TIP 1.4 Prewetting the pipet tip can help if you are pipetting liquids with a low surface tension, such as organics. They tend to drip out of the pipet tip the first time you load them. Draw up the organic, then blow it out. Draw up some more, and this time it should hold without dripping.

11%. That is why you should always choose the pipet that you can fill the most. It makes sense to use that 10-mL pipet to pipet anything from 5.1 to 10 mL, but if you want to pipet only 4 mL, then you should choose the common 5-mL pipet. If you want to pipet 2 mL, then the 5-mL pipet would not be so good because there is also a common 2-mL pipet. Make sure you know what pipets are available and use the ones that can be used near to capacity.

To use glass pipets correctly, follow this procedure:

1. Using a pipet pump or rubber bulb, draw the solution into the pipet up past the starting mark.
2. Depending on the device you use to draw up the solution, either leave the device attached or remove it and use your index finger to control the flow.
3. Wipe off the tip with a tissue.
4. Touch the tip of the pipet to the sides of the vessel you took the solution out of and let it run down until the meniscus is at the starting line.
5. Transfer the pipet to the vessel you want to put the solution into and touch it to the sides of the vessel.
6. Let the solution run down the sides until the meniscus is at the stopping line.

Glass Syringes

One of the most accurate devices is the glass syringe, the most common of which is the Hamilton syringe (Figure 1.17). These have a tight-fitting metal plunger in a glass tube and often are used for accurate dispensing of volumes as small as 1 μL.

To use a Hamilton syringe correctly, follow this procedure:

1. Draw the solution into the syringe and expel several times to wet the inside of the glass and the needle.

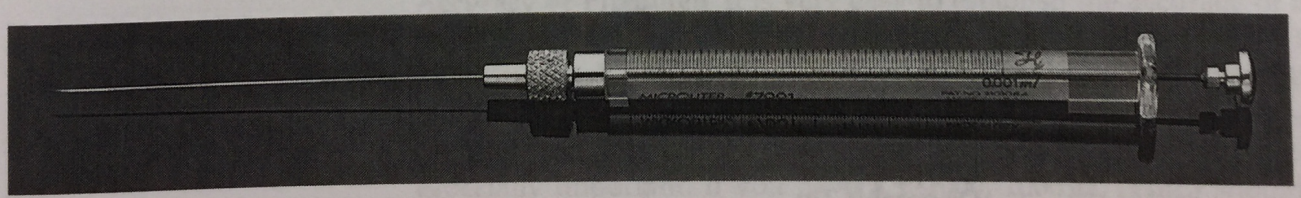

FIGURE 1.17 *The Hamilton syringe*
(Courtesy of Hamilton Co.)

2. Slowly draw up the solution until the plunger is at the line you want.
3. Watch carefully for bubbles forming. If you get bubbles between the solution and the plunger, expel the solution quickly to shoot out the bubble.
4. Repeat until you have no bubbles in the syringe.
5. Wash the syringe out repeatedly with water. Never leave salts or organics in the syringe, or the plunger will become permanently locked.
6. Be careful not to bend the plunger. They bend easily and once bent are useless.

Pipetmen

The generic term for these is air-displacement piston pipets, so you can see why we say Pipetmen. The three most common types in use these days are the Pipetman by Rainin Instruments (Figure 1.18), the Eppendorf pipettor

