Chapter 1 -- Biochemistry Boot Camp

Experiment 1 -- Use of Pipettors

Time Required

This brief experiment should take no more than 30 minutes, usually less if there is ample equipment.

Materials Required

P-100 or P-200 pipetmen (Rainin, Integrapette, Eppendorf)
P-1000 pipetmen
Top loading balances or others with a sensitivity of at least 0.01g
Deionized water

Hints for Preparation and Implementation

- If equipment is limiting, students can stagger which part they start first to better share the pipetmen.
- Be sure to give a prelab lecture about how to use a pipettor. Many students, including grad students, have never received any formal training in this. This lab, though brief, will establish the quality of results that will be seen for the rest of the semester.
- Make sure that students identify poorly calibrated pipets.
- If a student thinks that their pipettor draws up a low volume, make sure the pipet tips are on tight, the pipet is set to the correct volume, and that the lower barrel of the pipettor is screwed on tight. The latter is a common problem with Eppendorf pipettors.

Waste Disposal

None

Answers to Prelab Questions

1. What is the useable range of a P-1000 Pipetman?

100 to 1000 μL

2. What is the difference between accuracy and precision?

Accuracy is the relationship between the number you read and the true value. In other words, if you try to pipet 1 mL and you actually pipet 0.99 mL, then the pipetting was accurate. Its error was only 1%. Precision is how reproducible the numbers are, so if you

try to pipet 1 mL five times and you pipet 0.70, 0.70, 0.70, 0.70, and 0.70, then your pipetting was very precise although it was also very inaccurate.

3. What should 100 μL of water weigh?

Since water weighs 1 g/mL, the weight would be calculated thusly:

1 g/mL x 0.1 mL = **0.1 g**

4. What should 1000 μL of water weigh?

The answer is 1 g, since 1000 μL is the same as 1 mL.

Sample Data and Analysis of Results

Part A -- Precision of P-100 or P-200 pipettors

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

Weight #1 (x_1) 0.09
Weight #2 (x_2) 0.09
Weight #3 (x_3) 0.11

2. Average the three weights.

Average of three trials: 0.097

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

$$\% \text{ error} = \frac{|\, 0.097 - 0.100 \text{ g} \,|}{0.1 \text{ g}} \times 100 = 3\ \%$$

4. Calculate the mean deviation for the three trials:

$$\text{mean deviation} = \frac{\Sigma |\, x_i - x_{avg} \,|}{3} =$$

[-(0.09-0.097)+-(0.09-0.097)+(0.11-0.097)]/3 = **0.009**

Part B -- Precision of P-1000 pipettors

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

Weight #1 (x_1) 0.99
Weight #2 (x_2) 1.05
Weight #3 (x_3) 0.97

2. Average the three weights.

Average of three trials: 1.00

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

$$\% \text{ error} = \frac{|\text{avg. weight} - 1.00\text{ g}|}{1.00\text{ g}} \times 100 = 0$$

4. Calculate the mean deviation for the three trials:

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

$$[-(0.99-1.00)+(1.05-1.00)+-(0.97-1.00)]/3$$
$$= \mathbf{0.03}$$

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

Weight #1 (x_1) 0.09
Weight #2 (x_2) 0.08
Weight #3 (x_3) 0.12

6. Average the three weights.

Average of three trials: 0.10

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

$$\% \text{ error} = \frac{|\text{avg. weight} - 0.10\text{ g}|}{0.10\text{ g}} \times 100 = 0$$

8. Calculate the mean deviation for the three trials:

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

$$[-(0.09-0.10)+-(0.08-1.00)+ (0.12-0.10)]/3$$
$$= \mathbf{0.02}$$

Part C – Pipettors in the Lab

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

The % error is the measure of the accuracy of a pipetman. From the data presented, the P-1000 would appear to be the more accurate, since it had the smallest % error.

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

This is the trickier question. If you just look at the mean deviations, it appears that the P-1000 has the larger mean deviation of 0.02 when compared to the 0.009 of the P-100, when both were used to pipet 100 µL. However, one must bear in mind that the total weight expected is also important. For the pipetting of 1000 µL, if you calculated a % mean deviation by dividing by the expected weight and multiplying by 100, the P-1000 would, once again, have the smaller number.

3. What are the take-home messages from this exercise?

Take your pick from any of the following:

- There are different types of pipetmen that you must learn to use.
- Pipetmen are precise in the hands of a trained user, but not necessarily accurate.
- The accuracy of a pipet should be checked frequently.
- It is easy to check the accuracy and precision by doing a water weight test.
- Pipetmen have a range outside of which they are not accurate.
- You should learn what the correct volume of solution looks like in a pipet tip.
- Others that you can think of.

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?

