DEVELOPMENT OF A MICROFLOW PRIMARY STANDARD

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Abstract

The Volume Laboratory (LVO) of the Portuguese Institute for Quality (IPQ), in cooperation with the Department of Mechanical and Industrial Engineering (DEMI) of the Faculty of Science and Technology of The New University of Lisbon (FCT/UNL), developed a gravimetric [1] microflow standard with a range between 10 mL/min and 100 nL/min.
The design of the microflow standard was based on three main elements: a flow generator (syringe pump), a collector device and a data acquisition computer program.
The microflow standard was tested and validated by means of an ongoing intercomparison, at different flow rates, with uncertainties in the range of 0,1 % up to 0,5 %.

Introduction

With the development of science and the widespread use of nanotechnology, the measurement of fluid flow quantities is getting smaller, in the order of microliter per minute or even nanoliter per minute.
In order to pursuit the needs of industry and laboratories, in such fields as health, biotechnology, engineering and physics, giving traceability to its measurements, it was identified the need, not only national but also international [2] [3] [4] of developing a primary standard for microflow measurement.
The European Association of National Metrology Institutes, EURAMET, started, in 2007, the European Metrology Research Programme – EMRP, supported by the 7th Framework Program of the European Commission. This Program, which will end in 2017, opens a call every year for Joint Research Programs – JRPs, in strategic themes defined by EMRP. These JRPs are developed by the member countries and can be supported by Universities and other entities that work in scientific areas were measurements are critical and fundamental.
In 2011, one of the chosen JRP subjects was Metrology for Health. The choice of this subject had the purpose of developing science and technology in the field of health, specifically, to assure the traceability of clinical data, allowing the comparability of diagnostic and treatment information.
One of the JRP that was accepted in the subject of Metrology for Health was MeDD - Metrology for Drug Delivery. This project has the purpose of developing a primary standard for flow measurements between 150 microliter per minute and 1 nanoliter per minute and also the characterization of flow meters and flow generators already in the market, assuring the traceability of the syringe pumps measurements used in drug delivery. This work will allow in the future the increase of efficiency and trust in the flow delivered by the drug delivery systems, which are fundament instruments for patient therapy.
IPQ/LVO is one of the collaborators of the project. In order to give response to national needed in the field of micro flow, a national primary standard for micro flow measurement was developed in cooperation with FCT/UNL, DEMI.

Flow determination

Flow is the amount of fluid that goes through a specific area by a unit of time. The gravimetric dynamic measurement method is, by definition, the measurement of the mass of fluid obtained during a specific time period. The volumetric flow can be calculated using equation 1:

$$\boldsymbol{Q = \frac{1}{t_f - t_i}\left[\left((I_L - I_E)\right) \times \frac{1}{\rho_w - \rho_A} \times \left(1 - \frac{\rho_A}{\rho_B}\right) \times [1 - \gamma(T_0 - 20)] + \delta V_{evap}\right]} \quad (1)$$

where:

Q - Flow
t_f - Final time
t_i - Initial time
I_L - Final mass
I_E - Initial mass
ρ_W - Water density
ρ_A - Air density
ρ_B - Mass pieces density
γ- Expansion coefficient
T_0 - Measured water temperature
δV_{evap} – Evaporation

Design of the primary standard for micro flow measurement

Typical physics laws for the study of fluids at a macro scale level are not sufficiently accurate for the measurements at micro fluids level, as phenomenon like capillarity, thermal influences and evaporation plays a very important role in influence parameters.
Based on recent studies, [2] [3] [4], several parameters have to be taken into account, such as: thermal influence, dead volume, system for delivering and collection of the fluid, continuous flow, pulsation of the flow generator, evaporation effects, surface tension effects, drop and capillarity, balance effects (floatability and impacts), contamination and air bubbles, variation of pressure and time measurement.
Before starting the design of the microflow primary standard, an evaluation of the available conditions at LVO was carried out regarding the working area and the existing equipment. Then, it was decided that the gravimetric method was the one that best fitted the reality of the laboratory. Several design options for the project were analyzed, taking into consideration the target uncertainty of 0,5 %, namely, the flow generator, tubing (materials and geometry), type of liquid, balance and software for data acquisition.

Flow generation

Considering the values of the flow under study, 10 mL/min to 100 nL/min, and the needed stability, a syringe pump Nexus 3000 from Chemys was chosen as the flow generator. With 300 increments per step, this device allows a soft movement of the motor over the syringe, decreasing the pulsation effect of flow.

Tubing

After analysing several options for the tubes' material, namely glass, Teflon and stainless steel, the later was chosen due to its very low absorption rate [2], its appropriate thermal expansion coefficient value and good corrosion resistance.
The glass would also be a very good solution due to its lower thermal expansion coefficient; nevertheless, stainless steel has the advantage of being easy to bend in order to shape purposes, avoiding the use of connections and elbows that can lead to liquid loss and higher dead weight.

