23rd ABCM International Congress of Mechanical Engineering
December 6-11, 2015, Rio de Janeiro, RJ, Brazil

A STANDARD SYSTEM FOR METROLOGICAL TRACEABILITY ON LIQUID MICRO FLOW RATE MEASUREMENT

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Abstract. *The Bureau International des Poids et Mesures (BIPM) has encouraged National Institutes of Metrology to offer system for providing metrological traceability on micro flow rate measurement, since there is lack on the traceability chain in this field. Although to ensure reliability on quantifying fluid flow rate in micro scale is a challenging task, reference standards on systems and also measurement procedures need to be developed. In this way, the present work describes the assessment of a low flow rate system which is being developed by the Brazilian National Institute of Metrology (INMETRO) as part of the standardization in the traceability chain for microfluidic area. The system is based on the gravimetric method for fluid delivery quantification. Detailed apparatus characteristics, measurement techniques, mathematical modeling for volume conversion, performance and metrological validation are here presented and also discussed. The system measurement capability is within µl to ml scale and was found that it provides relative uncertainty of 0.21% in the 0.8 ml delivered volume at all tested flow rates.*

Keywords: *micro flow, microfluidic, metrological traceability, calibration*

1. INTRODUCTION

The microfluidic can be defined as the science which studies fluid flow in systems of micrometric dimensions, and where the fluid performance diverges of traditional theory to macrometric scale. Applications of microfluidic include several science areas, as chemistry, biotechnology, environmental, health, among others. Over the last decade has been growing the interest of Metrology Institutes in such topic, since dealing with the traceability and standardization in micro flow rate are a need and a big challenge. Nowadays, there is a gap in the chain of metrological traceability for very low flow rate. This lack is due to the difficult in controlling a large number of variables that play important role in the stability and measurement of the low flow rate. For a large number of microfluidic applications, an improved performance of a microfluidic system can be guaranteed if the impact of the behavior of the flow rate or total volume is known, as demanded by LOC-Lab on Chip technologies (Nuno *et al.*, 2014), TAS - Total Analitycal System and a µTAS (Micro Total Analitycal System) (Haswell, S.J.,1997). Such examples illustrate a drive reason for studying fluid flow in micro channels with metrological traceability.

Due to the standardization and traceability for fluid flow measurement in very low scales be a relevant need for industrial, technological and scientific fields, the *Bureau International des Poids et Mesures*-BIPM (BIPM, 2015) has been stimulating National Metrology Institutes to reach a good level of development in microflowrate standardization up to 2024, when is planned to run the first Key Comparison event in this area (BIPM; 2014-2024). Inmetro has been working aligned to this target.

Different methods, techniques and apparatus used for microflowrate standardization have been discussed in the metrology community (Wolf, H., 2010), as the apparatus for calibrating the fluid flow rate based on the volumetric pump working principle (Marinozzi *et al.*, 2005), or the investigate of types of microchannels using a finite difference approximation method. (Nath *et al.*, 2005), or the principle relies on using a microfluidic comparator to detect small pressure fluctuations in fluid flows (Green *et al.*, 2009), or the measurement of flow rate inside a microchannel by using a laser Doppler technique (König *et al.*, 2010), or calibration of volume syringe by gravimetric method (Reitz *et al.* 2010), or the method base a hygrometer for the calibration of syringe pumps (Hannu *et al.*, 2014), or a micromachine that enables precise measurement of the flow rate at the micron scale (µl/min) in microfluidic channel (Yi *et al.*, 2015). It evolves traditional methodologies up to sophisticated electronic systems, and eletrochemical, mechanical or optical mechanisms are principles for controlling, detecting or quantifying fluid and flow. In very low flow rate or small volume of fluid all these technologies claim for solution of challenges for providing accuracy, uncertainties reduction and confidence in the measurement results.

The present work describes the very low flow rate measurement system based on the gravimetric method for fluid delivery quantification. The volume is determined by conversion from mass to volume of liquid, thought this, it is possible traceability in micro flow rate. This system is being developed by the National Institute of Metrology, Quality and Technology (INMETRO), in the Fluid Dynamics Metrology Division. Besides seeking for metrological reliability of the experimental data, a detached goal of this project is to make possible the study of fluid flow in micro channels ensuring metrological traceability to Brazilian standards.