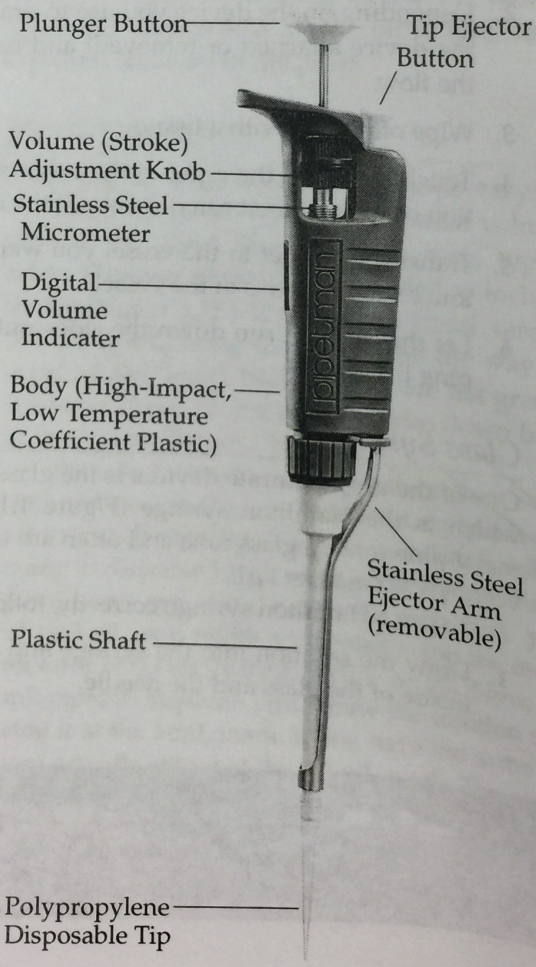

FIGURE 1.18 *Rainin Pipetman*
(Courtesy of Rainin Instruments, Inc.)

TABLE 1.5 *Volume Ranges of Rainin Pipetmen*

Pipetman Model	Range (μL)		Smallest Increment (μL)
	Adjustable	Recommended	
P-2	0–2	0.1–2	0.002
P-10	0–10	0.5–10	0.02
P-20	0–20	2–20	0.02
P-100	0–100	10–100	0.2
P-200	0–200	50–200	0.2
P-1000	0–1000	100–1000	2.0
P-5000	0–5000	500–5000	2.0

by Brinkman Instruments, and the Integrapette by Integrated Instrument Services. We use the Pipetman as an example throughout this section.

Pipetmen come in sizes from the P-2, with a maximum volume of 2 μL, to the P-5000, with a maximum volume of 5 mL. The most common sizes are the P-20, P-200, and P-1000. The instrument uses disposable plastic tips in various sizes depending on the size of the Pipetman. There is a color-coding system between the pipet and the tips used. For example, the P-1000 has a blue top to indicate that it should use blue pipet tips, which are the size that goes up to 1 mL. The P-20 and P-200 both have yellow tops and use yellow tips, which go up to 200 μL. Before you start, make sure you have the proper size Pipetman and the proper tips to use.

Most Pipetmen are infinitely variable from a lower limit up to the maximum. Never try to dial the Pipetman past its maximum because it will irreversibly damage the unit. As you set the volume on the Pipetman, keep in mind what the maximum is. The volume dial has three digits, and what those three digits mean depends on the total volume possible for the pipet. For example, if the dial reads 050, that is 500 μL for a P-1000 or 50 μL for a P-200. Table 1.5 shows the recommended range for the Rainin Pipetman, as well as a description of the dial readings for each one. The other brands of pipettors are similar.

Accuracy of Pipetmen It is very easy to establish the accuracy of a pipettor. All you have to do is pipet a known volume of water onto a balance and weigh it. If you have a digital top-loader balance in the lab, this takes but a few seconds and could save you hours of frustration later. In a teaching lab, verifying that the instrument you are about to use is accurate is especially important. If you have a P-1000, 1000 μL (1 mL) should weigh 1 g. If you have a P-200, 200 μL should weigh 0.2 g, and so on.

How to Use a Pipetman

1. Make sure you have the correct size Pipetman and tips.
2. Place the tip on the pipettor and tighten with your fingers, being careful not to touch the very tip. Wear gloves when appropriate.
3. Hold the pipettor vertically over the solution you want to draw up. Push the plunger down to the first stop. Be careful to not drive past the first stop.
4. Put the tip into the solution to a depth of a few millimeters.
5. Slowly let the plunger come up as the solution is drawn into the tip. After the plunger is all the way up, hold the tip in the solution for a couple of seconds to make sure that the solution is not still moving.
6. Look at the tip to make sure that you drew up the correct amount of solution.
7. Move the pipetman to the vessel that you want to dispense into. Touch the tip to the side of the vessel.
8. Slowly depress the plunger to the first stop and then past the first stop to the second stop. This will blow the solution out the tip. Any solution remaining should not be blown out further.
9. Use the tip ejector to dispose of the tip.

ESSENTIAL INFORMATION

Nearly all scientific calculations involve units. An answer without units is a wrong answer. We use units based on those of the MKS system. These units often have prefixes to tell you the power of 10 based on the standard unit. Learn how to convert from one prefix to another without needing to write long conversion factors. You will need to do this hundreds of times during a biochemistry course. Memorize the most used prefixes, such as kilo-, milli-, micro-, and nano-.