It is generally better to use a liquid transfer device closer to its maximum volume, so using the P-200 would be better.

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

It is more like a serological pipet, as you expel the liquid completely out of it all the way to the tip.

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?

One reason might be that the tip is not on tightly. In that case, just tighten the tip. Another might be that the liquid is an organic solvent, which might have a very low surface tension. To remedy that, draw up the liquid into the tip and then expel it. Then draw up the solution again. It usually will hold in the tip the second time after pre-wetting the tip.

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?

You need 0.2 liters of a 0.1 mole/liter solution or 0.02 moles of the solute. If the MW is 121.1 g/mol, you need 0.02 x 121.1 or 2.4 grams of solute. Thus, you weigh 2.4 grams of solute into a vessel and bring the volume up to 200 mL.

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 molar, millimolar, and micromolar?

The first dilution is a 10 to 1, since you start with 10 mL and end with 100 mL. The second dilution is a 5 to 1, since you took 5 mL and brought the volume to 25. Thus, the total dilution factor is 50 to 1. Since you started with 0.l M, the final concentration in molar is 0.1/50 or 0.002 M. this is 2 mM and 2000 µM.

Answers to Additional Problem Set

1. How many grams of solid NaOH are required to prepare 200 mL of a 0.05 M solution?
0.4 g

2. What would be the concentration from Problem 1 expressed in % w/v?
0.2 % w/v

3. How many mL of 5M NaCl are required to prepare 1500 mL of 0.002 M NaCl?
0.6 mL

4. What would be the concentration of the diluted solution from Problem 3 expressed in mM, µM, and nM?
2 mM, 2000 µM, 2 x 10^6 nM

5. A solution contains 15 g of $CaCl_2$ in a total volume of 190 mL. Express the concentration in terms of g/L, % w/v, M, and mM.
79 g/L, 7.9 % w/v, 0.71 M, 710 mM

6. Given stock solutions of glucose (1M), Asparagine (100 mM) and NaH_2PO_4 (50 mM), how much of each solution would you need to prepare 500 mL of a reagent which contains 0.05 M glucose, 10 mM Asparagine and 2 mM NaH_2PO_4?
25 mL glucose, 50 mL Asparagine, 20 mL sodium phosphate

7. Calculate the number of millimoles in 500 mg of each of the following amino acids: alanine (MW = 89), leucine (131), tryptophan (204), cysteine (121), and glutamic acid (147).
5.6 mmol Ala, 3.8 mmol Leu, 2.5 mmol Trp, 4.1 mmol Cys, 3.4 mmol Glu

8. What molarity of HCl is needed so that 5 mL diluted to 300 mL will yield 0.2 M?
12 M

9. How much 0.2 M HCl can be made from 5.0 mL of 12.0 M HCl solution?
300 mL

10. What weight of glucose is required to prepare 2 L of a 5% w/v solution?
100 g

11. How many mL of an 8.56% solution can be prepared from 42.8 g of sucrose?

500 mL

12. How many mL of $CHCl_3$ are needed to prepare a 2.5% v/v solution in 500 mL of methanol?

12.5 mL

13. If a 250 mL solution of ethanol in water is prepared with 4 mL of absolute ethanol, what is the concentration of ethanol in % v/v?

1.6 % v/v

Chapter 2 – Acids, Bases, and Buffers

Experiment 2 – Preparation of Buffers

Time Required

1.5 to 2 hours if there is no equipment constraint.

Materials Required

pH meters, preferably digital	
NaOH, 1 M	15 mL
HCl, 1 M	15 mL
NaAcetate	1 g
CAPS	"
HEPES	"
Na_2HPO_4	"
Tricine	"
TRIS	"
NaCitrate	"
NaH_2PO_4 unknowns,0.1 M, pH 5.6	5 ml
NaH_2PO_4 unknowns,0.1 M, pH 7	"
NaH_2PO_4 unknowns,0.1 M, pH 8	"

Sources for key ingredients

None of the chemicals used for this experiment need to be particularly pure. Use the cheapest source you can find. For the buffer compounds, it does not matter if you use the acid form or the basic form, as the students will adjust the pH anyway when making up the buffer. Sigma and VWR offer good sources of these compounds. For convenience, the Sigma materials are listed below:

Compound	**Catalog #**	**Price (2004)**
Acetate (sodium salt)	Sigma 24,124-5	$64.30 per Kg
CAPS	Sigma C 2632	$243 per Kg
Citrate (sodium salt)	Sigma C 3674	$27.60 per Kg
HEPES (sodium salt)	Sigma H 7006	$216.50 per 500 g
Tricine	Sigma T 0377	$86 per 250 g
TRIS (Trizma base)	Sigma T 1503	$84 per Kg

Hints for Preparation and Implementation

This is a fast lab that usually only slows down due to the logistics of acquiring the materials and using the balances and pH meters.