2. METHODS AND INSTRUMENTATION

2.1 Measurement Technique

The measurement technique in the Inmetro´s standard system is based on gravimetric method. This method provides good accuracy after converting mass value to volume quantity. It consists on measuring the mass of liquid delivered to (or collected in) a recipient whose volume was previously calibrated (traceable to SI). After measuring the mass of the liquid, the respective volume is determined by using the equation described in ISO 4787 (ISO 4787, 2010). By calculating the liquid volume by gravimetric method and estimating the associated components of measurement uncertainty, it is possible to ensure the traceability of the micro flow rate to SI.

Before deciding on the set up of the standard system, several tests and analysis of the influence of some variables were performed. In the next section a brief summary about that is shown, and also is presented the system assemblage.

2.2 Apparatus and setting up of the system

Several set out of gravimetric systems can be found for liquid flow rate determination. A classical system configuration for very low flow rate is basically composed by syringes and infusion pump. Tab. 1 and Fig. 1 show the main characteristics of the gravimetric apparatus discussed in the present work.

Table 1. Apparatus description

Syringe	Capacity: 1 ml Resolution: 1 µl Longer graduated lines:10 µl
Flow rate range tested	0.2 ml/min 0.05 ml/min 0.025 ml/min
Micro Pump (syringe pump)	Accuracy: ± 0.5% Resolution: 0.00001 ml/min
Balance	Capacity: 610 g Resolution: 0.1 mg
Fluid	Deionized water

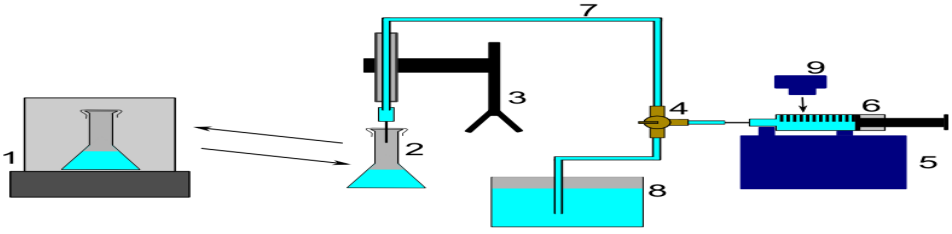

Figure 1. Schematic of the Gravimetric System Balance (1) Beaker covered with PVC film (2) Holder (3) On/off valve (4) Micro pump (5) Syringe filled of water (6) Hose filled of water (7) Water reservoir (8) Camera (9)

Although the configuration shown in Fig. 1 seems to be simple, some aspects of the assemblage condition, setting up and measurement procedure affect how well the system work. Examples are the disturbance that occurs during the time interval of measurement due to the neighborhood (environmental temperature oscillation, vibrations etc) or air bubbles trapping in the hydraulic circuitry and also water evaporation during the liquid weighing, among others.

Then, besides insulating the vibration in the environment, the room temperature, humidity and pressure were controlled, causing a thermal equilibrium. The instability and error in the mass value indication of the collected fluid due to evaporation was minimized by covering the beaker with PVC film (the plastic film was crossed by the needle of the syringe, as seen in the Fig. 1). In order to minimize the problem of air bubble trapping, before the hoses and syringe were connected to the system they were filled with water. Nevertheless, even doing such procedure, care was taken to avoid the presence of tiny air bubbles in the water during the measurement.

After acquiring a fluid sample for mass measuring (i.e. when the liquid injection into the beaker should be stopped) an on/off valve (see Fig. 1) connected to the water reservoir was opened, permitting the syringe be filled again. A camera was installed in this system for visualizing and improving the volume reading on the syringe scale. By using the camera, the resolution of the syringe reading scale is increased and parallax errors are avoided.

Other cautions related to the system handling were taken, as for example, the use of gloves during the measurement process in order to avoid the system contamination by hands. Deionized water was chosen as working fluid with the aim of reducing the contamination of the internal parts of the system by particles.

2.2.1 On some factors which affect the data

Air bubble formation

In this system, the presence of air bubbles is very difficult to deal with, since bubbles tend to be retainned on the beaker wall. Air bubbles can influence the flow rate quantification when using the gravimetric method. The air bubbles sometimes arise during the filling process of the system hoses and so, care is required to maintain it free from any influence that may cause entry of air. This result would affect the evaluation of a device under test, if it would depend on the flow rate value and its uncertainty.

