SOP 13

Standard Operating Procedure for Calibration of Volumetric Ware, Gravimetric Method[1]

1 Introduction

1.1 Purpose of Test

Volumetric flasks, pipets, graduated cylinders, burets, and similar glass and plastic volumetric ware, such as those used in chemical and clinical laboratories, need to be calibrated to ensure accuracy of measurement. This SOP describes procedures for calibration of such ware with capacities ranging from 0.1 cm^3 to 2000 cm^3. The method is extendable, in principle, to larger volumes, limited only by the capacity and physical dimensions of the weighing apparatus. It is not recommended for volumetric ware with capacities less than 0.1 cm^3. This procedure makes use of a single pan mechanical balance. For calibrations with an electronic balance see SOP 14.

1.2 Prerequisites

1.2.1 Verify that valid calibration certificates are available for the standards used in the test.

1.2.2 Verify that the mass standards to be used have sufficiently small standard uncertainties for the intended level of calibration. Reference standards should not be used for gravimetric calibration.

1.2.3 Verify that the balance used is in good operating condition with sufficiently small process standard deviation, as verified by a valid control chart or preliminary experiments, to ascertain its performance quality when a new balance is put into service. The accuracy of the balance and weighing procedures should be evaluated to minimize potential bias in the measurement process.

1.2.4 Verify that the operator is experienced in precision weighing techniques and has had specific training in SOP 2, SOP 4, SOP 29, GMP 3, GMP 10, and gravimetric calibrations.

1.2.5 Verify that the laboratory facilities meet the minimum conditions shown in Table 1 to meet the expected uncertainty that is achievable with this procedure.

1.2.6 Verify that an adequate supply of distilled or deionized water (see GLP 10) is available.

1 Based on the work of J. Lembeck "The Calibration of Small Volumetric Glassware," NISTIR 74-461 (1974).

Table 1. Laboratory environmental conditions

Procedure	Temperature	Relative Humidity
Gravimetric	20 °C to 23 °C, a set point ± 2 °C, maximum change 1 °C/h	40 % to 60 % ± 10 % max change / 4 h

2 Methodology

2.1 Scope, Precision, Accuracy

The procedure is applicable for the calibration of any size of measuring container that, when filled with water, will not overload the balance used. The precision of calibration will depend on the care exercised in adjusting the various volumes and strict adherence to the various steps of the procedure. The accuracy attainable will depend on the uncertainties of the standard weights and the air buoyancy and thermal expansion corrections that are made.

2.2 Summary

This procedure is based on a determination of the mass of water contained in or delivered from the vessel that is calibrated. The volumetric determination is calculated from the above measurements and a knowledge of the temperature, pressure, and relative humidity of the air, and the temperature of the water that is weighed.

2.3 Equipment and Standards

2.3.1 Balance having sufficient capacity to weigh the loaded vessel. The sensitivity of the balance will be a limiting factor in the accuracy of the measurement.

2.3.2 Mass standards or built-in weights calibrated with adequate accuracy and valid calibration certificates traceable to NIST. Ordinarily, meeting ASTM Class 2 or 3 weight specifications are required.

2.3.3 Calibrated thermometer accurate to ± 0.1 °C with recent calibration values that are traceable to NIST to determine water temperature.

2.3.4 Calibrated thermometer accurate to ± 0.5 °C with recent calibration values that are traceable to NIST to determine air temperature.[2]

2.3.5 Calibrated barometer accurate to ±135 Pa (1 mm Hg) with recent calibration values that are traceable to NIST to determine the air pressure.

2.3.6 Calibrated hygrometer accurate to ± 10 % with recent calibration values that are traceable to NIST to determine relative humidity.

[2] Values from the thermometer, barometer and hygrometer are used to calculate the air density at the time of the measurement. The air density is used to make an air buoyancy correction. The accuracies specified are recommended for high precision calibration. Less accurate equipment can be used with only a small degradation in the overall accuracy of the measurement.

2.3.7 Distilled or deionized water (See GLP 10).

2.3.8 Stopwatch or other suitable timing device (does not require calibration).

2.4 General Considerations

2.4.1 All glassware must be meticulously cleaned, prior to calibration. When clean, the walls will be uniformly wetted. Instructions for cleaning are given in GMP 7. An exception is plastic ware, which will not be wetted. Follow manufacturer's instructions for cleaning such vessels. Do not use cleaning agents that will attack, discolor, or swell plastic ware.