Your results will depend largely on how well you pipet. Learn how to use all the available glass pipets and automatic pipettors. You must know when to use a particular liquid-transfer device and when not to. Practice drawing up standard volumes into the Pipetmen. Then, during an experiment, you will know if you have pipetted the correct amount.

Study the section on graphing carefully. You cannot make a graph if you do not know what the relationship is between the x and y coordinates. Always use graph paper or a computer graphing program. Never scribble a graph on regular binder paper. You should rarely connect the dots on a graph. You should almost always draw a best-fit curve. Be wary of the default values on your computer program. Make the computer work for you, not the other way around.

Experiment 1

Using Pipettors

In this experiment, you will learn how to use Pipetmen of various sizes and measure their accuracy, precision, and calibration. The questions at the end will also review concentration and dilution.

Prelab Questions

1. What is the usable range of a P-1000 Rainin Pipetman?
2. What is the difference between *accuracy* and *precision*?
3. What should 100 µL of water weigh?
4. What should 1000 µL of water weigh?

Objectives

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

- Become familiar and adept at using pipettors.
- Check the calibration of the pipettors.

Experimental Procedures

Materials

- 100- or 200-µL pipettors
- 1000-µL pipettor
- Yellow and blue pipet tips (or otherwise correct size)
- Deionized water
- Balances

Methods

Part A: Precision of P-100 or P-200 Pipettor

1. Acquire a P-100 or P-200 pipettor with the correct size tips. Make sure that the color matches.
2. Set the pipettor to 100 µL.
3. Place weighing paper or a weighing boat on the balance and tare the weight to zero.
4. Draw up the 100 µL of deionized water and dispense it onto the weighing paper or weighing boat. Record the weight of the water.
5. Repeat the procedure twice more.
6. Draw up 10, 20, 50, and 75 µL just to see what they look like in the tip.

Part B: Precision of P-1000 Pipettor

1. Acquire a P-1000 pipettor and the correct size tips. Make sure that the color matches.
2. Set the pipettor to 1000 µL.
3. Place weighing paper or a weighing boat on the balance and tare the weight to zero.
4. Draw up 1000 µL of deionized water and dispense it onto the weighing paper or weighing boat. Record the weight of the water.
5. Repeat the procedure twice more.
6. Set the pipettor to 100 µL and check the weight of the liquid three times.
7. Draw up 200, 500, and 750 µL of water just to see what they look like in the tip.
8. Check the analysis of results questions, make sure that you have all the data you need, and put the pipettor away.

Name _____ Section _____

Lab partner(s) _____ Date _____

Analysis of Results

Experiment 1: **Using Pipettors**

Part A: Precision of P-100 or P-200 Pipettor

1. Record the weight you measured for the three trials of 100 µL:

   Weight 1 (x_1) _____

   Weight 2 (x_2) _____

   Weight 3 (x_3) _____

2. Average the three weights.

   Average of three trials: _____

3. Calculate the % error between the average of the three trials and the true value:

$$\% \text{ Error} = \frac{|\text{avg weight} - 0.100\text{ g}|}{0.1\text{ g}} \times 100 = \_\_\_\_\_$$

4. Calculate the mean deviation for the three trials:

$$\text{Mean deviation} = \frac{\Sigma |x_i - x_{\text{avg}}|}{3} = \_\_\_\_\_$$

Part B: Precision of P-1000 Pipettor

1. Record the weight you measured for the three trials of 1000 µL:

   Weight 1 (x_1) _____

   Weight 2 (x_2) _____

   Weight 3 (x_3) _____

2. Average the three weights.

   Average of three trials: _____

3. Calculate the % error between the average of the three trials and the true value:

$$\% \text{ Error} = \frac{|\text{avg weight} - 1.00\text{ g}|}{1.00\text{g}} \times 100 = \_\_\_\_\_$$

