Estimation of uncertainty in volume measurement

Example of the gravimetric method using balances

Naoto Izumo, Yuji Fukami A&D Company Limited
Higashi-Ikebukuro, Toshima-ku, Tokyo 170-0013 Japan

Abstract

Many kinds of volumetric meters, such as pipettes and dispensers, are used in research, clinical trials, industry, and so on.

Generally, the volume accuracy of such volumetric instruments is checked with the gravimetric method using a balance.

This report provides practical data and important points regarding volume measurement using the gravimetric method.

Keyword

Piston pipettes, Z correction factors, Evaporation trap, Human error, Density of liquid

1. Introduction

Pipettes are an example of a frequently used volumetric instrument. Pipettes are used in scientific experiments in fields ranging from medicine, pharmaceuticals, and biotechnology to food products and in situations ranging from research to clinical and student science experiments. There are currently over 30 pipette manufacturers worldwide. For micropipettes in particular, it is said that recent annual production numbers are 1 to 2 million worldwide and usage has been increasing about 5% a year. In fields ranging from food products, pharmaceuticals, and biotechnology to clinical testing in hospitals, individual locations and research departments use hundreds of pipettes. At the laboratory level, there are about 10 pipettes per person on average. In university chemical science departments, each department uses around 1,000 pipettes. Other than technical problems, they take up a substantial amount of time and money in administrative work.

In production industries, dispensers are frequently used for automation. They are widely used for the measurement of resist quantity in liquid crystal and LED manufacturing equipment, for the control of liquid ejection in battery production lines, and as important equipment in the application of cream solder, adhesives, grease, and oil. The performance of these products is maintained and quality is controlled.

Pipettes, dispensers, and other volumetric instruments used in liquid ejection work are obviously valuable for volumetric ejection performance. Still, because of the difficulty of introducing volumetric measurement technology to measure the liquid ejection performance, it cannot be said that there are established volumetric detection methods at locations using pipettes. Therefore, pipette experts typically have to arrange measurement environments for pipette management. While management by an expert is ideal, there exist differences with the actual usage environment and individual differences in user skill. As a result, the differences between the managed performance and actual ejection performance become a problem. This document presents an investigation of the gravimetric method, a standard method with a track record among the volume measurement methods currently proposed. It identifies points of caution during volumetric measurement with the gravimetric method and uses data obtained through testing to show the reality of volumetric measurement.

2. Measurement principles of the gravimetric method

The gravimetric method involves measuring volume as mass. This reflects that mass measurement is cheap and easy compared to other measurements of physical values yet has a high resolution. For example, for a general analytical balance with a capacity of 200 g and a minimum display of 0.1 mg has a resolution of 1/2,000,000, there are low-priced balances available at around 200,000 yen.

Fig.1
A pipette volume tester using a 10μg balance[4]

Meanwhile, among all pipettes, the micro-pipettes that are required for measurement of minute amounts with a measurement capacity of 20 to 1000 μL sell well. For example, testing of a pipette with a rated volume of 20 μL requires determination level of 0.1 μL so measurement requires another decimal place, or 0.01 μL (10 nL). Using a standard of distilled water with a density of 1 g/mL, 10 nL equals 10 μg. This means that a semi micro-balance is required as a weighing device. Since 10 μg is 100,000th of a paper clip, measuring a volume of 10 nL is obviously not easy.

Density correction is required to actually determine volume by mass measurement. Furthermore, the surrounding environment often increases measurement uncertainty when measuring mass at a level finer than semi micro-balances. It is known that control methods to reduce uncertainty require many technical cautions and can be expensive due to environmental adjustments.

Fig.1 shows an example of a volumetric tester that uses the gravimetric method with a balance that can measure to a level of 10 nL (µg). From the left, it comprises an operation section (display section), a weighing section, and a thermometer and container to measure the liquid temperature. An evaporation trap is set on the weighing section to minimize evaporation of liquid (distilled water). The evaporation trap limits evaporation of liquid ejected from the pipette using vapor pressure. It reduces uncertainty and ensures operability during weighing.

3. Factors for uncertainty with the gravimetric method

The main causes of uncertainty with the gravimetric method are as follows.

Correction coefficient: Z correction factor

In order to measure volume using mass, you must determine the mass of the sample you want to measure and then divide it by the density of the sample.

Volume: $V=M/\rho$ M: mass; ρ: density.

Accordingly, the uncertainty of the volume is determined by the uncertainty of the mass measurement and the uncertainty of the density determination, meaning that the factors constituting the uncertainty accrue at the time of each physical quantity measurement.

In volume measurements of distilled water ejected from a pipette, the factor related to density correction is called Z correction factor, which is defined as the coefficient for conversion from mass to volume.[1, 2]

Volume: $V=M \times Z$ M: mass; Z: Z correction factor (conversion coefficient)

The main factors of the Z correction factor are the density changes of water that accompany temperature changes and buoyancy changes from temperature and atmospheric changes.

If water at 20 ℃ changes 1 ℃, the density of water changes about 0.02%.

Changes in the mass value due to the evaporation of liquid

It is known that after liquid is ejected from the pipette to the container on the pan of the balance the vaporization and evaporation of the liquid create uncertainty in the mass measurement, which decreases the value.