2.4.2 Volumetric calibrations are critically dependent on the setting of a meniscus. See GMP 3 for guidance in reading a meniscus.

2.4.3 Use water that is temperature-equilibrated with the laboratory environment. Equilibration can be achieved by storing the water in clean containers in the laboratory.

2.4.4 All weighings (I_L, I_E) performed in this SOP should be determined with a mechanical balance that has verified calibration data available. A double substitution procedure(SOP 4) with air buoyancy correction may also be used. Equations must be modified if SOP 4 is used!

2.5 Calibration Procedure for Burets

2.5.1 Clamp the buret vertically on a support stand. Also clamp a plain glass test tube, large enough to hold a thermometer, in the vicinity of the buret.

2.5.2 Fill buret with water and test for absence of leaks from the tip and stopcock.

2.5.3 Drain and record delivery time, defined as the time of unrestricted flow from the zero mark to the lowest graduation mark with the stopcock fully open.

2.5.4 Fill the buret slightly above zero mark with temperature-equilibrated water and also fill the test tube that holds the thermometer. Record water temperature.

2.5.5 Set the meniscus on the zero mark and touch the tip with the wetted wall of a beaker to remove any excess water. The buret tip must be full.

2.5.6 Fully open the stopcock and discharge contents of buret into a previously weighed flask. The tip should be in contact with the wall of the flask. When the level in the buret is within a few millimeters above the line being calibrated, slow the discharge and make an accurate setting. When the setting is completed, move the flask horizontally to break contact with the tip. Recheck the setting.

2.5.7 Stopper and weigh the flask.

2.5.8 Measure and record the temperature of water in the test tube.

2.5.9 Test the next interval in the same manner - from the zero mark to the next interval of test.

2.5.10 For burets with a specified waiting time, empty as in 2.5.6 to within a few millimeters of the calibration mark. Pause for the specified waiting time (e.g., 10 s), then adjust the meniscus to the graduation line as in 2.5.6.

2.5.11 Make a duplicate determination.

2.5.12 Calculate the volume for each interval as described in Section 3.

2.6 Calibration Procedure for Pipets (One-Mark)

2.6.1 Fill the pipet to the index mark and measure the delivery time with the tip in contact with the internal surface of a beaker.

2.6.2 Refill the pipet by suction, slightly above the index line. Record the water temperature. Wipe tip with filter paper, then slowly lower level to the index line, using a stopcock or pinch clamp for fine control. The tip must be in contact with the wetted wall of a beaker while this setting is being made. Do not remove any water remaining on tip at this time.

2.6.3 Hold pipet in a vertical position and deliver water into a previously weighed weighing flask, with the tip in contact with the inside wall or neck.

2.6.4 After flow has ceased, wait two seconds then remove the pipet from contact with the flask.

2.6.5 Stopper the flask and weigh with its contained load.

2.6.6 Make a duplicate determination.

2.6.7 Calculate the volume as described in Section 3.

2.7 Calibration of Flasks (To Contain)

2.7.1 Clean and dry flask as described in GLP 13, then stopper and weigh the flask. Record the Empty Flask value.

2.7.2 Place an appropriate sized funnel in neck and fill flask to just below the reference graduation while maneuvering the flask to wet the entire neck below the stopper.

2.7.3 Let stand for two minutes then adjust the meniscus to the reference graduation line.

2.7.4 Determine the temperature of the water by putting some in a beaker or test tube containing a thermometer.

2.7.5 Weigh the filled flask and record the Filled Flask mass value.

2.7.6 Make a duplicate determination.

2.7.7 Perform volume calculations as described in Section 3.

2.8 Calibration of Flasks (To Deliver)

2.8.1 Clean but do not dry flask.

2.8.2 Place an appropriately sized funnel in the neck and fill the flask to just below the reference graduation while maneuvering the flask to wet the entire neck below the stopper.

2.8.3 Let the flask stand for two minutes, then adjust the meniscus to the reference graduation line, then weigh the full vessel, with cap or stopper.

2.8.4 Empty the flask over a 30-second period by gradually inclining it so as to avoid splashing. When the main drainage has ceased, hold the flask in vertical position for 10 seconds unless another drain time is specified, then touch off the drop of water adhering to the top of the flask.