- Make sure all available pH meters and balances are ready to go.
- Dispense the dry buffer powders so that there are several locations or several containers students can use to avoid a big line of students waiting to get started.
- Dispense unknowns in large test tubes with about 10 mL each.

Waste Disposal

All unknowns can be dumped down the sink at the week's end. Save the HCl and NaOH for future labs. Discard or save dry buffers depending on quality and quantity.

Answers to Prelab Questions

1. Calculate the weight of buffer you would use to make the buffer for part A for the six possibilities:

0.1 mol/liter x 0.1 liters = 0.01 moles. You need 0.01 moles of the buffer. Moles x MW = grams.

Acetate	136.1 g/mol x 0.01 mol = 1.36 g
CAPS	221.3 g/mol x 0.01 mol = 2.21 g
Citrate	294.1 g/mol x 0.01 mol = 2.94 g
HEPES	238.3 g/mol x 0.01 mol = 2.38 g
Phosphate	142.0 g/mol x 0.01 mol = 1.42 g
Tricine	179.2 g/mol x 0.01 mol = 1.79 g
TRIS	121.1 g/mol x 0.01 mol = 1.21 g

2. If we give you HEPES in the basic form and ask you to make a buffer of pH 8.0, will you add HCl or NaOH to adjust the pH? Why?

Calculate what would be the initial pH of a solution of HEPES base. Using the formula:

$pH = (pKa + 14 + \log[base])/2 = (7.55 + 14 + \log[0.1])/2 = 10.28$

We can see that the pH will be more basic than the desired 8.0. Therefore, we will have to add acid. In general, anytime you make up a buffer in its basic form, you will need to add acid to get to a useable buffer pH. This question could be answered philosophically as well. A buffer always needs to be a combination of the acid and basic form of the buffer. If we start with just the basic form of a buffer, we will always need to create the acidic form, so we would need to add HCl.

Sample Data and Analysis of Results

Data

Part A

Buffer #1: phosphate grams: 1.42 Original pH: 5.4

Buffer #2: Tricine grams: 1.79 Original pH: 10.2

Part B

Buffer #1 pH of 0.1 M 8.10 pH of 0.01 M 8.40 pH of 0.001 M 8.75

Buffer #2 pH of 0.1 M 8.10 pH of 0.01 M 7.99 pH of 0.001 M 7.95

Part C

Distilled water pH: 5-9 (depending on water source and cleanliness of beakers

Unknown # 21 pH: 7.0

Unknown # 36 pH: 5.6

Part D

Buffer chosen: Tricine pH: 8.10 pKa: 8.15

Acid or base added: NaOH

pH after adding acid or base: 8.18

pH of 50 mL of water: 7.0

pH after adding acid or base: 12.0

Calculations

1. (a)What is the ratio of A^-/HA in your buffer after you adjusted its pH to the required value?

This will depend on which buffer you used and at what pH. A good buffer was defined as one with a pH that was at most 1 pH unit away from the pKa.

As an example, consider a pH 8.5 TRIS buffer.

Since you made a 0.1 M solution and you have 100 ml, the total number of moles of buffer is calculated as the following:

0.1 mol/lit x 0.1 lit = 0.01 moles. Therefore HA + A^- = 0.01 moles.
Now you have to figure out what percentage is in each form at the pH you chose. To do that we use the Henderson-Hasselbalch equation.

$pH = pKa + \log([A^-]/[HA])$ $8.5 = 8.3 + \log([A^-]/[HA])$

$0.2 = \log([A^-]/[HA])$ $[A^-]/[HA] = 1.58$

(b) How many micromoles of A^- and HA are present in the solution?

Using two equations in two unknowns, we can solve for the moles of HA and A^-.

$A^- + HA = 0.01$ moles and $A^- = 1.58HA$ (from the last equation above)

$1.58\ HA + HA = 0.01$ moles $2.58HA = 0.01$ moles

$HA = 0.0039$ moles

$A^- = 0.01$ moles - 0.0039 moles = .0061 moles.

Therefore your initial conditions are the following:

$8.5 = 8.3 + \log(0.0061/0.0039)$

(c) If you now add 3 mL of 1M NaOH, will you still have a valid buffer?

You then add 3 ml of 1 M NaOH, which is 0.003 moles. For every mole of OH^- you add, the following reaction will occur:

$HA + OH^- \rightarrow A^-$, which uses up the OH^- until one of the reagents runs out.

Since you have 0.003 moles of OH^- and 0.0039 moles of HA, the OH^- will run out first. You will be left with 0.0009 moles of HA and will have created 0.003 more moles of A^-, so it will now be 0.0091 moles.

