CALIBRATION OF AUTO-PIPETTES AND DISPENSERS

1. SCOPE AND APPLICATION

In order to maintain the accuracy of analytical procedures, pipettes should be calibrated at least once a month.

2. SUMMARY OF THE METHOD

Pipettes and dispensers are calibrated using the known density of water. Aliquots of water are pipetted and weighed. The density of water is used to calculate the actual volume pipetted and this is compared to the expected volume. The accuracy (% difference) of the mean volumes checked and the precision (% RSD) of replicate measurements are evaluated.

3. APPARATUS AND EQUIPMENT

3.1. Weighing vessel

3.2. Deionized (DI) water

3.3. Analytical balance (accurate to 0.0001 g)

3.4. Calibrated thermometer

4. PROCEDURE AND DOCUMENTATION

4.1. All pipettes are assigned unique ID numbers. These ID numbers are documented on the pipette labels and in the pipette calibration worksheet. If you start using a new or different pipette, notify your supervisor and modify the worksheet accordingly.

4.2. There is a pipette calibration worksheet template for the Nutrients and General Chemistry workgroup located in the current year subfolder in the Pipette Calibration folder on the Nutrients drive at \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\. Open the assigned template. On the bottom left side of the file there are two tabs titled “Pipette Calibration” and “Corrective Action”. Make sure the Pipette Calibration tab is active. The worksheet is organized by type of pipette. Each chemistry is assigned a set of pipettes. Scroll to the top right of the sheet and read the instructions carefully.

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4.3. In the calibration worksheet, identify the pipette or set of pipettes that you are going to calibrate. For each pipette, the volumes to be used for the calibration must be entered. For an adjustable pipette or bottle-top dispenser, check the calibration at a low, middle, and high volume which bracket or equal the range of use. For a fixed-volume pipette or bottle-top dispenser, check the calibration at the volume used. If any changes to the worksheet are needed, contact your supervisor. Record the analyst, date, balance serial number, thermometer ID and temperature of the DI water used for the calibration. The DI water should be in a flask with the thermometer, which is located next to the balance.

Note: When using variable volume pipettes the accuracy at the maximum, middle and minimum values are checked. If only one value is calibrated the use of the pipette is restricted to this particular volume. The volume must be documented on the pipette label (see 4.7).

4.4. Check the calibration of the balance to be used (see DEP SOP CM-011). Bracket balance weight checks around expected measured mass values.

4.5. Locate a weighing boat /vessel that can hold the volume of DI water to be pipetted. Weigh the weighing vessel and tare balance. If you are calibrating a variable volume pipette make sure the pipette is set to the correct volume. Pipette an aliquot of DI water into the vessel, and **record the weight of the aliquot** in the appropriate cell. Tare the balance. Repeat for a total of five measurements of each volume checked. The volume in uL for each aliquot will be calculated and appear in the adjacent cell. The mean volume in uL, the RSD of the replicate measurements, and the % difference will be calculated automatically.

4.6. Check the "% Difference PASS/FAIL" and "% RSD PASS/FAIL" columns of the worksheet. The percent differences must not be greater than the manufacturer's specifications or laboratory guidelines for the pipette. If the calibration is acceptable "PASS" will automatically appear highlighted in green in the "Pass/Fail" column. If the calibration is not acceptable "FAIL" will automatically appear highlighted in red in the "Pass/Fail" column. If all the calibrations are acceptable, save the calibration worksheet and notify your supervisor to review the worksheet. If a calibration is not acceptable follow the corrective action procedure as described in section 5.

