4. Fluidic control systems

NOVEL GRAVIMETIC CALIBRATION METHOD FOR NANO LITER LIQUID HANDLING DEVICES

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ABSTRACT

A novel and simple liquid calibration method based on the gravimetric principle is presented. This method employs an ultra-microbalance and involves the gravimetric regression method (GRM) [1] for mass calculation as well as uncertainty analysis. The prefilled liquid in the weighing capsule is covered by a silicon oil layer to reduce the evaporation. In comparison to the GRM method with numerical compensation of evaporation this gravimetric regression method works with reduced evaporation and improves the expanded uncertainty ($U(V_{20}), k = 2$) from 12.5 nl to 2.6 nl. In 384 dispensing experiments over 16 volumes between 4.6 nl and 70 nl have been tested and a good repeatability (1% < CV < 15%) has been observed depending on the volume.

KEYWORDS

Gravimetric regression method, gravimetric volume measurement, volumetric calibration

INTRODUCTION

Gravimetry has been recognized as a standard method over half a century for laboratory and industrial liquid volume calibration. Adapted from the well-known standards ASTM-E542 and ISO8655-6 we have established in our previous work a gravimetric regression method (GRM) for gravimetric volume calibration [1]. Our previous work has shown a comprehensive uncertainty analysis of the GRM and proved that the gravimetric liquid calibration standard can be extended down to the sub-µl range. However, due to inevitable evaporation of liquid from the weighing capsule the balance never reaches equilibrium, thus generating an error that dominates in the uncertainty estimation. The expanded uncertainty of a measured volume referred to 20°C ($U(V_{20}), k = 2$) of the GRM is as high as 12.5 nl at 40 nl dosage volume. As the uncertainty describes how the true value deviates from the measured value, the GRM method can therefore not deliver highly precise measurement results for the volume below 100 nl.

The gravimetric regression method with reduced evaporation (GRM-R) presented here employs a silicon oil layer to reduce the evaporation from the liquid prefilled in the weighing capsule. GRM-R still involves the GRM method to numerically compensate the possible evaporation and makes reference to the ASTM-E542 standard. But due to the reduced evaporation this approach achieves an expanded uncertainty of $U(V_{20}), k = 2$ of about 2.6 nl in the volume range from 5 nl to 70 nl. Besides the description of the measurement setup and the GRM-R method, we also presented a full uncertainty analysis and experimental evaluation of this method in the following.

SETUP OF GRM-R

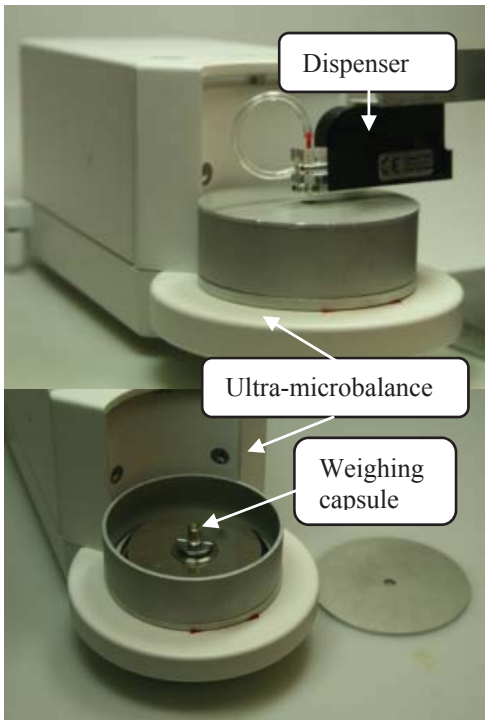

Figure 1: Gravimetric measurement setup.

The GRM-R employs an ultra-microbalance with a readability of 0.1 µg as central measuring equipment. Such an ultra-microbalance provides currently the smallest readability among all the commercial available analytical balances. In our setup an ultra-microbalance XP2U/M (Mettler Toledo Inc, Switzerland) is placed directly under a PipeJet P9 (BioFluidix, Germany) non-contact micro dispenser, as shown in Figure 1. The dispensed nanoliter droplet enters through a 6 mm-diameter hole in the windshield and hits an aluminum weighing capsule (ID x H 6mm x 8mm, elemental Microanalysis). The setup is shielded from the surrounding with a transparent plastic cover and placed on one vibration isolated granite table (Johann Fischer Aschaffenburg DIN876-126 hardstone surface plate and Newport VH3660W-OPT vibration isolated workstation).

The presented GRM-R method employs DOW Corning® 200-Fluid 50-cs silicon oil to build an oil layer on top of the liquid in the weighing capsule (Figure 2). The initial preparation of the weighing capsule is shown in Figure 2. It begins with pipetting about 50 µl test liquid into weighing capsule with contact to the capsule bottom, then goes on with the slow pipetting of about 100 µl silicon oil in contact with the capsule wall on top of the test liquid. This preparation procedure avoids contamination of liquid on the capsule wall that could result in evaporation and drift of the balance signal. The dispensed liquid droplet can pass the oil layer and merges with the prefilled test liquid (Figure 3). The prefilled liquid ensures that with increasing liquid volume the oil layer can always stay on top. Therefore, the density of the measured liquid always has to be larger than the applied oil (0.96 g/cm³) which is the case for most liquids in life-science and diagnostic applications.