The use of clean water free from contamination is also very important because it prevents bacterial growth and even air bubbles. The ideal is to use deionized and filtered water, reducing the contamination with particles which come from the water source.

Thermal influence

The temperature exerts the main influence on the results of the measurement system and it is the major contribution to the experimental uncertainties. Its effects are difficult to control. A temperature variation can change the fluid properties, as well as evaporation level and thermal disequilibrium. The fluid temperature must be monitored since the fluid can provoke heat transfer to or receive heat from the system walls. Such problems are very common on flow measurement to avoid potential problems it is necessary to use calibrated instruments to monitor the temperature, pressure and humidity of the environment, and wait for the correct condition to perform the measurements. In the system presented here, a temperature variation induces a thermal expansion of the syringe material, changing its volume and so, a probable influence in the measurement results by gravimetric method.

Evaporation Effects

When measuring water flow rate in micro scale, difficulty of controlling evaporation increases, and deep problems take place. Evaporation can be a critical influence in gravimetric systems, since no stability of the balance indication occurs. The control of the environmental conditions also helps in reducing evaporation In the present experiment, although a cover was put on the beaker overture for minimizing the evaporation of the collected water, this problem should be investigate and assessed, in order to identify in more details the implications and reduce the potential effects on the measurements.

Influences related to measuring instruments

These influences are related to nonlinearity of instruments, repeatability, resolution, drift and eccentricity. The ideal is to use calibrated instruments. The information provided by the calibration certificates are applied for corrections or accounting of the influence of the instruments behavior on the measurement results. Depending on the situation, the

knowledge of an instrument behavior can reduce the total uncertainty of an experiment, since it can avoid an overestimation of an uncertainty of certain component contribution.

Effects related to system installation

Another influence to a microfluidic system performance is that related to system installation mode, for instance, the fluid leakage out of the system. In the presence of leakage, the total delivered volume is lower than the expected. To avoid this problem, a sealed system is required and all the connections must be checked in the device.

2.3 Measurement Procedure

As described in Fig. 1, the syringe is connected to the infusion pump. Through the three way valve and hoses the syringe is linked both to the beaker and to the water reservoir. First of all, attempting for disturbance sources as commented in the previous section, all the hydraulic circuitry is filled of working fluid (deionized water), and both for withdraw and infusion process of the pump. Following, after set the flow rate range in the pump, the infusion is started and the initial mass of water and respective temperature inside the beaker are measured. Finally, the total mass of liquid flowed into the beaker is measured. After that, considering a reference temperature of 20ºC, a conversion from mass to volume of liquid is performed, according to the mathematical model (Eq. 1). The water density was determined by using an equation recommended by BIPM, the Tanaka Equation (Tanaka *et al*., 2001).

In this work, three liquid flow rate levels were tested: 0.2, 0.5 and 0.025 ml/min. six samples of fluid were collected in each flow rate, in order to estimate the instrument repeatability. The syringe capacity was 1ml, but in each run the totalized volume was 0.8 ml, since within this limit the syringe plunger couldn't reach the barrel end (besides, in this manner, a best visualization of the delivered volume of water by reading the barrel scale using the camera was achieved).

3. MATHEMATICAL MODEL

The mathematical model used for determining the volume of liquid by the gravimetric method is presented in Eq. 1 :

$$\Delta Ve = \frac{Mc - Mv + M_E}{\left(\rho_L[T_{Lt}] + \delta\rho_L[T_{Lt}] - \rho_{ar}\right)} \cdot \left(1 - \frac{\rho_{ab}}{\rho_b}\right) \cdot \left(1 + \alpha_v \cdot \left(T_r - T_t\right)\right) + \delta V \tag{1}$$

- ΔV_e is the volume of the liquid at the reference temperature T_r (ml)
- M_c is the apparent mass of the beaker full of the sample of liquid (g)
- M_v is the apparent mass of the beaker before receiving the sample of liquid (g)
- M_E is the evaporated mass (g)
- ρ_L is the liquid density at temperature T_L (ºC)
- T_L is the liquid Temperature (ºC)
- δT_L error due to the liquid temperature variation (in space and time) (ºC)
- ρ_A is the air density during the apparent water mass measurement (kg/m^3)
- ρ_{ab} is the air density during balance calibration (kg/m^3)
- ρ_b is the density of the standard weight used in balance calibration (g/m^3)
- α_V is the volumetric thermal expansion coefficient (1/ ºC)

- T_d is the syringe (barrel) temperature (ºC)
- Tr is the reference temperature = 20ºC
- δV error due to repeatability of volume measurement

The indicated data of the apparent mass, environment temperature, water temperature, pressure and humidity were corrected in accordance to their respective calibration certificated.

