

Biological buffer systems for maintaining physiologic pH



**ASSIGN
BUSTER**

The preparation of 250ml of 0.200 M phosphate buffer with pH of 7.40 was conducted and its buffering was tested afterwards. Exactly 5.31g of K_2HPO_4 and 2.65g of KH_2PO_4 were mixed to form the buffer. The initial pH was measured at 7.09 which is 4.19% lower than the theoretical pH. A fixed amount of acid (0.100 M HCl) and base (0.100 M NaOH) were added separately to the buffer which resulted to pH of 6.69 and 7.46 respectively. These values resulted to 5.11% and 4.85% error from addition of HCl and NaOH respectively. When such procedures were repeated using distilled water in exchange of the buffer, the results show a drastic change in pH. The experimental buffer shows to be a good buffer, resisting a drastic change in pH unlike the plain distilled H_2O . This makes it a suitable buffer in maintaining physiologic pH and thus preventing the disruption of biomolecules and biochemical reactions.

INTRODUCTION

Buffers are highly stable acid-base solution that resists a drastic change in pH, especially when combined with an acid or base solution. It is made up of a weak acid and weak base. A weak acid and its conjugate base can remain stable, without neutralizing each other. The same is true with a weak base and its conjugate acid. (2) Buffers are important in biological studies due to the fact that most biomolecules and biochemical reactions are sensitive to pH conditions. Human body has a physiologic pH of 7.40; any major changes here could be fatal to the body. By having buffer in the biological system, a drastic and fatal change in pH is being avoided by the human body and other biological organisms. In this experiment buffer was made from K_2HPO_4

(weak base) and KH_2PO_4 (weak acid) where sample size were calculated to attain a 250 ml of 0.200 M $\text{H}_2\text{PO}_4\text{-HPO}_4\text{-}2$ buffer with pH of 7.40. (1)

MATERIALS AND METHODS

The weights of the K_2HPO_4 (weak base) and KH_2PO_4 (weak acid) used were calculated. They were weighed nearly exact at 5.307g and 2.652g respectively using analytical balance. Next, the two compounds were transferred qualitatively in a 250ml volumetric flask which then got half-filled with distilled H_2O and covered with a stopper. Then, the flask was swirled to allow the solid particles to dissolve. The flask, after being swirled, got filled up to the 250ml mark with distilled H_2O and vented for 20 times. The pH of the made buffer was measured and compared to the theoretical value and percent error was calculated. Using a volumetric pipette, two 25ml of the buffer were transferred to separate Erlenmeyer flask. One got an addition of 10ml 0.100 M HCl and the other with 10ml 0.100 M NaOH, and both were swirled. The pH of the separate mixture was measured and got compared to the theoretical pH, and percent error was then calculated. The procedure got repeated, but uses distilled H_2O in exchange of the buffer. Lastly, all data were recorded. (1)

RESULTS AND DISCUSSION

Phosphate buffer was made from dipotassium hydrogen phosphate (K_2HPO_4) and potassium dihydrogen phosphate (KH_2PO_4). (1) It was included in the methodology to calculate the specific or exact weight of the buffer made which was 250 ml of 0.200 M $\text{H}_2\text{PO}_4\text{-HPO}_4\text{-}2$ with pH of 7.40. As part of the experiment, the buffer's pH of 7.40 serves as the theoretical or the constant variable where the experimental buffer would be compared of. Theoretical
<https://assignbuster.com/biological-buffer-systems-for-maintaining-physiologic-ph/>

values for the pH after adding 10ml of HCl and NaOH separately was also calculated. The formula, calculations and results of the experiment are as follows:

