

Optimizing Biological Buffers: How Adding Different Volumes of Acidic and Basic Components Effects the pH of them.

Target Audience: This scientific inquiry is intended for first year university students who are being introduced to buffers and have limited knowledge about what biological buffers are and what makes a buffers optimal pH.

Estimated Time: 10-20 minutes

Big Inquiry Question: How does adding different ratios of acidic and basic components change the pH of biological buffers and what is a buffers optimal pH?

Learner Activity:

1)Observe/Read: Imagine you were a biochemist in lab, and you were working on a study on the analysis of enzymes. However, in your research you have been having problems with pH fluctuations of the enzyme samples. You want to prepare a new phosphate buffer solution, and you have your acidic and basic components. Unfortunately, you have never made a buffer before so you have no idea what the ratio of two components should be. Luckily, you found a scientific study that use the same reagents with the same concentration of your solutions.

2) Predict: Without looking at the table or diagram guess what volume of NaH_2PO_4 and Na_2HPO_4 would provide the most optimal pH for the buffer. More of NaH_2PO_4 than Na_2HPO_4 , equal amounts of NaH_2PO_4 and Na_2HPO_4 or less NaH_2PO_4 than Na_2HPO_4 .

3) Examine the Evidence: Examine the table and try to identify what range of volumes of NaH_2PO_4 and Na_2HPO_4 would give the most optimized pH.

Solution "A" (mL)	Solution "B" (mL)	0.1 M Phosphate buffer (pH)	
(0.1 M NaH_2PO_4)	(0.1 M Na_2HPO_4)	Experimental	Back calculated
6.80	93.20	8.00	8.000
10.40	89.60	7.80	7.799
15.50	84.50	7.60	7.601
22.60	77.40	7.40	7.399
31.60	68.40	7.20	7.200
42.30	57.70	7.00	7.000
53.70	46.30	6.80	6.800
64.80	35.20	6.60	6.600
74.50	25.50	6.40	6.399
82.20	17.80	6.20	6.201
88.00	12.00	6.00	6.000
92.10	7.90	5.80	5.800

Table 1: Volume of NaH_2PO_4 and Na_2HPO_4 and the corresponding pH of 0.1M Phosphate Buffer (Ganesh et al.,2017).

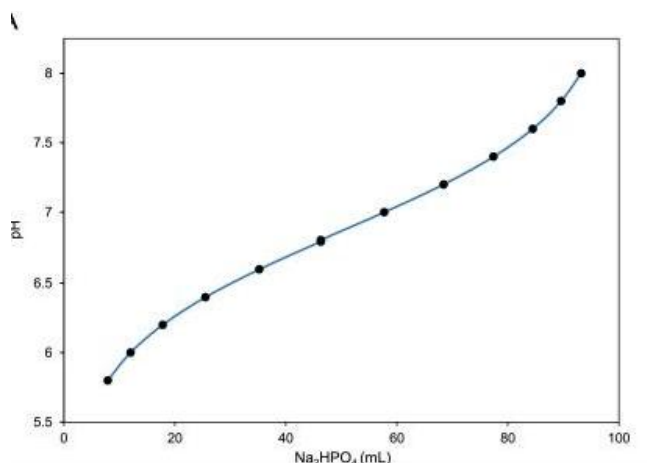


Figure 1: Curve of the corresponding data from Table 1 (Ganesh et al., 2017).

4) Explain: Get students to explain why they think the range would be the most optimal and circle the points on the provided pH diagrams of the phosphate buffer. As well, they should explain if their pH was outside this range what effect would this have on their enzyme samples.

5) Reflect/ Extend: The concentration of the phosphate buffer for this example was 0.1M, what would happen to the pH if the concentration of the buffer was increased?

Instructor/ Teacher Guide:

1. Purpose of the activity: The purpose of the activity is to introduce to students what biological buffers are and what volumes of the components allow for the most optimal pH. As well, it gives them a chance to understand how school topics can be applied to the real world.