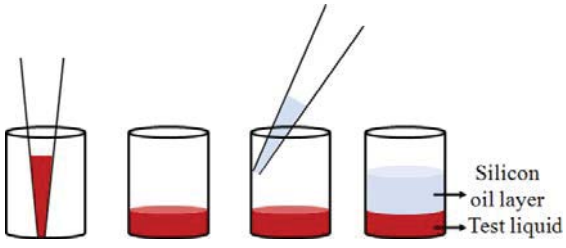

Figure 2. Initial preparation of weighing capsule.

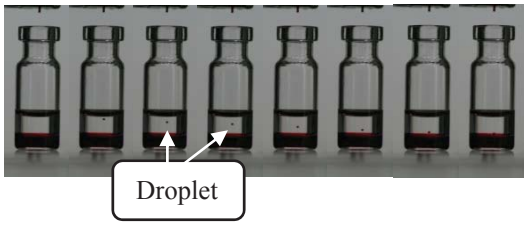

Figure 3. Image sequence (from left to right) of the transition and the merging of a dispensed droplet into a glass vessel (this vessel was used for visualization only, because the used weighing capsule is not transparent). The vessel was prefilled with test liquid and silicon oil layer like shown in Figure 2. The test liquid is a red dyed water solution.

The measurement procedure of the GRM-R including dispensing control is carried out automatically by the Software GraviDrop (BioFluidix, Germany). GraviDrop calculates the dispensed mass with the linear regression method to compensate the possible evaporation loss numerically and provides an estimation of uncertainty based on the prognosis interval [1]. A typical measurement procedure is shown in Figure 4. Thanks to the silicon oil layer almost no evaporation occurs.

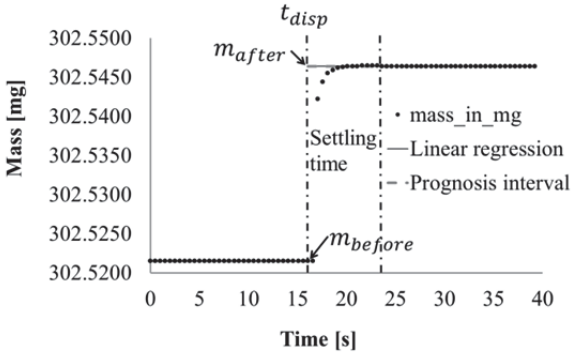

Figure 4. Typical balance readouts of one dispense measured with the GRM-R method. m_{before} and m_{after} : denote regression values "before" and "after" the liquid is dispensed onto the balance. t_{disp}: is the time when the dispense occurs.

UNCERTAINTY ESTIMATION

The GRM method as described in [1] employs similar equations as used in the well-known ASTM E542 standards to calculate liquid volumes at reference temperature 20 °C V_{20}:

$$V_{20} = m \cdot \frac{1}{\rho_w(T) - \rho_a} \cdot \left(1 - \frac{\rho_a}{\rho_c}\right) \cdot [1 - \gamma(T - 20)] \quad (1)$$

Where T is temperature during measurement, ρ_w is the density of the measured liquid at measurement temperature T. ρ_a is air density and ρ_c is the density of the weights used to calibrate the balance. γ is the thermal coefficient of expansion of the measured liquid and m the mass of the dispensed liquid which is equal to $m_{after} - m_{before} + \delta m$. m_{after} and m_{before} are regression values "before" and "after" the liquid is dispensed onto the balance. δm is the correction to the statistical random weighing errors from the used balance.

The standard uncertainty of V_{20} is then calculated as [1]:

$$u(V_{20})^2 = \left(\frac{\partial V_{20}}{\partial m}\right)^2 u^2(m_{after}) + \left(\frac{\partial V_{20}}{\partial m}\right)^2 u^2(m_{before}) + \left(\frac{\partial V_{20}}{\partial m}\right)^2 u^2(\delta m) + \left(\frac{\partial V_{20}}{\partial \rho_w}\right)^2 u^2(\rho_w) + \left(\frac{\partial V_{20}}{\partial \rho_a}\right)^2 u^2(\rho_a) + \left(\frac{\partial V_{20}}{\partial \rho_c}\right)^2 u^2(\rho_c) + \left(\frac{\partial V_{20}}{\partial \gamma}\right)^2 u^2(\gamma) + \left(\frac{\partial V_{20}}{\partial T}\right)^2 u^2(T) \quad (2)$$

$u(m_{after})$ and $u(m_{before})$ are estimated according to the prognosis interval to the regression line based on the data acquisition "before" and "after" dispensing. In case of using only the regression method to compensate the evaporation like the normal GRM [1], $u(m_{after})$ and $u(m_{before})$ are dominating in the calculation of $u(V_{20})$ because of evaporation and the oscillations of weighing readouts.

