begin{aligned}
 pH &= pK_a + \log\left(\frac{[\text{carboxylate}]}{[\text{carboxylic acid}]}\right) \\
 7.3 &= 5 + \log\left(\frac{[\text{carboxylate}]}{[\text{carboxylic acid}]}\right) \\
 2.3 &= \log\left(\frac{[\text{carboxylate}]}{[\text{carboxylic acid}]}\right) \\
 199 &= \frac{[\text{carboxylate}]}{[\text{carboxylic acid}]}
 \end{aligned}$$

Therefore at physiological pH, the carboxylate anion is found with a 199-fold higher concentration than the protonated carboxylic acid. Naturally, changing the pH will alter this ratio.

? EXERCISE 20.3.1

What is the ratio of acetate ion to neutral acetic acid when a small amount of acetic acid ($pK_a = 4.8$) is dissolved in a buffer of pH 2.8? pH 3.8? pH 4.8? pH 5.8? pH 6.8?

Answer

We use the Henderson-Hasselbalch equation (Equation 20.3.1) and let the base to acid ratio be x .

For pH = 2.8:

$$2.8 = 4.8 + \log x$$

$$x = 0.01 \text{ to } 1$$

- pH 3.8, the ratio is 0.10 to 1
- pH 4.8, the ratio is 1.0 to 1
- pH 5.8, the ratio is 10 to 1
- pH 6.8, the ratio is 100 to 1

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