4. Calculate the mean deviation for the three trials:

$$\text{Mean deviation} = \frac{\Sigma |x_i - x_{avg}|}{3} = \_\_\_\_\_$$

5. Record the weight you measured for the three trials of 100 µL using the P-1000:

Weight 1 (x_1) _____

Weight 2 (x_2) _____

Weight 3 (x_3) _____

6. Average the three weights.

Average of three trials: _____

7. Calculate the % error between the average of the three trials and the true value:

$$\% \text{ Error} = \frac{|\text{avg weight} - 0.100\text{ g}|}{0.1\text{ g}} \times 100 = \_\_\_\_\_$$

8. Calculate the mean deviation for the three trials:

$$\text{Mean deviation} = \frac{\Sigma |x_i - x_{avg}|}{3} = \_\_\_\_\_$$

Part C: Pipettors in the Lab

1. Which of the two pipettors that you used was the more accurate? Explain.

2. Which of the two pipettors that you used was the more precise? Explain.

3. What are the take-home messages from this exercise? Give three specific things that you learned from this lab.

4. Without checking the accuracy of a given Pipetman, would you predict that it is better to use a P-200 or P-1000 to pipet 100 µL? Why?

5. Is a Pipetman more like a serological pipet or a Mohr pipet? Why?

6. If you are trying to pipet an unknown liquid with a Pipetman and the liquid keeps running out of the tip before you can transfer it, what are two possible reasons for this? What can you do to remedy the situation?

7. How do you make 200 mL of a 0.1-M solution of a substance that has a molecular weight of 121.1 g/mol?

8. If you take 10 mL of the solution you made in Question 7, add 90 mL of water, mix, and then take 5 mL of the mixture and bring it to 25 mL, what will be the concentration of the final solution in molars, millimolars, and micromolars?

Additional Problem Set

1. How many grams of solid NaOH are required to prepare 200-mL of a 0.05 M solution?
2. What will be the concentration from Problem 1 expressed in % w/v?
3. How many milliliters of 5 M NaCl are required to prepare 1500 mL of 0.002 M NaCl?
4. What will be the concentration of the diluted solution from Problem 3 expressed in millimolars, micromolars, and nanomolars?
5. A solution contains 15 g of $CaCl_2$ in a total volume of 190 mL. Express the concentration in terms of grams/liter, % w/v, molars, and millimolars.
6. Given stock solutions of glucose (1 M), aparagine (100 mM) and NaH_2PO_4 (50 mM), how much of each solution do you need to prepare 500 mL of a reagent that contains 0.05 M glucose, 10 mM asparagine, and 2 mM NaH_2PO_4?
7. Calculate the number of millimoles in 500 mg of each of the following amino acids: alanine (MW = 89), leucine (MW = 131), tryptophan (MW = 204), cysteine (MW = 121), and glutamic acid (MW = 147).
8. What molarity of HCl is needed so that 5 mL diluted to 300 mL will yield 0.2 M?
9. How much 0.2 M HCl can be made from 5.0 mL of 12.0 M HCl solution?
10. What weight of glucose is required to prepare 2 L of a 5% w/v solution?
11. How many milliliters of an 8.56% solution can be prepared from 42.8 g of sucrose?
12. How many milliliters of $CHCl_3$ are needed to prepare a 2.5% v/v solution in 500 mL of methanol?
13. If a 250-mL solution of ethanol in water is prepared with 4 mL of absolute ethanol, what will be the concentration of ethanol in % v/v?

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 web page. You can access this page as http://www.brookscole.com. Webconnections are in the biochemistry portion of the chemistry page under the title of this manual.

References and Further Reading

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Total
= 50 ml

5:1

= 40ml H2O
= 10ml reagent

Lab 2

Follow

Chapter 2

Acids, Bases, and Buffers

calculation

TOPICS

Introduction

All biochemical reactions occur under conditions of strict control over the concentration of hydrogen ion. Biological life cannot withstand large changes in hydrogen-ion concentration, which we measure as the pH. When chemicals that keep the pH from changing are present, we say that the system is buffered. Whether in your body or in a test tube, the reactions that will be important to you will be buffered. The proper choice and preparation of a buffer is paramount to your success in a biochemistry lab.