4.7. After the calibration is completed a LIMS generated label must be printed and attached to the pipette. Open the LIMS program. Go to the "Laboratory" menu and under "Technical" select the "Prep Worksheet Manager". There are six icons to select from. Choose the third one from the left, depicting a small label, with the name "Print Prep Labels". After selecting it a separate window opens. In this new window, under the "Options" menu, select "Four Lines of Text". Enter the text of the first line of the label in the box at the bottom of the "Print Prep Labels" window. Each time the "Enter" key is clicked a small new window appears

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where you can enter the next three lines of text. The label must contain the pipette ID, calibration date, the chemistry the pipette is assigned to, and the initials of the analyst. Additionally, if a variable pipette was only checked at one volume, add the volume checked. Example labels are shown below:

PIP ID 2301847 CAL DATE 11/10/15 250 uL only PO4 JS	PIP ID 3992086 CAL DATE 12/20/15 TP prep CAA

4.8. Save the calibration worksheet in the current month's subfolder using the filename format "chemistry_pipette cal_MMDDYY_initials", for example: "PO4_pipette cal_111015_JS". Notify supervisor when the worksheet is complete for review.

5. QUALITY CONTROL AND CORRECTIVE ACTION

5.1. Do not over-write calibration results. If a calibration result is not acceptable the "PASS/FAIL" column of the calibration worksheet shows "FAIL" highlighted in red. In this case the pipette should be inspected for maintenance, adjustment, or repair, and the calibration must be repeated.

5.2. Activate the "Corrective Action" tab of the worksheet. The Title cell of this sheet is clearly marked in red. Perform a new calibration as described in section 4, and enter the data in the Corrective Action worksheet. If the calibration passes at this point, a comment will be added to the worksheet indicating the pipette was adjusted and placed back into service.

5.3. If the calibration fails again, a comment will be added to the worksheet indicating that the pipette cannot be adjusted and is removed from service. Notify your supervisor if a pipette could not be calibrated successfully. It must be repaired and re-calibrated, or taken out of service. During review and repair, the failing pipette should be clearly labeled "OUT OF SERVICE". Pipettes that cannot be repaired should be removed from the laboratory.

5.4. All uncalibrated pipettes that are used in the lab for purposes like transferring concentrated acids or soaps/surfactants must be approved for the intended use by the supervisor. They must be clearly labeled "NOT CALIBRATED" and state the approved use.

5.5. Use the assigned template for performing the monthly pipette calibrations. The monthly pipette calibration worksheets are due by the 20th of each month. A supervisor will check the calibration worksheet for correctness and completeness.

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This allows for enough time until the end of the month to correct any problems that may be observed.

5.6. Hand-written pipette labels are not acceptable. Please refer to section 4.7 for details.

5.7. After the calibration worksheet is reviewed by the supervisor, it is converted to pdf format for archival on the chemistry drive at \\FLDEP1\DEAR\Labs\Lab5\Chemistry\Inorganic\Nutrient\Pipette calibration\.

6. CALCULATION

The following equation is used to calculate the percent difference between the nominal volume pipetted and the calculated volume. The worksheet will calculate this value automatically. The result is recorded in the "% Difference" column.

$$\%D = |(V_e - V_a)/V_e| * 100$$

V_e = Volume of water according to pipette setting (µL)

V_a = Average volume of water (µL)
$= \sum[V_n]/n$

V_n = Volume of n^{th} aliquot of water (µL)
$= (W_n/\text{Density of water at given temperature}) * 1000$

n = Number of aliquots pipetted

W_n = Weight of n^{th} aliquot of water (g)

%D = Percent difference between volume of water according to the pipette setting and the calculated volume

The Density of water is used as reported in: CRC Handbook of Chemistry and Physics, 75th ed., Ed. David R. Lide, CRC Press Inc., 1994

Example: 20 ºC: 0.9982 g/mL

The %RSD is also calculated automatically in the worksheet as the standard deviation of the replicate measurements in µL divided by the mean volume in µL. The result is recorded in the "RSD of Vol. (µL)" column.

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7. REFERENCES

7.1. CRC Handbook of Chemistry and Physics, 75th ed., Ed. David R. Lide, CRC Press Inc., 1994

7.2. DEP SOP CM-011, Gravimetric Analysis: Analytical Weight and Balance Calibration

Appendix of Significant Changes

01/03/2011 Section 4 was extensively modified, including instructions on the use of the new pipette calibration worksheets, to describe the calibration procedure in more detail. Section 5 was added to the SOP.

06/01/2016 Sections 2, 4, and 6 were updated to describe the precision calculations required in the calibration worksheet. The corrective action procedure was updated in Section 5.

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