Uncertainty from pipette usage

The main causes of uncertainty caused by the user are excessive or insufficient suctioning, residual water on the tip of the pipette when water is suctioned and ejected, and convective flow caused by temperature changes when the user holds the measurement container.

Of these causes, density changes and evaporation volume are mainly the uncertainty due to the measurement system. Uncertainty caused by water suction and residual water, as well as body heat, depends on the knowledge and experience of the user. Since density changes can be simply corrected after calculating the water and air density changes, our testing is focused on the remaining two items: the evaporation of liquid and user-created uncertainty.

4. Measurement results

4-1. Evaporation amount of liquid

When using the gravimetric method, the medium is generally distilled water, which has a fixed density in relation to temperature. Since water has a large specific heat and a high boiling temperature, it does not evaporate easily. Nevertheless, water starts to evaporate after it is ejected from the pipette. Table 1 shows the measurement results for the amount of evaporation from the container.

When water is left in a 30 mL container at a room temperature of 30 °C and a humidity of 50%, the amount of evaporation reaches about 0.3 mg per minute (0.3 µL (300 nL) per minute). This amount will be problematic in determining the results when testing 20 µL, or 10% of a pipette with a rated capacity of 200 µL.

To improve the volume measurement environment, an evaporation trap like the one shown in Fig. 2 was introduced. The trap reduced evaporation to 0.07 mg per minute (0.07 µL (70 nL) per minute), which is a reduction of about 75%. If a measurement is completed in 10 seconds or less, evaporation is further reduced to about 10 nL. This indicates that an evaporation trap can reduce the effect of evaporation to about 0.05%, even when measuring 20 µL.

Evaporation of water per unit time

Container	Evaporation trap: No	Evaporation trap: Yes
5 mL (Inner diameter: 13 mm)	0.14 mg per minute	0.05 mg per minute
30 mL (Inner diameter: 30 mm)	0.26 mg per minute	0.07 mg per minute

Table 1. Amount of evaporation

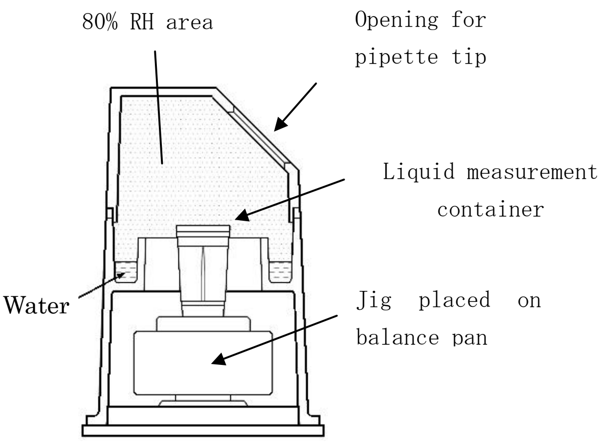

Fig. 2. Evaporation trap

4-2. Convection flow caused by user holding container

It has been proposed that a cover should be used with the container to reduce the effect of evaporation, particularly with regard to measurement of small volumes.[1, 3] However, if the user holds the container for a certain amount of time under some conditions, body heat is transferred to the container and its temperature is raised. It was confirmed that this temperature change caused convection around the container and changed the weighing value.

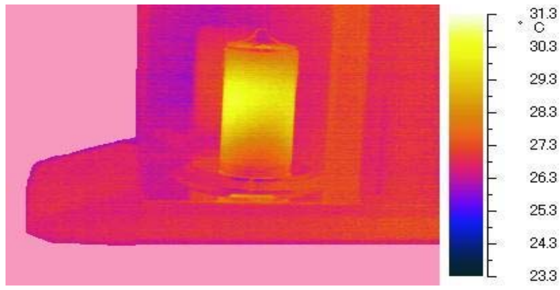

Fig. 3. Thermograph immediately after the container was held by hand

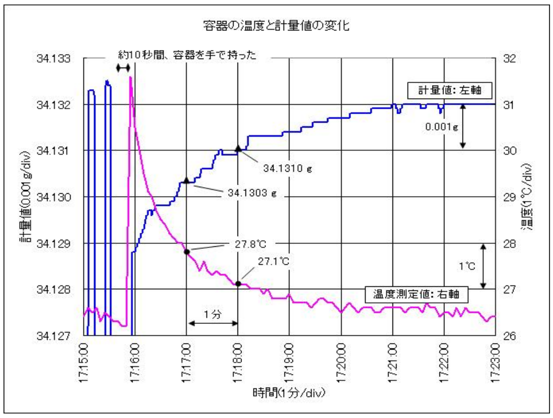

Fig. 4. Changes of container temperature and weighing values

Fig. 3 shows temperature measurement results from a thermograph immediately after the container was held by hand for a period of time (about 10 seconds). The thermograph shows the analytical balance from the side. The operation section and display section are on the left and the white section in the middle is the container on the weighing pan (diameter: 53 mm, length: 100 mm, material: steel). Fig. 4 shows the measurement results for the container temperature and weighing values at this time. The results of a test in which the container was held for a certain amount of time and then left at room temperature confirmed that the temperature of the container dropped about 0.7 ˚C per minute and the weighing value increased about 0.7 mg per minute after one minute at room temperature.

