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 < 1 mL?

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 (e.g. 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**
You have the opportunity to learn or practice the basics of pipetting. It is very easy, when under pressure from intense pipetting workloads, to forget the simple principles that ensure the right results. Your goal today is to:

   *Design and 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 – you can discuss problems with your group, show your work to instructor when done.

Next Week:
- Design an experiment for checking the precision and accuracy of a P1000 micropipette with your group. Each student writes down the experimental plan as a step-by-step protocol. Hand the protocol in at the end of class.
- Practice the use of a micropipette; check each other’s work within your group.
- Conduct the experiment you designed 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 µL 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 example, to convert 2 mL to units of uL:

$$2 \text{ mL} \times (1000 \text{ uL/1 mL}) = 2000 \text{ uL}$$

Notice how the mLs cancelled out and you were left with uL.

Notice that you are multiplying by 1 because $1000 \text{ uL} = 1 \text{ mL}$ and $1000 \text{ uL/1 mL} = 1$.

- 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 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
\(\bar{x}\) is the mean
\(n\) is the sample size.

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

Percent Error

- There is useful material in Chapters 1A, 1D and 1E (also 1B about lab notebook recommended) 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 experimental design activity that follows. 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?

![Graph](image)

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 so this is at 50 % of the maximum volume (maximum volume of P1000 is 1000 uL). 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 your 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. You want to check the calibration of a P1000 micropipette at 20% volume. What volume do you set the micropipette to in uL? Draw a picture to show how this looks on the P1000 volume display that has 3 digits shown.

12. How many stops does a micromicropipette plunger have?

13. Why should the micromicropipette plunger never be allowed to snap back in place?

14. Why should the pipette tip not be immersed more than a few millimeters below the surface of the fluid?
15. What is the volume dialed on each micropipette? Write the volumes with its unit on the lines below.

P10 and P20 the last digit is a decimal

<table>
<thead>
<tr>
<th>P10</th>
<th>P10</th>
<th>P20</th>
<th>P200</th>
<th>P1000</th>
<th>P1000</th>
</tr>
</thead>
<tbody>
<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. **Experimental Design**

With your group, plan out how you will conduct an experiment to meet your goal. Your goal is to check the calibration of a P1000 micropipette for 100% volume (1000 uL), 80% volume (800 uL), 60% volume (600 uL), 40% (400 uL) and 20% volume (200 uL). Write a 1-page experimental protocol (step-by-step directions). Consider the number of times each experiment should be repeated (minimum of 3 or 5 times but more if you get outlier results) for accurate results. It is best to set up a grid that shows all the measurements you plan to do. Each group will design the protocol together, discussing it, and come to a consensus. Each student will write down the protocol on the provided form individually (see at the end of the document). The protocol will be handed in at the end of today’s class. Include your name, the names of all group members, the date, class, section, title, module number and instructor name. At the end of the protocol, write down a few notes about what was confusing or difficult about designing this experiment.

8. **Help for Getting Started with Experimental Design**

Think how you can find out that the amount you are pipetting is correct. Thus, if you were supposed to pipet 1000 uL, how would you confirm that aspirated amount is actually 1000 uL? Remember that you are supposed to use the gravimetric method, which involves using a scale. Also, remember that the density of water is 1 g/mL. Could weighing the liquid you pipette give you information about the volume? Remember, your goal is to write a step-by-step protocol (similar to a recipe) of what to do. You can start your protocol with just a few steps and then revise and provide more details as you go. You can brainstorm with your group. You can try drawing a cartoon of what you plan to do to help you visualize your plan.

9. **Practicing using a micropipette**

Try using a micropipette. Adjust the volume (try 1000 uL, 800 uL, 600 uL, 400 uL, and 200 uL 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 uL and 20 uL) and the P200 (for several volumes between 10 uL and 200 uL).

10. **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.

11. Vocabulary

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

12. 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.

13. 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.

14. Data Sheet (homework)

Each group prepares a data sheet (one per group). Please, provide only a title and your data on a single page that shows data in neat tables (remember units etc.), graphs. Please, do not provide an introduction, methods section or conclusion. Please, show sample calculations for mean, percent error and standard deviation. Include a graph that shows the relationship between weight and volume for water for your P1000 micropipette, remember axis labels including units. Is the weight a dependent or independent variable? Determine the slope of this line by fitting the data to a linear equation (add trendline y = mx+b) and provide units for the slope. Remember to format your data sheet professionally and include your names, class, section, instructor, date, and title. Include a number and a caption (brief paragraph describing what is shown) for each table and figure. Remember to include the following items:

• Table with all your measured weights (multiple values for each volume)
• Average weights for each volume (one value for each volume)
• Standard deviation for each volume (one value for each volume)
• Percent error for each volume (one value for each volume)
• Prepare a graph in Excel that shows the relationship between weight in mg and volume in uL 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 and \( R^2 \) value. You should include the 0,0 data point as by definition 0 uL of water should weigh 0 mg.