Before we can truly understand buffers, however, we must understand the more basic concepts of strong versus weak acids and pH. Throughout this text, we will use the Brønsted definition of acid *as a substance that can donate a hydrogen ion and a* base *as a substance that can accept a hydrogen ion.*

2.1 Strong Acids and Bases

An acid is a compound that has a hydrogen ion that it can give up to the solution. Common strong acids that you are familiar with are hydrochloric acid (HCl), sulfuric acid (H_2SO_4), and nitric acid (HNO_3). When strong acids are dissolved in water, they dissociate completely into their ions:

$$\mathbf{HCl(aq) \rightarrow H^+(aq) + Cl^-(aq)}$$

A more formal way of writing this equation would indicate that the hydrogen ion is not really "hanging out" loose. There are no "naked" protons, as we say; rather, the hydrogen ion is attached to a water molecule to give a hydronium ion:

$$\mathbf{HCl + H_2O \rightarrow H_3O^+(aq) + Cl^-(aq)}$$

Let's use the abbreviated formulas for simplicity's sake.

Strong bases, such as NaOH, KOH, and $Ca(OH)_2$ likewise dissociate completely into ions. For example,

$$\mathbf{NaOH(aq) \rightarrow Na^+(aq) + OH^-(aq)}$$

The strength of an acid is determined by how much of the hydrogen ion dissociates when the acid is put into water. This can be determined from the K_a:

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

The complete dissociation is indicated by a unidirectional arrow. If we calculate the K_a for the HCl, it would be much greater than 1. If we did the same thing for the NaOH (using the corresponding K_b), it would also be very large.

Because strong acids and strong bases break down completely into their ions, it is relatively easy to calculate the pH. As long as you know how many moles are in the starting compound, you will know how many ions you will get. To calculate the pH of a solution of a strong acid or strong base, we use the following procedures.

PRACTICE SESSION 2.1 What is the pH of 0.01 M HCl?

Because HCl dissociates completely, the concentration of the H^+ is also 0.01 M.

$$\text{pH} = -\log[H^+] = -\log 0.01 = 2$$

Answer pH = 2

PRACTICE SESSION 2.2 What is the pH of 0.01 M NaOH?

NaOH is a strong base, so it dissociates completely into Na^+ and OH^- ions. Therefore, the concentration of OH^- is also 0.01 M.

This is also easy because we know that the product of the concentration of hydrogen ion and the concentration of hydroxide ion is always equal to 10^{-14}. This is called the *water equation*.

$$[H^+][OH^-] = 10^{-14}\ M^2$$

$$[H^+] = \frac{10^{-14}\ M^2}{0.01\ M} = 10^{-12}\ M$$

$$\text{pH} = -\log 10^{-12} = 12$$

Answer pH = 12

2.2 Weak Acids and Bases

Determining the pH of solutions of weak acids or bases is a little trickier. Because they do not dissociate completely, determining the $[H^+]$ is more difficult, and an equilibrium expression with K_a must be used. The K_a tells us the strength of the acid, so it can be used to calculate how much H^+ dissociates from the acid. We would soon tire of writing equilibrium constants such as 10^{-8}, 4.3×10^{-6}, . . . , so we have simplified matters by using pK_a's. Basically the "p" of anything is −log of that quantity, just as the pH is $-\log[H^+]$. Therefore,

$$\mathbf{pK_a = -\log K_a}$$

So if $K_a = 10^{-12}$, $pK_a = 12$. Since all reactions can be written in two ways, remember that when you use K_a's or pK_a's, you *must* write the reaction as an acid dissociation.

How you calculate the pH depends on which chemical species you have in solution. There are three possibilities: **weak acid only, weak base only,** or **buffer.** We consider only the first two here.

Weak-Acid-Only Situations

If we have 0.002 mol of the weak acid, acetic acid ($pK_a = 4.76$), and we bring the volume up to 100 mL with pure water, we will have a solution of HAc at 0.02 M. Some of that HAc will break down via the following equation:

$$\mathbf{HAc \rightleftharpoons H^+ + Ac^-}$$

How much will break down? We don't know because it is a weak acid and doesn't break down completely. The pK_a is a measure of acid strength and indirectly will tell us how much breaks down. There are shortcut formulas available to do these calculations:

$$\mathbf{pH = \frac{pK_a - \log[HA]}{2}}$$

$$\mathbf{= \frac{4.76 - \log(0.02)}{2}}$$

$$\mathbf{= 3.23}$$

Remember, use this equation when the problem gives you the amount or concentration of a weak acid and you have no way of determining the amount of H^+ or A^-. This shortcut formula works for most reasonable concentrations of acids that you will see in biochemistry. The shortcut formulas are valid for concentrations up to about 0.1 M and for pK_a values as low as about 3. Once the acid becomes much stronger than that or the total concentration is higher, the shortcut formulas start to break down. (See Section 2.11 for more information on these.)

