Inquiry Module 1: Checking the calibration of a micropipette

1. Introduction
Larger volumes (1mL and more) are usually measured using pipets or measuring cylinders. Such cylinders and pipets are labelled with given fixed volume marks:

But what do we use to measure out volumes that are < 1mL?

Here, adjustable micropipets come in handy (See picture on right).

They use movable mechanical parts like a small piston inside a cylinder to dispense small volumes by positive displacement. However, it is easy to see how these mechanical parts can get damaged, bent or misaligned (eg when a micropipet is accidentally dropped).

So what is a good way to check the accuracy and calibration of such adjustable micropipets?

2. Purpose of the lab

Conduct an experiment to determine the precision and accuracy of a micropipette using the gravimetric method.

3. Agenda for this module

Today:
- Form groups, 3 students per group.
- Presentation: Using micropipettes, designing experiments, error calculations.
- Complete Math Moment Practice Problems –discuss problems with your group, show your work to instructor when done.

Next Week:
- Practice the use of a micropipette; check each other’s work within your group.
- Conduct the experiment with your group, each student records data in their notebooks individually.
4. **Background:**

- You will need to be able to convert between units of mL and uL and between units of g and mg for this experiment. See the table below for most commonly used prefixes in this lab. As a science student, it is recommended that you memorize these prefixes.

<table>
<thead>
<tr>
<th>Prefix</th>
<th>Factor</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>kilo</td>
<td>$10^3$</td>
<td>k</td>
</tr>
<tr>
<td>centi</td>
<td>$10^{-2}$</td>
<td>c</td>
</tr>
<tr>
<td>milli</td>
<td>$10^{-3}$</td>
<td>m</td>
</tr>
<tr>
<td>micro</td>
<td>$10^{-6}$</td>
<td>µ</td>
</tr>
<tr>
<td>nano</td>
<td>$10^{-9}$</td>
<td>n</td>
</tr>
</tbody>
</table>

Note: The larger or smaller unit is denoted by attaching the prefix to the unit name. For example, a centimeter (cm) is $10^{-2}$ meter (m), a millivolt (mV) is $10^{-3}$ (V), and a L is $10^6$ microliters (uL).

For everyday use in the biochemistry lab, the easiest approach is to simply memorize the following:

- 1L = 1000mL; 1 gram = 1000 mg
- 1mL = 1000 uL; 1 mg = 1000 ug
- 1 uL = 1000nL; 1 ug = 1000 ng

- The gravimetric method is based on the measurement of mass.
- Density = mass/volume
- The density of water is 1 gram/mL. This means that 1 mL of water weighs 1 gram.
- Every µL of water thus weighs 0.001 grams or 1 mg.
- Standard deviation measures precision. This is the uncertainty in a measured quantity. Standard deviation has the same units as the quantity itself.
- Percent error provides the deviation from the “actual value” and provides information about accuracy. This indicates how close the experimentally determined value is to the expected or actual value. For most experiments, there is no authoritative, expected value. For this calibration experiment, since the density of water is 1 g/mL, 1 mL of water should theoretically weigh 1 g. In other words, the actual mass of 1 mL of water is 1 g.

\[
s = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}
\]

Where, \(x\) = data item
\(n\) = number of measurements
\(\bar{x}\) = average of data items

\[
\% error = \frac{\text{Actual - Experimental}}{\text{Actual}} \times 100
\]

Percent Error

Standard Deviation

- There is useful material in Chapters 1A, 1D and 1E (also read 1B about lab notebook keeping) in Rodney Boyer’s Book. You should learn about micropipette use, accuracy, precision, how to determine average (mean), standard deviation, and percent error.
- The instructor will likely have checked the calibration of the balances using a set of standard weights. It is important to check the calibration of the balances for this experiment.
5. **Math Moment**
   Note: The goal of these calculations is to prepare you for the experiment. Show instructor your work when done.

1. How many mL is 250 uL equal to?

2. How many mg is 4.58 g equal to?

3. How much does 250 uL of water weigh? Density of water is 1 g/mL.

4. For a perfectly balanced scale and micropipette, fill in the expected weights in the bottom row of the table below.

<table>
<thead>
<tr>
<th>Volume of water measured (uL)</th>
<th>1000</th>
<th>800</th>
<th>600</th>
<th>400</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. For data from question 4, draw data points into the graph below. What is the slope of the line formed by the points? What are the units of the slope?

6. A student weighed 500 uL of water. What do you expect the mass to be if the micropipette and scale are in excellent calibration?

7. She does 5 replicate measurements of 500 uL of water. She is using the P1000 micropipette. The masses are 0.512 g, 0.561 g, 0.466 g, 0.501 g, and 0.477 g. Calculate the mean (average) mass and standard deviation of these values. Express you answer as mass +/- standard deviation and remember to include units.

8. For the measurements in question 7, what is the “expected” or “actual” value? In other words, how much do you expect 500 uL of water to weigh? Hint: remember that the density of water is 1 g/mL.