Thanks to the silicon oil layer the evaporation in the GRM-R is strongly reduced. The ultra-microbalance can therefore more easily reach force equilibrium, which results in smaller values for $u(m_{after})$ and $u(m_{before})$. The calculation of $u(V_{20})$ and $U(V_{20}), k = 2$ based on the data in Figure 4 is shown in Table 1.

The estimated $u(V_{20})$ of the GRM-R by measuring about 25 nl liquid volume is about 1.3 nl. From Table 1 it can be obviously deduced that $u(V_{20})$ is mainly determined by $u(\delta m)$, which is related to the conventional weighing process and the accuracy of the used balance. In contrast to the GRM, the $u(m_{after})$ and $u(m_{before})$ values can be neglected. $u(\delta m)$ in the calculation was determined by Deutscher Kalibrierdienst (DKD-K-14701) for the used balance. "The DKD calibration certificate documents the traceability to national standards, which realize the units of measurement according to the International System of Units (SI)" [2].

Table 1. Uncertainty calculation for the data in Figure 4.

Input Quantity (*)	Unit	Value	Standard Uncertainty u(*)	Sensitivity Coefficient
m_{before}	µg	302521.5	0.0195	1.0029
m_{after}	µg	302546.4	0.0265	1.0029
δm	µg	0	1.30	1.0029
ρ_w	µg/nl	0.997391	1.52E-06	-25.0683
ρ_a	µg/nl	0.0012	2.89E-07	21.6161
ρ_c	µg/nl	8.006	0.01	0.0005
γ	°C^{-1}	0.000207	2.89E-07	-89.9693
T	°C	23.6	0.05	-0.0052
V_{20}	nl	25.0		
$u(V_{20})$	nl	1.3		
$U(V_{20}), k = 2$	nl	2.6		

EVALUATION

To evaluate the GRM-R procedure a nanoliter dispenser (PipeJet P9, BioFluidix GmbH, Germany) is positioned above an ultra-microbalance (XP2U, Mettler Toledo, Switzerland) (Figure 4). The testing liquid is double distilled water as recommended in ASTM-E542 and ISO8655-6. The weighing capsule stays in the center of the weighing cell of the balance. The inlet hole of the balance's windhield is about 5 mm below the nozzle. The whole setup is covered by an additional wind shield and isolated from vibrations like described before. The data acquisition during the experiments as well as the volume calculations are automatically executed by the software GraviDrop (BioFluidix GmbH, Germany).

The results of the 384 experiments performed in the range from 5 nl to 70 nl are shown in Figure 5. The CV of each 24 measurements at volumes down to 5 nl remains below 10% (except for the smallest value of 4.6 nl) and the value of $U(V_{20}), k = 2$ remains below 10% for volumes larger than 24 nl. Therefore, GRM-R is able to deliver a reliable measurement precision down to 5nl volumes and a reliable absolute accuracy down to 24 nl.

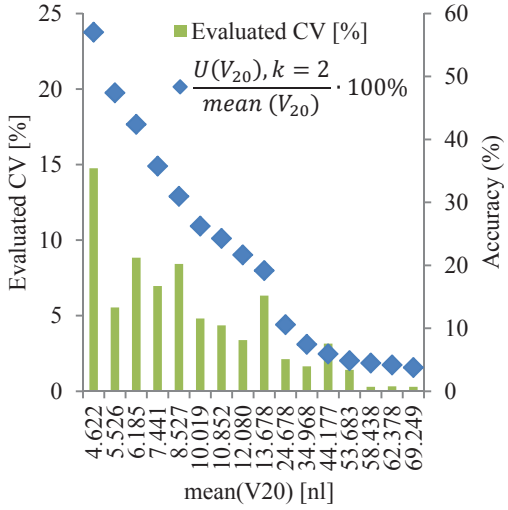

Figure 5. GRM-R evaluation results. The mean V_{20} of each of 24 dispensing experiments at 16 different droplet sizes is shown on the x-axis in [nl]. The coefficient of variation in [%] of each 24 dispenses is shown as green bars and the relative expanded uncertainty ($\frac{U(V_{20})k=2}{mean(V_{20})} \cdot 100\%$) is shown as blue diamonds.

CONCLUSION

An improved method, the gravimetric regression method with reduced evaporation (GRM-R), for precise and accurate gravimetric low volume calibration has been presented. It is derived from the ASTM E542 standard and prevents evaporation errors by averaging balance readings before and after the droplet impact as well as by applying an oil layer to the weighing capsule to significantly reduce errors caused by evaporation. In contrast to our previous work the GRM-R improves the expanded uncertainty ($U(V_{20}), k = 2$) from 12.5 nl to 2.6 nl at volumes of 40 nl. Thus, the gravimetric liquid calibration methods and standards can be extended to the lower nanoliter range with high precision.

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[2] Deutscher Kalibrierdienst DKD Calibration certificate, 350DE83, DKD-K-14701, 2012-07

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