4. RESULTS AND DISCUSSION

For each flow rate (0.200 ml/min, 0.025 ml/min, and 0.050 ml/min) six measurements were performed with a syringe. The results are presented in the following tables and figure:

Table 2. Results at flow rate of 0.200 ml/min 0.05 ml/min and 0.025 ml/min

Calculated Volume (ml)	Nominal Flow Rate (ml/min)	Reading Correction (%)	Expanded Uncertainty (ml)	Relative Uncertainty (%)
0.8070	0.200	0.0316	0.00173	0.21
0.8065	0.050	0.0157	0.00171	0.21
0.8027	0.025	0.0234	0.00172	0.21

The volume was calculated by using the Eq. 1 described above and the reading correction is the difference between the calculated volume and the volume of fluid delivered (read with the camera on the barrel surface after the liquid was transferred to the beaker). The Expanded Uncertainty and Relative Uncertainty (GUM, 2008) (VIM, 2008) were calculated considering all the variables of influence mentioned before. According to Tab. 2, it was found that the relative uncertainty of the volume at the three levels of the nominal flow rate experimented are in the same range. This is a significant achievement for the system in a metrological point of view.

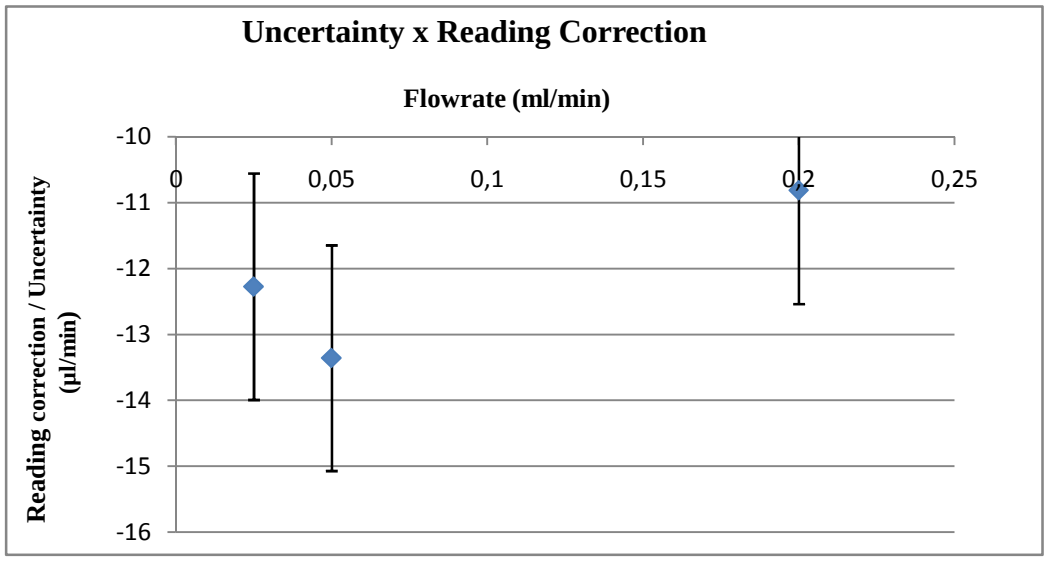

Figure 2. Association of correction with uncertainty

The relative contributions to the uncertainty of most important variables are presented in the Tab. 3:

Table 3. Relative contribution to uncertainty of each variable of influence

Variable of influence	Relative Contribution at flow rate 0.2 ml/min (%)	Relative Contribution at flow rate 0.05 ml/min (%)	Relative Contribution at flow rate 0.025 ml/min (%)
VLi	32.165	33.367	32.765
M_c	20.085	20.836	20.459
M_v	20.085	20.836	20.459
Me	20.085	20.836	20.459
δV	7.177	3.705	5.433
Others	0.403	0.421	0.424

- VLi : Reading of the syringe volume

While the development of the system was going on, some aspects were discussed to be investigated later. It includes, the following points, among others, more investigation for detailing the evaporation effects and its suppression, improvement of the barrel scale visualization method, research and refinement of the uncertainty budget, review and improvement of the gravimetric assemblage configuration (the use of an analytical balance with lower capacity than that used in the present work certainly will promote best results).

