CALIBRATION OF MICROPIPPETTE BY GRAVIMETRICAL METHOD

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Abstract

Micro Pipette is the one of the most important tool for various procedures in a laboratory. The accuracy and precision of pipette decides the quality of test result performed in particular laboratory. Micropipette is used to accurately measure small volumes of liquids (volumes typically vary from 1 to 1000 µl).

Micro pipette does assist laboratory technician in handling liquids with precise measurement. It is a superior edition of medicine dropper or pasture pipette or serological pipette and works on the equal principle of making equivalent vacuum for drawing up the liquid. With the purpose to make sure correctness at all times and therefore micro pipette requires to be calibrated.

The calibration of pipette is carried out by gravimetric method. The volume delivered by the pipettes is determined by weighing the amount delivered and dividing this by the density of water, when determining the volume of water, the accuracy of measurements is affected by various factors such as ambient temperature, atmospheric pressure and relative humidity. These factors are usually combined to give the Z factor, used to convert from a mass reading to a volumetric reading. Then the calculated volume compared with the theoretical volume to determine the accuracy and precision of the micropipette.

1.0 Introduction
Volume measurement is an important step in most industrial and analytical measurement operations. Volume instruments are used in many fields like chemistry, health, biology and pharmacy. Laboratories must ensure that results obtained using these instruments are reliable. In order to reduce and identify possible errors in liquid handling, it is necessary to calibrate volume instruments using correct methods.

At the highest level of traceability chain, the volume can be determined by the primary method by weighing the contents of a suitable liquid by known temperature and density (Gravimetric method) [5]

Micropipettes are used extensively in laboratory to accurately and precisely deliver small volumes of liquid. The correct use of micropipettes is essential to ensure accurate volume delivery.

Molecular biologists frequently use much smaller volumes of liquids in their work, even getting down to 0.1µL (that’s one ten thousandth of a milliliter, or one ten millionth of a litre). For such small volumes, they need to use a micropipette.[2]

Micropipettes are called a lot of different names, most of which are based on the companies which manufacture. For example, you might hear them called “Gilsons” or “Eppendorf.”
2.0 Classification and design of micropipette

2.1 Fixed volume
Those which are designed and supplied by manufacturer to dispense a specific, fixed volume of liquid. This volume cannot be altered.

2.2 Variable volume
Those in which the user can adjust the volume of liquid to be dispensed over a range specified by the manufacturer [1].

3.0 Equipment and supplies

Equipment: Balance with resolution of 0.1 µg thermometer, and stopwatch and thermohygrometer.
Supplies: Distilled water or deionized water, and Glad wrap.
4.0 **Calibration process**

The figures below describe the process for calibration of micropipette.

![Figure 2; operation steps of micropipette](image)

Perform the balance check, at expected weight of filled receiving container. Record the results on the data sheet. Always make sure that the balance displays zero before weighing anything.

Weigh the empty dry clean receiving container covered with a glad wrap in which the distilled water from the micropipette has to be transferred to, tare the balance.

![Figure 3; Container covered by glad wrap to reduce evaporation](image)
To improve accuracy air displacement pipettes are usually pre-wetted by filling them several times with the liquid being dispensed and expelled to the waste. This reduces the chance of air bubbles being aspirated. However, low volume air displacement pipettes of less than 10µl are usually designed to be used without pre-wetting.

In filling the micropipette immerse the tip approximately 2-3mm for pipette volume of 1µl to 100µl, 2-4mm for pipette of volume of 100µl to 1000µl and 2-5mm for volume of 1000µl to 5000µl into the distilled water reservoir vessel.

Set to the required point to be calibrated usually the minimum point10% , 50% of volume and the nominal volume and then attach the tip to tip holder to suck the water from the reservoir vessel.

![Figure 4; changing volume](image)

In a 2-20µL micropipette (e.g. a Gilson P20) the first digit is tens of µL (it should never go past 2), the second is units and the third red digit is tenths. Therefore 20µL would read as 200, while 2.5µL would read a 025

Hold pipette comfortably in one hand with thumb resting on plunger. Then press the plunger down smoothly to first top position, at this stage the pipette tip should not be immersed in the liquid.

Immerse the tip of the pipette in the liquid to correct depth in a vertical position and release the plunger in a smooth and uniform manner to expel the liquid, ensure that the plunger extends fully to its upper top position and wait one or two seconds before withdrawing the tip of the pipette from the liquid. [2]

After withdrawing the tip from the liquid touch it against the edge of the reservoir to remove excess liquid

Touch the pipette against the wall of the receiving vessel at an angle of about 30° to 45° be careful not to immerse the tip in any liquid already in the receiving container.

Carefully expel the liquid from the pipette by pressing the plunger down steadily and evenly to first top position while keeping the tip in contact with the wall of the vessel, push the plunger down to second top. This will force air through the tip to expel any remaining liquid.

Remove the tip from the solution and release the plunger.

Record the mass of water (take the reading after the balance has stabilized), if not stabilizing take the readings within 10 seconds and this should be kept constant in all other runs.
5.0 Volume calculation

\[ \rho_a = 0.34848 p - 0.009024 h e^{0.0612 t} / 273.15 + t \]

\[ V_{20} = m^* (1/\rho_\omega - \rho_a)^* (1 - \rho_a / \rho_s)^* (1 - \alpha (t_w - 20)) \]

Where:

- \( \rho_a \) is the density of air
- \( p \) is the air pressure
- \( h \) is the humidity
- \( t \) is the ambient temperature
- \( m \) is the mass of water
- \( \rho_\omega \) is the density of water
- \( \rho_s \) is the density of standard mass
- \( \alpha \) is the thermal coefficient of water
- \( t_w \) is temperature of water

6.0 Uncertainty of measurement

Parameters that affect the uncertainty in gravimetric determination of volume are as follows:[5]

6.1 Weighing

Weighing is the most important step in gravimetric calibration. The weighing results are influenced by several factors such as the resolution and sensitivity of the balance (eccentricity, linearity and repeatability), the class and density of the weights used to calibrate an electronic scale or balance.

6.2 Water density

Mass is converted into volume by determining the value of the calibration liquid density. This value can be obtained from the literature.

6.3 Water temperature

Water temperature influences the determination of water density; thus it should be carefully measured in each measurement. Methods of estimating the temperature of the water without changing the volume have to be established.

6.4 Ambient conditions

The ambient conditions influence gravimetric measurement during the air density determination.

6.5 Instrument characteristic

The characteristics of the instrument under calibration e.g scale or the expansion coefficient of the material must be considered.

6.6 Operator

The operator can directly influence the measurement in emptying or in the handling of the equipment.
7.0 Uncertainty budget

<table>
<thead>
<tr>
<th>Source of uncertainty</th>
<th>Probability distribution</th>
<th>Value</th>
<th>Standard uncertainty</th>
<th>Divisor</th>
<th>Sensitivity coefficient</th>
<th>Uncertainty contribution(nl)</th>
<th>Degrees of freedom</th>
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Comb. Uncert 950.66
Expanded uncert 1902.00

8.0 References

1. Measurement Good Practice Guide No. 69, The Calibration and Use of Piston Pipettes
3. ISO 8655-2:2002 (E); Piston-operated volumetric apparatus-piston pipettes
4. ISO/TR 20461: 2000; Determination of uncertainty for volume measurements made using gravimetric method
5. EURAMET: Guidelines on the determination of uncertainty in gravimetric volume calibration, Euramet cg-19 version 2.1 (03/2012)