Air removal and purge system

In order to minimize the existence of impurities and bubbles in the fluid, that can introduce measurement errors, the primary standard was designed with a purge system that allows water draining before the beginning of the measurements. The water is also purified by heating and submitted to ultrasounds.

Evaporation and drop fall control

The main water evaporation occurs during the collection of the sample in the weighting vessel located inside the balance. Hence, an evaporation trap system was used allowing the air saturation in the weighing vessel and decreasing the evaporation influence. This solution is recommended for the calibration of micropipettes where the effect of evaporation can be reduced up to 50 times [5]. Also, in order to reduce evaporation, but mainly to minimize the effects of the dripping (formation of drop) the tube was placed below the liquid's surface.

Balance

The balance used to measure the mass of the fluid was a Mettler Toledo AX26, due to its resolution of 1 μg, and due to the fact that the balance already was installed at the laboratory. In addition, the equipment had the physical characteristics suitable for the project's proposes. The experimental tests carried out allowed the confirmation of the use of the "Dosing" and "Unstable" weight method as the most appropriate.

Data acquisition system

A data acquisition system was developed using "LabView" graphical development environment. Different modules were developed to automate the acquisition, validation, online visualization of measured data, statistical treatment and uncertainty calculation. The data acquisition is done directly from the balance every 50 ms and the measurement of time is done simultaneously. All data is stored for future use.

Assembly of the micro flow primary standard's parts

After choosing all the equipments and software, the parts were modelled and a first "virtual" assembly was made, see figure 1.

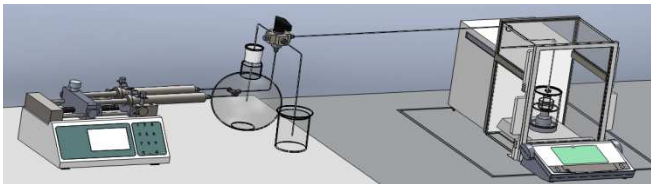

Figure 1 – Assembly of the micro flow primary standard measuring system: (1) *Syringe Pump*; (2) T connection; (3) 4 ways distribution valve; (4) Weighing vessel; (5) Purge; (6) Balance with *evaporation trap*

The final assembly of the microflow primary standard measuring system was then performed, as represented in figure 2.

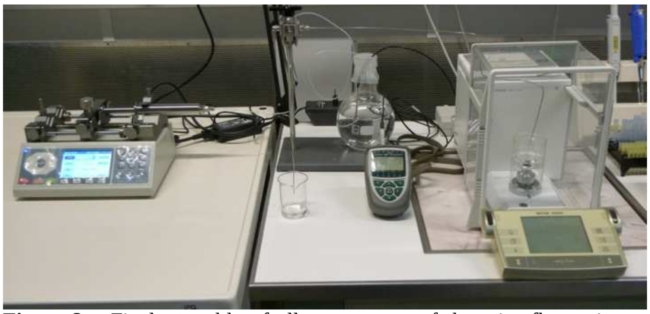

Figure 2 – Final assembly of all components of the microflow primary standard

Experimental procedure

Initial preparation of the system

The syringe was connected to a 1/16" stainless steel tube up to the distribution valve. The distribution valve allows choosing the active circuit, depending on the handle position and three different options are allowed; purge, filling of the syringe and delivery for the fluid's weight.

The water used is ultra-pure without air entrapment and this was achieved by heating the water at 80 ºC and then submitted it to an ultrasound bath for 10 minutes. After this, the water is stored in a vessel for thermal stabilization during 24 h.

The temperature of the water is measured using a Pt100 before each measurement.

The end of the tube is positioned below the surface of the water, in the collecting vessel, inside the balance.

Flow measurements

Depending on the flow under study, the capacity of the syringe is chosen: 20 mL, 10 mL, 5 mL or 1 mL. In order to remove any air from the system, a purge is done before starting the measurements. When the syringe and all tubes are full with the calibration liquid (water) the measurements can start. The acquisition routine is started and the flow is directly calculated with the dedicated developed routine.

Uncertainty calculation

In the uncertainty analysis of the calibration for the gravimetric system, the GUM approach was used [6] and the calculation was based on equation 1. The uncertainty components identified and the correspondent evaluation are presented in table 1.

