

## Calibrating Pipettes by the Spectrophotometric Method

### Principle

The ideal and most-often used method for calibrating pipettes is the gravimetric method, which: (1) utilizes an analytical balance to measure the mass of pipetted fluid; (2) converts the mass to volume; and (3) ascertains whether the *measured* volume is acceptably close to the *expected* volume. Sometimes, however, an analytical scale will not be available. And so, in such instances, an alternative method will be required. One such method utilizes a standard laboratory spectrophotometer.

The spectrophotometric method for calibrating pipettes requires only yellow food coloring dye and a few other basic materials. In aqueous solution, this dye (which can be obtained at the market) is known to follow Beer's Law when measured between 400 and 460 nm. The method is both simple and elegant, and can be summarized as follows: (1) prepare two equivalently diluted yellow dye solutions using both a gravimetrically standardized pipette and a non-calibrated pipette; and (2) compare the optical density (OD) of each. For example, to calibrate a 20 uL pipette by this method, one first would make a yellow dye solution. 100uL of this dye would be dispensed in water via a gravimetrically standardized 100 uL pipette. Next, one would prepare an equivalent dye solution using five aliquots from the 20uL pipette (i.e.,  $5 \times 20 \text{ uL} = 100 \text{ uL}$ ). Finally, one would measure the OD values for each solution, and compare the two.

### Materials

Spectrophotometer and two mL cuvettes  
Yellow food dye  
Deionized H<sub>2</sub>O  
Two (2) test tubes  
One (1) standardized 100 uL pipette  
The pipette which is to be calibrated  
Transfer pipette

### Method

*Step 1:* Place two drops of yellow dye into 10 mL of deionized H<sub>2</sub>O.

*Step 2:* Using a volumetric pipette, transfer 2.0 mL of H<sub>2</sub>O into each of two test tubes.

*Step 3:* (a) Using the 100 uL gravimetrically standardized pipette, add 100 uL of the dye solution (from Step 1) to one of the tubes described in Step 2.  
(b) Using the pipette to be calibrated, add 100 uL of the dye solution (from Step 1) to the other tube described in Step 2.

*Step 4:* Set the spectrophotometer to 400 nm and blank with H<sub>2</sub>O using a 2.0 mL cuvette (Note: the [Russian] spectrophotometer has a tendency to drift; therefore, each time a measurement is to be made, the instrument should be blanked immediately beforehand).

*Step 5:* Measure the OD values for both of the solutions described in Step 3 (Note: Both of these solutions should read in the 0.3 to 0.6 range. If not, adjust the ratio of the solutions accordingly).

*Step 6:* Ascertain whether the OD value for the solution prepared using the uncalibrated pipette is within 5% of the OD value for the solution prepared using the standardized pipette. For example . . .

If the OD value of the solution prepared by the standardized pipette were 0.500, then multiply this value by both 0.95 and 1.05, respectively (which yields a range of 0.475 to 0.525, respectively). Then, so long as the OD value for the solution prepared by the (formerly) uncalibrated pipette is within this range, you can be certain that your pipette volume is correct, within  $\pm 5\%$ .

*Step 7:* Date and initial (on a label attached to the pipette) that the pipette is within the acceptable tolerance range.

*Step 8:* Fill out the pipette calibration log [Form "LAB.PipCalibr"] to document the calibration as having been performed.

### Frequency

Spectrophotometric pipette calibrations should be performed on a quarterly basis.

### Authors

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