A. MOLARITY

Step 1: Step 2:

$$\text{pH} = \text{pKa} + \log \left(\frac{[\text{A}^-]}{[\text{HA}]} \right) \quad [\text{A}^-] + [\text{HA}] = 0.200 \text{ M}$$

$$7.40 = -\log(6.2 \times 10^{-8}) + \log \left(\frac{[\text{A}^-]}{[\text{HA}]} \right) \quad [\text{A}^-] = 0.200 \text{ M} - [\text{HA}]$$

$$7.40 = 7.20 + \log \left(\frac{[\text{A}^-]}{[\text{HA}]} \right) \quad \text{Step 3: (substitute)}$$

$$7.40 - 7.20 = \log \left(\frac{[\text{A}^-]}{[\text{HA}]} \right) \quad 1.55[\text{HA}] = 0.200 \text{ M} - [\text{HA}]$$

$$\text{Antilog}(0.19 = \log \left(\frac{[\text{A}^-]}{[\text{HA}]} \right)) \quad (2.55[\text{HA}] = 0.200 \text{ M}) / 2.55$$

$$1.55 = \frac{[\text{A}^-]}{[\text{HA}]} \quad [\text{HA}] = 0.078 \text{ M}$$

$$1.55[\text{HA}] = [\text{A}^-] \quad [\text{A}^-] + [\text{HA}] = 0.200 \text{ M}$$

$$[\text{A}^-] + 0.078 \text{ M} = 0.200 \text{ M}$$

$$[\text{A}^-] = 0.200 \text{ M} - 0.078 \text{ M}$$

$$[\text{A}^-] = \mathbf{0.122 \text{ M}}$$

B. MOLECULAR WEIGHT



$$\text{K} = 2 \times 39 = 78 \quad 1 \times 39 = 39$$

$$H = 1 \times 1 = 1 \quad 2 \times 1 = 2$$

$$P = 1 \times 31 = 31 \quad 1 \times 31 = 31$$

$$O = 4 \times 16 = 64 \quad 4 \times 16 = 64$$

174g/mol 136g/mol

C. WEIGHT

$$g_{\text{base}} = M \text{ MW VL} \quad g_{\text{acid}} = M \text{ MW VL}$$

$$= (0.122 \text{ M}) (174\text{g/mol}) (0.250\text{L}) = (0.078\text{M}) (136\text{g/mol}) (0.250\text{L})$$

$$g_{\text{base}} = 5.31\text{g} \quad g_{\text{acid}} = 2.65\text{g}$$

D. THEORETICAL pH AFTER ADDITION OF ACID AND BASE

$$+\text{base pH} = \text{pKa} + \log \left(\frac{[A^-] + B}{[HA] - B} \right)$$

$$= (-\log 6.2 \times 10^{-8}) + \log \left[\frac{((0.122\text{M} \times 0.025\text{L}) + (0.100\text{M} \times 0.010\text{L}))}{((0.078\text{M} \times 0.025\text{L}) - (0.100\text{M} \times 0.010\text{L}))} \right]$$

$$= 7.2076 + 0.6297$$

$$\text{pH} = 7.8373 \sim 7.84$$

$$+\text{acid pH} = \text{pKa} + \log \left(\frac{[A^-] - B}{[HA] + B} \right)$$

$$= (-\log 6.2 \times 10^{-8}) + \log \left[\frac{((0.122\text{M} \times 0.025\text{L}) - (0.100\text{M} \times 0.010\text{L}))}{((0.078\text{M} \times 0.025\text{L}) + (0.100\text{M} \times 0.010\text{L}))} \right]$$

$$= 7.2076 + (-0.1581)$$

pH = 7.0495 ~ 7.05

Figure 1. Step by Step Calculations for Buffer Preparations and Theoretical Values

Meanwhile, Table 1 summarizes the pH Values of the phosphate buffer against distilled water. The buffer made in the experiment gains a pH of 7.09. In contrast to the theoretical pH of 7.40 the experimental buffer resulted into 4.19% error. This is not that significant since the difference between theoretical and experimental was only in decimal. The same thing occurs in the addition of 10 ml 0.100 M HCl and 0.100 M NaOH separately with an error of 5.11% and 4.85% respectively, as seen in table 1. This was expected since the premade buffer gained an error. Although the error was not that big and could also be considered correct, there are several factors that may have affected these errors. First, in weighing the buffer the exact amount of the reagents was not attained. K_2HPO_4 use was 5.315g instead of exactly 5.307g and 2.650g of KH_2PO_4 was use instead of exact 2.652g. This could have affected the results partially which causes the error. Another thing that is possible is the fact that some reagents got stocked in the weighing paper lessening the amount that went into the volumetric flask.

