Experiment 3
Calibration of a Pipet

Objective: You will determine the accuracy of a 25 mL pipet and the precision of your technique using a 25 mL volumetric pipet.

Introduction: According to a table in Harris (Table 2-5, 7th edition) a 25 mL volumetric pipet has an accuracy of ± 0.3 mL and a precision of ± 0.05 mL. In this experiment you will seek to measure the accuracy and precision of one of the 25 mL volumetric pipets in the Chem 311 lab.

Accuracy is always evaluated by comparing it to an established standard. In this experiment the established standard will be the weight of water at a measured temperature. The data tabulated below comes from a trusted source; the CRC Handbook.

Densities of water at different temperatures

Temperature (°C)	Density (g/mL)
18.50	0.998531
18.75	0.998483
19.00	0.998435
19.25	0.998385
19.50	0.998335
19.75	0.998285
20.00	0.998234
20.25	0.998182
20.50	0.998129
20.75	0.998076
21.00	0.998022
21.25	0.997968
21.50	0.997913
21.75	0.997857
22.00	0.997800
22.25	0.997743
22.50	0.997686
22.75	0.997627
23.00	0.997568
23.25	0.997509
23.50	0.997449
23.75	0.997388
24.00	0.997327
24.25	0.997265
24.50	0.997202
24.75	0.997139
25.00	0.997075
25.25	0.997011
25.50	0.996946

Proper Pipeting Technique
From Harris, 7th Ed pg 28

Using a Transfer Pipet

Using a rubber bulb or other pipet suction device, *not your mouth,* suck liquid up past the calibration mark. Discard one or two pipet volumes of liquid to rinse traces of previous reagents from the pipet. After taking up a third volume past the calibration mark, quickly replace the bulb with your index finger at the end of the pipet. Gently pressing the pipet against the bottom of the vessel while removing the rubber bulb helps prevent liquid from draining below the mark while you put your finger in place. (Alternatively, you can use an automatic suction device that remains attached to the pipet.) Wipe the excess liquid off the outside of the pipet with a clean tissue. *Touch the tip of the pipet to the side of a beaker* and drain the liquid until the bottom of the meniscus just reaches the center of the mark, as shown in Figure 2-10. Touching the beaker draws liquid from the pipet without leaving part of a drop hanging when the liquid reaches the calibration mark.

Transfer the pipet to a receiving vessel and drain it by gravity *while holding the tip against the wall of the vessel.* After the liquid stops, hold the pipet to the wall for a few more seconds to complete draining. *Do not blow out the last drop.* The pipet should be nearly vertical at the end of delivery. When you finish with a pipet, you should rinse it with distilled water or soak it until you are ready to clean it. Solutions should never be allowed to dry inside a pipet because removing internal deposits is very difficult.

Procedure:

Wear gloves. Fingers leave fingerprints and will impact your results!

1. You will obtain about 500 mL of the lab water in the large containers at the ends of the aisles in a large beaker.
2. You will measure the temperature of the water. Record the measurement in your notebook.
3. Place a clean and dry plastic 250 mL beaker on an analytical balance and tear the balance to zero.
4. Take the beaker off the balance and pipet a 25 mL aliquot of the water into the clean and dry 250 mL beaker. Your instructor will demonstrate proper technique. One of the purposes of this experiment is to learn and practice proper technique.
5. Weigh the 25 mL aliquot on the analytical balance to the nearest ± 0.0001 g. Record the measurement in your notebook.
6. Tear the balance to zero. Take the beaker off the balance and pipet a 25 mL aliquot of the water into the clean and dry 250 mL beaker.
7. Weigh the 25 mL aliquot on the analytical balance to the nearest ± 0.0001 g. Record the measurement in your notebook.
8. Repeat steps 6-7 until you have at least 20 measurements of the mass of the 25 ml aliquot of water delivered by your pipet that are in reasonable agreement (10 from you and 10 from your partner).

Data Analysis and Discussion Points

Calculate the volume of water delivered using the pipet for each of the replicate measurements using the mass of the aliquot and the density from the table that corresponds to the measured temperature of the water. Produce a table of these results. (10 pts)

Calculate the mean volume delivered and its standard deviation (s_e) and its standard deviation of the mean (s_m) for your set of ten replicates and again for your partner's set of ten replicates. Provide a table that summarizes the mean, se and 95 % CL for your data and your partner's data. (15 pts)

Using the mean and the standard deviation of the mean of your set of ten replicates, evaluate the accuracy of your pipet. Does your data fall within the tolerance of the 25 mL pipet, as given in Harris? Why is the confidence limit a good metric to use in evaluating accuracy in this case? (15 pts/Discussion)

Using the s_e of your set of ten replicates, evaluate the precision of your pipeting technique with your 25 mL pipet. Does your precision match well with the expected precision according to Harris (see above)? Why is the standard deviation a good metric to use in evaluating precision? (15 pts Discussion)

Use the F-test [Comparison of Standard Deviations (4-4 in Harris)] to determine whether your precision was different than your partner's precision to the 95 % confidence limit (F_{table} = 3.18, if you both did 10 replicates) (10 pts/Data, 5 pts /Discussion)

Was your pipet functioning optimally? Was it draining properly of were there beads of water clinging to the walls of the pipet? (5 pts/Discussion)

REPORT

Abstract (10 pts)

Procedure (5 pts)

Spreadsheet (10 pts)

Data (35 pts)

Discussion (40 pts)