This change in value was about double that of the evaporation amount in 4-1 (in which no evaporation trap was used). While the actual container used is smaller and made of plastic, repeatedly holding it by hand can lead to increased temperatures. The difference of the room temperature and the container temperature causes convection on the surface of the container, which in turn leads to changes in weighing values. This indicates that directly holding the container by hand for long periods can be major disturbance.

4-3. The effect of pipette usage proficiency

Next, we will report on measurement results when three new engineers (Engineer A, B, and C) at our company used pipettes. The three engineers received a pipette with a rated capacity of 200 µL and measured a volume of 100 µL under the following three conditions: 1. No instructions 2. Brief instructions about suction and ejection procedures 3. Instructions about all aspects of stable measurement, including tip exchange, pre-rinsing, suction angle, tip submergence depth, and procedure speed. Table 2 shows the results. While an extreme case, Engineer B improved in accuracy/repeatability from 5.8%/22% to 0.20%/0.15% and then to 0.11%/0.18%. Even Engineer C, who had experience using pipettes in experiments as a student, showed improvement in accuracy from 0.36 to 0.34 and then to 0.04%.

Training level	A (novice)		B (novice)		C (experienced)	
	Accuracy	Repeatability	Accuracy	Repeatability	Accuracy	Repeatability
1. No training	-3.9%	0.46%	5.8%	22%	-0.36%	0.13%
2. Instruction about pipette procedures	-0.26%	0.24%	0.20%	0.15%	-0.34%	0.22%
3. Instruction about stable measurement procedures	-0.15%	0.25%	0.11%	0.18%	-0.04%	0.10%

Accuracy: Deviation of the mean value from a set volume; Repeatability: Coefficient of variation (standard deviation/mean); measurement repeated 10 times

Table 2. Improvement by training level

5. Conclusion and summary

○ Introducing an evaporation trap effectively limits the evaporation of water ejected from a pipette. When a trap is used, data shows that it is possible to perform volume measurements in which evaporation is limited to around 10 nL for each measurement (10 second period), even when there is an opening for the tip of the pipette. It has been shown that this method can improve the inefficiencies caused by handling containers and uncertainty caused by the body temperature of the user (the trouble caused by the method of covering the container before measurement as proposed for measurement of small volumes in JIS K0970-1989 and ISO8655-6).

○ In volumetric measurement using the gravimetric method, measurement system uncertainty includes (1) uncertainty of mass measurement by a balance, and (2) uncertainty caused by the environment measurement (temperature, atmospheric pressure) required for conversion from mass to volume. The standard uncertainty of mass measurement was investigated based on the specifications of the balance used in the test in 4-3, considering the following as the main causes of uncertainty with mass measurement by balance: repeatability, linearity, rounding error, and sensitivity drift.

The results showed that when the temperature change at measurement is assumed to be 1 ˚C, the uncertainty when measuring 100 µL (100 mg) is 0.058 µL. Furthermore, the main cause of the uncertainty in environment measurement was the temperature change of the distilled water. When the uncertainty of temperature measurement was assumed to be 1 ˚C, from the relation of the temperature and density of distilled water (deviation of 0.02 for each 1 ˚C), the uncertainty related to conversion from mass to volume when measuring a volume of 100 µL is 0.012 µL. Accordingly, the standard uncertainty of measurement in the weighing system is 0.059 µL and the extended uncertainty is 0.118 µL (coverage factor k=2). For a measurement volume of 100 µL, the values are 0.06% and 0.12%, respectively.

○ It was learned that the repeatability depends on user handling when the effects of evaporation are controlled. As shown by the test results of 4-3, repeatability when measurement is performed by a novice without training was 0.46% (Engineer A) and 22% (Engineer B). The estimated standard uncertainty obtained by dividing these by the square root of the number of measurements (10) for 100 µL were 0.15% (Engineer A) and 6.9% (Engineer B) respectively. This is more than double the standard uncertainty of the measurement system, and further training improved the repeatability.

○ The results proved that operator skill causes individual differences when determining volumes using pipettes. In addition, training (even with novice users) and the addition of measuring instruments that can numerically demonstrate training results reduce uncertainty (accuracy, repeatability), which makes it possible to measure volumes with better stability. A method must be established to confirm and acknowledge these effects to follow the cycle of “plan-do-check-action” in volume measurement, and the results here clearly demonstrate that introducing measurement devices based on the gravimetric method is effective in this regard.

○ As an example of future work, we would like to propose methods to make procedures easier, such as a method to reduce residual liquid on the tip during ejection, and further the development of instruments that can help reduce uncertainty in a wide range of volume measurements.

<u>References</u>

[1] ISO8655 1-6: 2002(E) Piston-operated volumetric apparatus
[2] ISO/TR20461: 2000(E) Determination of uncertainty for volume measurements made using the gravimetric method
[3] JIS K 0970-1989 Piston operated micro-volumetric apparatus
[4] A&D http://www.aandd.co.jp