Weak-Base-Only Situations

What if we start out with sodium acetate, NaAc. This is the weak base formed from acetic acid. If we start with a weak base, some of it will react with water via the following equation:

$$\mathbf{NaAc + H_2O \rightleftharpoons Na^+ + HAc + OH^-}$$

How much will react this way? Again, if this had been a strong base, the calculation would be simple; however, because it is a weak base, we are not sure to what extent the reaction occurs. The pK_a indirectly tells us this. There is another equation to use for this situation:

$$\mathbf{pH} = \frac{\mathbf{pK_a + 14 + \log[A^-]}}{\mathbf{2}}$$

$$= \frac{\mathbf{4.76 + 14 + \log(0.02)}}{\mathbf{2}}$$

$$\mathbf{= 8.53}$$

2.3 Polyprotic Acids

The use of polyprotic acids, those with more than one hydrogen that can dissociate, creates one other complication. Each dissociation of a hydrogen has its own dissociation constant. If you look at Table 2.2, you will see several weak acids with more than one pK_a listed. This means they are polyprotic acids. Not to worry! The beauty of the system is that you normally have to worry about only one of them at a time. For example, if you start out with H_3PO_4, you have a weak-acid-only situation, and you use 2.12 for the pK_a. If you start out with Na_3PO_4, you have a weak-base-only situation, and you use 12.32 for the pK_a.

There is unfortunately one other possibility, and that is an **intermediate of a polyprotic acid.** If you put 0.01 mol of NaH_2PO_4 into water, what type of solution do you have? The active species is $H_2PO_4^-$, but what is it? It could be the acid in

$$\mathbf{H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{2-}}$$

or it could be the base in

$$\mathbf{H_2PO_4^- + H_2O \rightleftharpoons H_3PO_4 + OH^-}$$

As it turns out, when you have an intermediate of a polyprotic acid, the pH is controlled solely by the two pK_a values and is independent of the concentration of the solution. The pH is calculated by using the following equation:

$$\mathbf{pH} = \frac{\mathbf{pK_a1 + pK_a2}}{\mathbf{2}}$$

2.4 Buffers

What happens if we add both HAc and NaAc to the same solution? This is the definition of a buffer because it has both the weak acid and the weak base in the same solution. Some of the HAc would tend to break down via the equation

$$\mathbf{HAc \rightleftharpoons H^+ + Ac^-}$$

and some of the NaAc would tend to break down via the other equation:

$$\mathbf{NaAc + H_2O \rightleftharpoons Na^+ + HAc + OH^-}$$

This would be very confusing except that with weak acid systems these two competing reactions tend to cancel each other out and these reactions do not happen to any great extent because they are so weak.

The *Henderson–Hasselbalch equation* can be used to calculate the pH when you have a buffer:

$$\mathbf{pH = pK_a + \log\left(\frac{A^-}{HA}\right)}$$

So, if we add 0.001 mol of HAc to 0.002 mol of NaAc and bring the volume up to 100 mL with pure water, the pH will be

$$\mathrm{pH} = 4.76 + \log\left(\frac{0.002}{0.001}\right)$$

$$= 5.06$$

You may have noticed that, although we used three different equations for three different situations, we used the same pK_a each time. Why? The pK_a is really a number that is a constant for a reaction rather than a particular molecule. In each case, we dealt with acetic acid and acetate. The pK_a of 4.76 is the pK_a of the reaction

$$\mathbf{HAc \rightleftharpoons H^+ + Ac^-}$$

Why a Buffer Is a Buffer

Buffers resist pH changes because they use up excess hydrogen ion or hydroxide ion. If we have a solution with both a weak acid and its salt and we add some H^+, then the following reaction occurs:

$$\mathbf{A^- + H^+ \rightarrow HA}$$

TIP 2.1 A buffer is a solution that contains both the weak acid form and the weak base form. Your success with the write up for this chapter will depend on how well you recognize whether a solution is a weak acid, a weak base, or a buffer.