2.8.5 Place a stopper or cap on the flask and reweigh.

2.8.6 Make a duplicate determination.

2.8.7 Calculate the volume of the flask as described in Section 3.

2.9 Calibration of Other Volumetric Glassware.

2.9.1 Measuring Pipets - Calibrate in a manner similar to that used to calibrate burets (2.5).

2.9.2 Graduated Cylinders - Calibrate in a manner similar to that used for flasks (2.7, 2.8).

3 Calculations

3.1 Calculate the volume at 20 °C from the weight of water, contained or delivered, for each run, as follows:

$$V_{20} = (I_L - I_E)(Q)\left(1 - \frac{\rho_a}{\rho_B}\right)\left[\frac{1}{(\rho_w - \rho_a)}\right][1 - \alpha\,(t - 20)]$$

Table 2. Variables for volume equation

Variable	Description
I_L - I_E	the difference, in grams, obtained from the balance indications associated with the loaded vessel and the empty vessel.
Q	the apparent mass correction factor defined by the expression $Q = \frac{\rho_B (D_{20} - 0.0012)}{D_{20}(\rho_B - .0012)}$ where ρ_B is the density of the balance weights in g/cm^3, 0.0012 is the density of *normal air* in g/cm^3 and D_{20} is the apparent mass to which the weights are adjusted. The factor has a maximum value of 1.000013 so it may be considered as unity for most calibrations.
ρ_B	density of the balance weights in g/cm^3
ρ_w	density of water at the temperature of measurement in g/cm^3
ρ_a	density of air at the conditions of calibration in g/cm^3
α	the thermal cubical coefficient of expansion for the vessel being calibrated (in per degree units that match temperature units)
t	water temperature of calibration, °C

3.2 Air density should be calculated using formula provided in SOP 2. Water density should be calculated as described in GLP 10. Look up the cubical coefficient of expansion for the vessel material in NIST IR 6969, Table 9.10 or obtain it from the manufacturer. Calculations of these intermediate values are preferred. However, values for the density of water and air, and for the cubical coefficient of expansion may be found in Tables 9.8, and 9.9 in NISTIR 6969. These values are used in connection with equation 3.1.

3.3 Calculate the average volume at 20 °C. If a reference temperature other than 20 °C is needed, the equation provided in 3.1 must be appropriately modified.

4 Measurement Assurance

4.1 Duplicate the process with a suitable check standard (See GLP 1, SOP 30, and NISTIR 6969, Sec. 7.4)

4.2 Plot the check standard value and verify that it is within established limits OR a t-test may be incorporated to check the observed value against an accepted value.

4.3 The mean of the check standard is used to evaluate bias and drift over time.

4.4 Check standard observations are used to calculate the standard deviation of the measurement process, s_p.

5 Assignment of Uncertainty

The limits of expanded uncertainty, *U*, include estimates of the standard uncertainty of the mass standards used, u_s, plus the uncertainty of measurement, s_p, at the 95 percent

level of confidence. See SOP 29 for the complete standard operating procedure for calculating the uncertainty.

5.1 The standard uncertainty for the standard, u_s, is obtained from the calibration report. The combined standard uncertainty, u_c, for the standard is used and not the expanded uncertainty, U, therefore the reported uncertainty for the standard will usually need to be divided by the coverage factor k.

5.2 Standard deviation of the measurement process from control chart performance (See SOP 17 or 20.).

The value for s_p is obtained from the control chart data for check standards or estimated based on the range of duplicate measurements over time. This value will incorporate a repeatability factor related to the precision of the weighings and the setting of the meniscus, but not related to the uncertainty of internal weights in the balance or errors in reading the meniscus.

5.3 Other standard uncertainties usually included at this calibration level include uncertainties associated with water temperature measurements, thermometer accuracy, calculation of air density, standard uncertainties associated with the density of the standards used, improper observance of drainage times, viscosity or the surface effects on the volume of liquid clinging to vessel walls after draining and the lack of internal cleanliness.

Additional References:

Bean, V. E., Espina, P. I., Wright, J. D., Houser, J. F., Sheckels, S. D., and Johnson, A. N., NIST Calibration Services for Liquid Volume, NIST Special Publication 250-72, National Institute of Standards and Technology, Gaithersburg, MD, (2006).