Table 1 – Uncertainty components

Uncertainty source	Standard uncertainty	Evaluation process	Evaluation type	Distribution
Final mass	$u(I_L)$	1/2 mse (mean square error)	A	Normal
Initial mass	$u(I_E)$	1/2 mse	A	Normal
Density of water	$u(\rho_w)$	Literature	B	Rectangular
Density of air	$u(\rho_A)$	Literature	B	Rectangular
Density of the mass pieces	$u(\rho_B)$	Calibration certificate	B	Rectangular
Temperature	$u(T)$	Calibration certificate	B	Rectangular
Expansion coefficient	$u(\gamma)$	Literature	B	Rectangular
Evaporation	$u(\delta V_{evap})$	Polynomial adjustment	B	Rectangular
Final time	$u(t_f)$	Estimation (1 µs)	B	Rectangular
Initial time	$u(t_i)$	Estimation (1 µs)	B	Rectangular

The combined uncertainty was determined using equation 2:

$$\boldsymbol{u(Q)} = \left[\begin{array}{c} \left(\frac{\partial Q}{\partial I_L}\right)^2 \boldsymbol{u^2(I_L)} + \left(\frac{\partial Q}{\partial I_E}\right)^2 \boldsymbol{u^2(I_E)} \\ + \left(\frac{\partial Q}{\partial \rho_W}\right)^2 \boldsymbol{u^2(\rho_w)} + \left(\frac{\partial Q}{\partial \rho_A}\right)^2 \boldsymbol{u^2(\rho_A)} + \left(\frac{\partial Q}{\partial \rho_B}\right)^2 \boldsymbol{u^2(\rho_B)} \\ + \left(\frac{\partial Q}{\partial \gamma}\right)^2 \boldsymbol{u^2(\gamma)} + \left(\frac{\partial Q}{\partial T}\right)^2 \boldsymbol{u^2(T)} + \left(\frac{\partial Q}{\partial \delta V_{evap}}\right)^2 \boldsymbol{u^2(\delta_{Vevap})} \\ + \left(\frac{\partial Q}{\partial t_f}\right)^2 \boldsymbol{u^2(t_f)} + \left(\frac{\partial Q}{\partial t_i}\right)^2 \boldsymbol{u^2(t_i)} \end{array}\right]^{\frac{1}{2}} \quad (2)$$

The expanded uncertainty is determined using equation 3, for a *k* = 2, considering a confidence level of 95%.

$$U = k \times u(Q) \quad (3)$$

Experimental results

After the assembly of the microflow primary standard's parts it was necessary to verify the performance of the system. Several tests were performed, at different flow rates, using two different stainless steel syringes of 20 mL and 5 mL. The obtained results are presented in table 2 and table 3.

Table 2 – Repeatability results using a stainless steel syringe of 5 mL

	Nominal flow (mL/min)	Obtained flow (mL/min)	Error (%)	Standard deviation (%)	Expanded uncertainty (%)
Syringe 5 mL	**0,033**	0,0349	5,6153	0,25	0,50
		0,0346	4,7480		0,50
		0,0347	5,2219		0,50
	0,1	0,1050	5,0089	0,20	0,11
		0,1048	4,8249		0,11
		0,1043	4,3289		0,11
	0,33	0,3448	4,4962	0,10	0,11
		0,3439	4,2008		0,11
		0,3448	4,4762		0,11
	1	1,0286	2,8563	0,07	0,11
		1,0296	2,9633		0,11
		1,0309	3,0943		0,11

Table 3 – Repeatability results using a stainless steel syringe of 20 mL

	Nominal flow (mL/min)	Obtained flow (mL/min)	Error (%)	Standard deviation (%)	Expanded uncertainty (%)
Syringe 20 mL	**0,1**	0,1007	0,7018	0,15	0,12
		0,1002	0,1978		0,11
		0,1005	0,5428		0,12
	0,33	0,3331	0,9511	0,15	0,11
		0,3336	1,0856		0,11
		0,3319	0,5847		0,12
	1	1,0017	0,1703	0,005	0,12
		0,9982	0,1816		0,12
		0,9983	0,1658		0,12
	3,3	3,2602	1,2068	0,13	0,12
		3,2714	0,8659		0,12
		3,2742	0,7811		0,12
	10	9,9349	0,6512	0,018	0,12
		9,9167	0,8333		0,13
		9,9796	0,2043		0,12

After analyzing the results, one can verify that the uncertainty and standard deviation obtained for small flows, namely lower than a 0,33 mL/min, are the higher ones.

Conclusions

The IPQ microflow primary standard was developed and tested at different flow rates, namely: 10 mL/min, 3 mL/min, 1 mL/min, 330 µL/min, 100 µL/min and 33 µL/min. The uncertainty calculated for the flow system was comprehended between 0,1 % and 0,5 %.
The results obtained showed that some improvements can still be made to the system, namely: the use of two parallel syringes, which can generate a more uniform flow and the use of an automatic valve that will reduce the direct influence of the operator. It is also necessary to make more detailed studies regarding evaporation and tube immersion influence.
IPQ has participated in an international comparison in the field of microflow, that is still on-going, and therefore results can be then validated.

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Note: Brand names used in this article, reflect the factual use of equipment without intent to endorse or suggest any commercial statement.