2. Required Materials: Printed Copies of the table 1 and figure 1. Writing utensils to highlight and draw on the figure.

3. Factuality Notes: This inquiry should be used as introduction to students about what buffers are and spike students' interests on the topic. It should be first briefly introduced what buffers are then this activity should be shown immediately after. First, allow them to read the prompt and make a prediction about the volumes. Next, show them the figure and table and allow them to examine table. They should pick their desired pH and explain why they chose it. As well, if they were that biochemist what do they think would happen to the enzymes. After some discussion with classmates and comparing answers, they should get into partners and try to guess what increasing the concentration would do.

4. Expected Response: The most stable pH would be when the NaH_2PO_4 and Na_2HPO_4 are close as possible to equal volumes due to the concentration of both components using Henderson-Hasselbalch equation (Gunawardena, 2015). This is due to when the concentration of the acid and conjugate base is equal, $\text{pH} = \text{pK}_a$ (Gunawardena, 2015). For this article the optimized pK_a of phosphates 6.8 (Ganesh et al., 2017). The correct range of pH for where the resistance to change of the buffer is least would be 6.8-7.0. If the pH were to drop outside of this optimal range the effects

on the enzyme samples would change the amount of ionization of enzymes acid and basic side groups (“*Factors affecting enzyme activity*”, 2017). This would have a large effect since the groups require a charge or it will change the catalytical activity of the enzyme (“*Factors affecting enzyme activity*”, 2017). A larger concentration of the buffer would not change the optimal range since it is the ratio of concentrations between NaH_2PO_4 and Na_2HPO_4 . However, it would change the buffer capacity, which is the quantity of acid or base that can be added prior to drastic change in the pH (“*Maintaining pH using buffer solutions.*”, 2024).

5. Safety Notes: No safety notes are required as there is no lab component for this activity.

6. Accessibility: Print off the required papers for students and provide students will need writing utensils. Braille copies should provide if required or students could be read the inquiry. Provide a quiet space for students that require, such as doing the activity outside of the classroom.

Connection to SDG 4: This Scientific Inquiry does reflect the principles of SDG 4 since this activity has got students to using their problem-solving skills with real life examples rather than using textbook questions. Furthermore, students are improving there understanding by trying the question first rather than just memorizing that the ratio is 1:1 of the concentration of the conjugate base and acid for optimized pH. Furthermore, the activity very low cost and allows any student to be to participate in the activity.

References

Ganesh, K., Soumen, R., Ravichandran, Y., Janarthanan. (2017). Dynamic approach to predict pH profiles of biologically relevant buffers. *Biochemistry and Biophysics Reports*, 9, 121–127. <https://doi.org/10.1016/j.bbrep.2016.11.017>

Gunawardena, G. (2015, November 19). *Henderson-hasselbach equation*. Chemistry LibreTexts. https://chem.libretexts.org/Ancillary_Materials/Reference/Organic_Chemistry_Glossary/Henderson-Hasselbach_Equation

Libretexts. (2024, August 17). *13.3: Maintaining pH using buffer solutions*. Libretexts. Chemistry LibreTexts. [https://chem.libretexts.org/Courses/Pasadena_City_College/Chem_2A%3A_General_Organic_and_Biological_Chemistry_\(Ku\)/13%3A_Acids_and_Bases/13.03%3A_Maintaining_pH_Using_Buffer_Solutions](https://chem.libretexts.org/Courses/Pasadena_City_College/Chem_2A%3A_General_Organic_and_Biological_Chemistry_(Ku)/13%3A_Acids_and_Bases/13.03%3A_Maintaining_pH_Using_Buffer_Solutions)

LibreTexts Chemistry. (2017, August 5). *19.5: Factors affecting enzyme activity*. Chemistry LibreTexts. [https://chem.libretexts.org/Bookshelves/Introductory_Chemistry/Fundamentals_of_General_Organic_and_Biological_Chemistry_\(LibreTexts\)/19%3A_Enzymes_and_Vitamins/19.05%3A_Factors_Affecting_Enzyme_Activity](https://chem.libretexts.org/Bookshelves/Introductory_Chemistry/Fundamentals_of_General_Organic_and_Biological_Chemistry_(LibreTexts)/19%3A_Enzymes_and_Vitamins/19.05%3A_Factors_Affecting_Enzyme_Activity)

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