9. What is the percent error of the measurements in question 7?

10. Do you consider the micropipette in question 7 to be well calibrated? Why or why not?
11. Why should the pipette tip not be immersed more than a few millimeters below the surface of the fluid?

12. What is the volume dialed on each micropipette? Write the volumes with its unit on the lines below.

<table>
<thead>
<tr>
<th>P10</th>
<th>P10</th>
<th>P20</th>
<th>P20</th>
<th>P1000</th>
<th>P1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

6. **Supplies Provided**

- P1000 micropipette
- Micropipette tips (large)
- Analytical Balance
- Weigh boats (the plastic containers to hold measured material)
- Deionized water
- Water with food color - do not use this for the experiment. This is for seeing the liquid better when practicing aspirating
- P200 and P20 and appropriate tips are also available for pipetting practice.

7. **Practicing using a micropipette**

Here are the steps to be followed when using a micropipette:

- Press plunger down to first stop
- Immerse tip in liquid to be taken up
- Slowly aspirate liquid
- Dispense liquid into receptacle, depressing plunger to second stop
- As a rule, do not dispense liquid into air: either touch tip to liquid already present in receptacle tube or touch tip to side of tube

Try using a micropipette. Adjust the volume (try 1000 µL, 800 µL, 600 µL, 400 µL, and 200 µL in a P1000), aspirate the liquid (the first stop) and dispense (the second stop, or “all the way down”). Use colored water and watch the volume level in the tip. Try using the P20 (for several volumes between 2 µL and 20 µL) and the P200 (for several volumes between 10 µL and 200 µL).

8. **Experimental Protocol**

1. Set a P1000 pipettor to 1000 µL.
2. Put a weigh boat on a balance and tare to zero.
3. “Prime” the pipette tip once by drawing up ddH2O and dispensing it back into the water container.
4. Pipet 1000 µL of distilled water and put it on the weigh boat. Record the weight.
5. Repeat the steps 4 and 5 about 4 more times for total of 5 trials. If you encounter outliers, do more trials.
6. Take the average (mean) of the weights.
7. Calculate the % error between the average of the 5 trials and the true value (for true value, think about density).
8. Calculate the standard deviation for the 5 trials (see CH1 in your book).
9. Set a P1000 pipettor to 750 µL.
10. Put a weigh boat on a balance and tare to zero.
11. Pipet 750 µL of distilled water and put it on the weigh boat. Record the weight.
12. Repeat the steps 10 and 11 about 4 more times for total of 5 trials
13. Take the average (mean) of the weights.
14. Calculate the %error between the average of the 5 trials and the true value.
15. Calculate the standard deviation for the 5 trials (see CH1 in your book). Record the pipettor number and barcode for your results.
16. Repeat process (steps 9-15) for 500 µL, 250 µL, and 100 µL.

9. **Common Mistakes and Some Advice**

- Remember to include units with all your numbers and calculations.
- Insufficient number of replicate measurements (repeat measurements). You must always do a minimum of 3 – 5 measurements. If the values are very different from each other or you observe an outlier, do more measurements.
- Pipetting errors. Remember to check the volume of the micropipette before you aspirate. You need to check the volume every time you use it! Keep an eye on the liquid level in the tip to make sure that you draw in and expel the correct volume each time.
- You must use an analytical balance. You can reuse a weigh boat during this experiment: tare it, add water, weigh, tare again etc.

10. **Vocabulary**

Density, micropipette, plunger, accuracy, reproducibility (precision), standard deviation, standard error, mean, expel.

11. **Safety**

You must wear safety glasses when conducting the experiment. You must never eat or drink in the laboratory. Be patient when waiting to use the analytical balances to avoid bumping into each other. Any observed violations of these rules will result in lower final grade and/or removal from the lab. These safety items are solely the responsibility of the student.

12. **Clean-up**

Tips should be discarded in the regular trash. Do not use the biohazard trash unless instructed to do so. Return micropipettes in the correct boxes, the last person puts the boxes away. If you are the last user, make sure balance is clean and dry, the glass doors are closed, and the balance is turned off. Place all other items where you got them from. Make sure they are clean. Leave your bench and the balances and areas around the balances ready for the next class to start working.

13. **Data Sheet (homework)**

Each person prepares a data sheet. Provide the following:

- Title, name, section, date
- Table showing all the data collected, average, standard deviation, percent error
- Graph: prepare a graph in Excel that shows the relationship between weight in mg (y-axis) and volume in uL (x-axis) for your micropipette, show linear fit to data and slope on the graph. You will plot the points, then add a
linear trendline. Show the equation (generic format $y = mx+b$) and $R^2$ value. You should include the 0,0 data point as by definition 0 uL of water should weigh 0 mg.

- NO introduction, methods section or conclusions are needed.

- Show sample calculations for mean, percent error and standard deviation. Is the weight a dependent or independent variable?