The final step is to run this new ratio through the Henderson Hasselbalch equation:

$pH = 8.3 + \log(0.0091/0.0009) = 8.3 + (0.63) =$ **9.30**

Since the new pH is 1 unit from the pKa, you are right on the border. In this case, you would still have a valid buffer, but just barely.

2. Calculate the theoretical pH of one of your buffers at 0º C. Assume that room temperature is 22 º C.

Using TRIS at pH 8.0, the change in pKa with temperature is -.031 per degree C. Going from 22 degrees down to 0 degrees is a -22 degree change.

-22 x -.031 = 0.682. If the original pH were 8.0, lowering the temperature would change the pH to 8**.7.**

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

Since 8.5 is on the basic side of the pKa of 7.55, you would start with HEPES in the basic form and add HCl to bring the pH down to 8.5.

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?

The pKa for acetic acid is 4.76. Thus, a buffer using acetic acid and acetate as its buffering species would only be effective at a pH of 3.76-5.76. By making up a solution of sodium acetate at pH 8.0, the solution was not a buffer at all, rather just a weak base solution.

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

For a weak acid only solution, there is an equation to use:

$$pH = \frac{pKa - \log[HA]}{2} = \frac{8.3 - \log(0.1)}{2} = \mathbf{4.65}$$

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

50 ml is 0.05 L. If you have 0.05 L of 0.1 M TRIS, you have 0.005 moles of TRIS, which all started out in the acid form, so you used the acid only equation above. Now you add some base, which will create the basic form. Two ml of 1 M NaOH is 0.002 moles of NaOH or 0.002 moles of OH^-. The OH^- will react with the TRIS acid to create TRIS base until you run out of something, which will be the hydroxide.

$TRIS_a + OH^- \rightarrow TRIS_b$

When the reaction is over, you will have 0.002 moles of $TRIS_b$ and 0.003 moles of $TRIS_a$ left over. Now you have a buffer and can use the H-H equation:

$pH = pKa + \log(A^-/HA) = 8.3 + \log(0.002/0.003) = 8.1$

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

For a weak base only problem, use the following equation:

$$pH = \frac{pKa + 14 + \log[A^-]}{2} = \frac{7.55 + 14 + \log(0.1)}{2} = 10.3$$

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

3 mL of 1M HCl is 0.003 moles of H^+. This will react with the A^- to create HA. The new amounts will be 0.003 moles of HA and 0.007 moles of A^-. Plug these into the Henderson-Hasselbalch equation:

$pH = 7.55 + \log(0.007/0.003) = 7.9$

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

This will vary depending on the results for the experiment, but if the pH electrode is sensitive enough, there will always be a change in the pH. The change should be much greater when dilution from 0.01M to 0.001M, especially if the water source is not pure. Acidic buffers, like phosphates, tend to have the pH rise with dilution, while basic buffers like tricine have the pH fall with dilution.

Answers to Additional Problem Set

1. Calculate the pH of a 0.1M HCl solution.
1

2. Calculate the pH of a 0.1M NaOH solution.
13

3. What is the concentration of $[H^+]$ in M, mM, and μM for a solution of pH 5?
1×10^{-5} M, 1×10^{-2} mM, 10 μM

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?
12.52

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.
pH = 2.12, $K_a = 2.3 \times 10^{-4}$

6. What is the pH of a 0.05 M solution of TRIS acid (pKa = 8.3)?
4.8

7. What is the pH of a 0.045 M solution of TRIS base?
10.48

8. If you mixed 50 mL of 0.1 M TRIS acid with 60 mL of 0.2 M TRIS base, what would be the resulting pH?
8.68

9. If you added 1 mL of 1 M NaOH to the solution in 6 above, what would be the pH?
8.81

10. How many total mL of 1 M NaOH could you add to the solution in 6 above and still have a good buffer (i.e. within one pH unit of the pK_a)?
3.5 mL

11. If you were making 100 mL of a 0.1 M HEPES buffer starting from HEPES in the basic form, would it be prudent to get 50 mL of 1 M HCl from the community reagent bottle to use for your titration?

No, because you would never need 0.05 moles of strong acid to titrate 0.01 moles of buffer

12. An enzyme-catalyzed reaction is carried out in 100 mL of a 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 moles of H^+ were produced. What was the ratio of TRIS base to TRIS acid at the start of the experiment? What was the ratio at the end of the experiment? What was the final pH?

The ratio of A^-/HA at the start was 0.5 to 1
The ratio of A^-/HA at the end was 0.15
The final pH was 7.47

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 mL that would be needed to make 300 mL of 0.2 M HEPES buffer at pH 7.2.

14.3 g HEPES acid and 18.5 mL of 1 M NaOH required