Experiment 2

Preparing of Buffers

In this experiment, you will learn how to choose and prepare a buffer. You will then see how the pH of the buffer responds to dilution and compare how buffered and unbuffered systems respond to addition of acids and bases.

Prelab Questions

1. Calculate the weight of the buffers you will use to make the buffers for Part A for all the buffer possibilities listed under procedures in Part A, step 1. In other words, how many grams do you need to make 100 mL of a 0.1 M buffer?

2. If we give you HEPES in the basic form and ask you to make a buffer of pH 8.0, will you have to add HCl or NaOH? Why? (With commercial buffers, there is always an acid form and a basic form that can be bought. It is not obvious from the name of the compound, so look to see if it is acid or basic. If HEPES is bought in the acid form, then write the equation

$$\text{HEPES} \rightarrow \text{HEPES}^- + \text{H}^+$$

Objectives

Upon successful completion of this lab, you will be able to

- Calculate the pH of solutions of strong acids or bases, weak acids or bases, buffers, and/or combinations of these.
- Explain how a buffer resists change in pH.
- Prepare an appropriate buffer for a given pH.
- Calculate the theoretical pH of the buffer after adding a known quantity of acid or base.
- Predict the effect of changing temperature and concentration on buffer pH.
- Standardize and operate a pH meter.

Experimental Procedures

Materials

Solid buffers
Standard buffers: pH 4, 7, and 10
pH meters
1M HCl and NaOH
Solutions of unknown pH

Methods

Part A: Preparation of Buffers

Make two buffers starting with solid material, which is the most common way to make buffers. You will be given a desired pH, and your task is to prepare 100 mL of two appropriate buffers at a concentration of 0.10 M. One of the buffers will be a phosphate or citrate buffer, and the other will be one of the others (not phosphate or citrate).

1. Using the following table, choose the most appropriate buffer compounds for your pHs. Proceed with steps 2–7 for both buffers.

Buffer	pK_a1	pK_a2	pK_a3	*Formula Weight (g/mol)*
Acetate	4.76			136.1
CAPS	10.4			221.3
Citrate	3.06	4.74	5.40	294.1
HEPES	7.55			238.3
Phosphate	2.12	7.21	12.32	142.0
Tricine	8.15			179.2
TRIS	8.3			121.1

2. Calculate the weight of the buffer you would need to make 100 mL of a 0.100 M solution. Weigh out the correct amount and dissolve in 50 mL water.
3. Standardize the pH meter at pH 4, 7, and 10. Set up the beaker with your buffer solution on a stirplate such that you can stir the solution and read the pH continuously. If you have no stirplate, just swirl the beaker often while adding acid or base.
4. Use 1 M NaOH or 1 M HCl to titrate to the desired pH. Add the acid or base a drop at a time. By doing this, you effectively change some of the acid form to the basic form or vice versa until the ratio is the correct one to give you the pH you want.
5. Add water until the volume is about 99 mL.

6. Recheck the pH to make sure it has not changed. If it has, correct it with NaOH or HCl. **Warning! You might want to use a lower concentration of NaOH or HCl.**
7. Bring the volume to 100 mL and save this solution for later.

Part B: Effect of Concentration on pH

For this part, you must have a digital pH meter.

1. Take 10 mL of each of your two buffers and dilute with deionized water to give a final concentration of 0.01 M. Save these solutions.
2. Take 10 mL of your diluted buffers from step 1 and dilute to a concentration of 0.001 M. Save these solutions.
3. Measure the pHs of the undiluted and diluted solutions.

Part C: pH Measurement of Other Solutions

Measure the pH of the following solutions:

Distilled water
Unknown _____
Unknown _____

Part D: Why a Buffer Is a Buffer

1. Put 50 mL of one of your original 0.1 M buffers in a beaker. If your buffer has a pH higher than its pK_a, add 0.5 mL of 1 M HCl to it. Record the new pH. If your buffer has a pH lower than its pK_a, add 0.5 mL of 1 M NaOH to it. Record the new pH.
2. Repeat step 1, but use 50 mL of water instead of your buffer. Add either acid or base, depending on what you did in step 1.