In the experiment, the buffer attained a pH of 7.09 which is not that far from pH of 7.40 (required in the experiment). A buffer was defined to be resistant to drastic change in the pH, upon adding specific acids or bases. (1) This property of buffer to resist a drastic change in pH is due to its composition; weak acid and weak base that doesn't neutralize each other but rather the base in the buffer neutralizes hydrogen ions (from addition of acid) and same

thing happens to the acid in the buffer neutralizing the hydroxide ions (from addition of base). (2)

The buffer made in the experiment was proven an effective buffer, supported by the proof in table 1, where addition of 10ml 0.100 M of HCl only decrease the pH from 7.09 to 6.69, which is only a difference of 0.4, on the other hand, addition of the 10ml 0.100 M of NaOH increases the buffer's pH slightly from 7.09 to 7.46 which have a difference of 0.37. The experiment was repeated but using distilled water instead of the made buffer. And as expected there was a drastic change in pH. Buffers are important, most specially in biological bodies. If there's no buffer in the system a drastic change in the pH will occur which could cause the fatality of cells and thus the body.

Examples of synthetic organic buffers are PIPES Buffer (Piperazine) and MES Buffer (Morpholino ethane sulfonic acid). (4) (5) Their structures are in Figure 2. There are advantages of using synthetic organic buffers. These includes solubility (they freely soluble in water and poorly soluble in other solvents, simpler means it is easy to prepare), permeability (these buffers are not permeable to plasma membranes, avoiding concentration in cell organelles), ionic strength (they doesn't alter ionic strength of the system) and others. (6)

Human body has its own buffer system to maintain its physiological pH of 7.40. Bicarbonate buffer system is one of the biological buffers in our body. They are present as sodium bicarbonate located in the blood plasma, where the sodium serves as the main positive ion in extracellular fluids or ECF. This

regulates the physiologic pH through these processes: (The processes listed below is not owned nor made by the author of this paper; it is an excerpt from an electronic reference listed at the references (3).)

- A. The bicarbonate ion (HCO_3^-) can combine with a proton (H^+) to form carbonic acid (H_2CO_3), thus absorbing protons from solution and raising blood pH.
- B. Carbonic acid, which can be formed from CO_2 and water, can dissociate into H^+ and HCO_3^- in order to provide H^+ and lower blood pH.
- C. Carbonic acid, which can be formed from bicarbonate, is converted to CO_2 and water via a very fast enzymatic reaction.
- D. CO_2 , being volatile, can be rapidly expelled from the body at varying rates by respiration.

Through these simple processes physiological pH of 7.40 is maintained in our body.

Acidosis is a condition where there are too much acid to the body fluids. (7) In contrast alkalosis is too much base in the blood. (8) Acidosis occurs when an acid build-up arise or when bicarbonate (a base) is lost. Acidosis has two types, namely respiratory acidosis and metabolic acidosis. Respiratory acidosis happens when the body has too much carbon, an acid (body can't release CO_2 fast enough). On the other hand metabolic acidosis happens when excessive acid is produced or the kidneys cannot remove enough acid from the body. (7) Acidosis is caused by chest or lung diseases or injury, liver failure, excessive exercise (lactic acid build-up), kidney problems and other <https://assignbuster.com/biological-buffer-systems-for-maintaining-physiologic-ph/>

factors related to production of acid in the biological body. Effects of acidosis include mental confusion, fatigue and lethargy, breathing difficulty and shock or even death. (9) Alkalosis has different types including respiratory alkalosis (low CO₂ in the blood), metabolic alkalosis (excessive bicarbonate in the blood), hypochloremic alkalosis (lack of too much chloride), hypokalemic alkalosis (lack of too much potassium), and compensated alkalosis (body returns to acid-base normal balance but bicarbonate and CO₂ remains not normal). Alkalosis could cause from fever, being at a high altitude (depleted O₂), liver disease, lung diseases and Salicylate poisoning. It may cause confusion, hand shaking, and light-headedness, and muscle palpitation, nausea accompanied with vomiting, numbness and prolonged muscle spasm. (8)