5. CONCLUSION

The present article deals with initial results of studies aiming the development of a standard bench for a liquid micro flow rate measurement, which is intended to be employed as part of the metrological traceability chain for microfluidics applications. The system works by gravimetric method, and was found that it provides relative uncertainty of 0.21% in the delivered volume at all tested flow rates. As an initial work, the achieved uncertainty is within the expected range, and it could be satisfactory for using, for example, as reference in experiments when the microPIV (micro Particle Image Velocimetry) technique is applied, since the repeatability in the measurement was good. The system development and validation still are in progress, and here are presented only the first achievements and main results.

6. ACKNOWLEDGEMENT

The authors are grateful to CNPq, FAPERJ, FINEP, PETROBRAS, PFRH-PETROBRAS and INMETRO for their support and sponsorship which have become possible the development of this research

7. REFERENCES

BIPM, 2015. Bureau Internation of Poids at Mesure. 15 May 2015. <http://www.bipm.org >.

Bureau International des Poids et Mesures- BIPM, Strategy 2014-2024, *Consultative Committee for Mass and Related Quantities (CCM)*

Evaluation of measurement data.; 2008. *Guide to the expression of uncertainty in measurement* – GUM

Green R. and Vanapalli, S.A.; 2009. *Quick assessment of the stability of flow generatedn by a syringe pump in a Microfluidic device, Lab on Chip.*

Hannu A. Sairanen, Marti, O. Heinonem.; 2014. *MicrofluidicNanoFluidic, 16 :187-193, Hygrometer-based calibration method for syringe pumps providing ultra-low liquid flows.*

Haswell, S.J., 1997. *Development and operating characteristics of micro flow injection analysis systems based on eletroosmotic flow, Analyst.;*Vol 122 (1R-10R).

International Vocabulary of Metrology.; 2008. *Basic and General concepts and associated terms (VIM)* 3rd edition.

ISO 4787:2010 – *Laboratory glassware – volumetric instruments methods for testing of capacity and for use*

König, J., Voigt, A., Büttner, L. and Czarske, J., 2010. *Precise micro flow rate measurements by a lase Doppler velocity*

profile sensor with time division multiplexing, Meas. Sci. Technol., vol 21.

M. Tanaka, G. Giard, R. Davis, A. Peuto and N. Begnell.; 2001. *Recommended table for the density of water between 0ºC and 40ºC based on recent experimental reports.*

Marinozzi, F., Bini, F. and Cappa, P., 2005. *Calibrator for micro flow delivery systems, Rev. Sci. Instr.*, 76.

Nath, P., Roy, S., Conlisk, T. and Fleischman, A.J., 2005. *A system for micro/nano fluidic flow diagnostics, Biomedical Devices*, vol 7(3)

Nuno Miguel Matos Pires, Tao Dong, Ulrik Hanke and Nils Hoivik.; 2014. *Recent Developments in Optical Detection - Technologies in Lab-on-a-Chip Devices for Biosensing Applications*, Sensors, 14, pp15458-15479

Reitz, S., Kummrow, A., Kammel, M. and Neukammer, J., 2010. *Determination of micro-litre volumes with high accuracy for flow cytometric blood cell counting, Meas. Sci. Technol.*, vol 21.

Wolf, H., 2010. *Microflow meets metrology*, Meas. Sci. Technol., vol 21

Yi-Jui Liu ,Juin-Yi Yang , Yung-Mau Nie, Chun-Hung Lu, Eric Dowkon Huang, Chow-Shing Shin, Patrice Baldeck, Chih-Lang Lin.; 2015. *A simple and direct reading flow meter fabricated by two-photonpolymerization for microfluidic channel,* MicrofluidNanofluid 18:427–431.