Name _____ Section _____

Lab partner(s) _____ Date _____

Analysis of Results

Experiment 2: **Buffers**

Data

Part A

Buffer 1: _____ Weight (g): _____ Original pH:* _____

Buffer 2: _____ Weight (g): _____ Original pH:* _____

Part B

Buffer 1 pH of 0.1 M _____ pH of 0.01 M _____ pH of 0.001 M _____

Buffer 2 pH of 0.1 M _____ pH of 0.01 M _____ pH of 0.001 M _____

Part C

Distilled water pH: _____

Unknown _____ pH: _____

Unknown _____ pH: _____

Part D

Buffer chosen: _____ pH: _____ pK_a: _____

Acid or base added: _____

pH after adding acid or base: _____

pH of 50 mL of water: _____

pH after adding acid or base: _____

* When buffer powder was added to water.

Calculations

1. This problem will be done for one of the two buffers you made. (Your lab partner should do these calculations for the other one.)
   a. What is the ratio of A^-/HA in your buffer after you adjusted its pH to the required value?
   b. How many micromoles of A^- and HA are present in the solution?
   c. If you now add 3 mL of 1 M NaOH, will you still have a valid buffer?

2. Calculate the theoretical pH of one of your buffers at 0°C. Assume that room temperature is 22°C. If none of your buffers is listed on the table of changing pK_a with temperature, do this problem for TRIS at pH 8.0.

3. What is the most efficient way to make up a HEPES buffer at pH 8.5? What starting compounds and reagents will you use?

4. When Dr. Farrell was a graduate student, he once made up a pH 8.0 sodium acetate buffer. Why would the casual observer to this buffering faux pas come to the conclusion that he had the intellectual agility of a small soap dish?

5. If you make up a solution of 50 mL of 0.1 M TRIS in the acid form, what will be the pH?]

6. If you add 2 mL of 1 M NaOH to the solution in step 5, what will be the pH?

7. If you make up a solution of 100 mL of 0.1 M HEPES in the basic form, what will be the pH?

8. If you add 3 mL of 1 M of HCl to the solution in step 7, what will be the pH?

9. What can you conclude about the effect of dilution on the pH of a buffer?

Additional Problem Set

1. Calculate the pH of a 0.1 M HCl solution.
2. Calculate the pH of a 0.1 M NaOH solution.
3. What is the concentration of $[H^+]$ in molars, millimolars, and micromolars for a solution of pH 5?
4. If you mix 10 mL of a 0.1 M HCl solution with 8 mL of a 0.2 M NaOH solution, what will be the resulting pH?
5. If a weak acid, HA, is 3% dissociated in a 0.25 M solution, calculate the K_a and the pH of the solution.
6. What is the pH of a 0.05 M solution of TRIS acid ($pK_a = 8.3$)?
7. What is the pH of a 0.045 M solution of TRIS base?
8. If you mix 50 mL of 0.1 M TRIS acid with 60 mL of 0.2 M TRIS base, what will be the resulting pH?
9. If you add 1 mL of 1 M NaOH to the solution in 6 above, what will be the pH?
10. How many total milliliters of 1 M NaOH can you add to the solution in Problem 6 and still have a good buffer (that is, within 1 pH unit of the pK_a)?
11. If you are making 100 mL of a 0.1 M HEPES buffer starting from HEPES in the basic form, is it prudent to get 50 mL of 1 M HCl from the community reagent bottle to use for your titration?
12. An enzyme-catalyzed reaction is carried out in a 50-mL solution containing 0.1 M TRIS buffer. The pH of the reaction mixture at the start was 8.0. As a result of the reaction, 0.002 mol of H^+ were produced. What is the ratio of TRIS base to TRIS acid at the start of the experiment? What is the ratio at the end of the experiment? What is the final pH?
13. The pK_a of HEPES is 7.55 at 20°C, and its MW is 238.31. Calculate the amounts of HEPES in grams and of 1.0 M NaOH in milliliters that would be needed to make 300 mL of 0.2 M HEPES buffer at pH 7.2.

Webconnections

For a list of websites related to the material covered in this chapter, go to **Webconnections** at the *Experiments in Biochemistry* site on the Brooks/Cole Publishing website. You can access this page at http://www.brookscole.com and follow the links from the chemistry page.

References and Further Reading

Boyer, R. F. *Modern Experimental Biochemistry*. Menlo Park, CA: Addison-Wesley, 1993.

Campbell, M., and S. Farrell. *Biochemistry*, 4th ed. Pacific Grove, CA: Brooks/Cole, 2002.