

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 μ L of water weigh?

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

$$1 \text{ g/mL} \times 0.1 \text{ mL} = \mathbf{0.1 \text{ g}}$$

4. What should 1000 μ L of water weigh?

The answer is 1 g, since 1000 μ L 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 μ L:

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.

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

4. Calculate the mean deviation for the three trials:

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

$$[-(0.09-0.097)+-(0.09-0.097)+(0.11-0.097)]/3 = \mathbf{0.009}$$

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:

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

4. Calculate the mean deviation for the three trials:

$$\begin{aligned} \text{mean deviation} &= \frac{\sum |x_i - x_{\text{avg}}|}{3} = \\ &= \frac{[-(0.99-1.00)+(1.05-1.00)+-(0.97-1.00)]/3}{0.03} \end{aligned}$$

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:

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

8. Calculate the mean deviation for the three trials:

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

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

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 0.02×121.1 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 0.1 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 , 2×10^6 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