Traceability of pulsed flow rates consisting of constant delivered volumes at given time interval

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Abstract

Very low flow calibrations are important in several areas of pharmaceutical, microfluidic and health care applications where volumetric dosage or delivery at given flow rates are crucial for the process. Not only continuous flow rates are commonly used in the health sector, but also pulsed flow rates consisting of constant delivered volumes at given time intervals. One known application is the delivery of Insulin with tethered or patch pumps. These constant volumes can be of the order of several tens or hundreds of nanoliters. As the delivery times can vary up to several minutes, it is not appropriate to determine an average flow rate of the delivered volume. It is more advisable to determine the average volume and the average time interval of delivery.

The METAS Microflow facility has been upgraded to perform measurements with insulin pumps delivering a volume of 500 nl at a given time interval of several minutes. The updated design and new aspects of the discontinuous volume collection from the tethered or patch pumps are discussed in this paper. First calibration results of insulin pumps are also presented.

1. Introduction

Dosing small amounts of liquids at a well-defined interval in time are implemented in several areas of pharmaceutical, microfluidic and health care applications. Examples for this application are the tethered pump and patch pump for the delivery of insulin. The characterization of one of each pump for flow rates from 70 nL/min to 500 nL/min is presented in this paper. Very low flow rates are often determined by collecting the water continuously in a beaker placed on a balance for the gravimetric method. However, in this case the flow is not generated by a continuous method, but by the stepwise delivery of a constant volume per unit time.

2. Microflow facility

2.1 Gravimetric method

METAS has developed the Microflow facility for flow rates applied in microfluidics covering flow rates from 50 nl/min up to 1 ml/min [1]. Only the main component being the beaker for continuous water collection is emphasized in this paper. The beaker is placed in the weighing environment shown in Figure 1. To control the evaporation rate, the conventional method of adding water in an evaporation trap in the weighing zone is applied to saturate the air with humidity.

The weighing data are continuously collected by a Real Time system (RT), which communicates with the balance at 20 Hz and pairs the weight value directly with the time stamp of the RT.
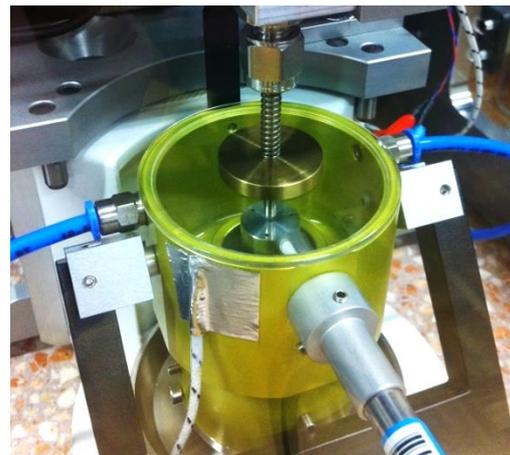
Figure 1: Insight in the weighing zone with its evaporation trap filled with water and the holes at the top of the weighing zone housing acting as humidity exchanger. Details can be found in [1].

2.2 Standard flow rate determination

The collected weighing data are then fitted by means of a least square linear fit to obtain the mass flow rate. A fixed time window of the order of 3600 s or larger is

chosen for the data presented in this paper. By increasing the starting time of this fixed time window by time steps of the corresponding data the moving average of the flow rate in time can be followed. More details of this procedure are beyond the scope of this paper and can be found in the references [1] and [2].

2.3 Volume increment per unit time
As the investigated insulin pumps deliver a certain amount of liquid per unit time, it is more appropriate to determine the increment of volume per unit time. The increment of volume per unit time can also be expressed as a flow rate if desired.

3. Setup for the insulin pumps

3.1 Setup for the tethered pump
The tethered pump is like a small syringe pump with a fixed tubing at the outlet of the small syringe. This tubing is connected to the gravimetric method of the Microflow facility. The setup is shown in Figure 2, where also a thermal mass flow meter is connected in between to follow the flow rate variation with a second technique.

**Figure 2:** Setup of the tethered pump connected to the METAS Microflow facility.

3.2 Setup for the patch pump
The patch pump has a fixed tubing of a length of 12 mm, where the fluid is extruded. Therefore, a special holder for the patch pump was constructed to position the tubing in the beaker on the glass filter. Details of the water collecting beaker can be found in the reference [1]. A picture of the setup is shown in Figure 3.
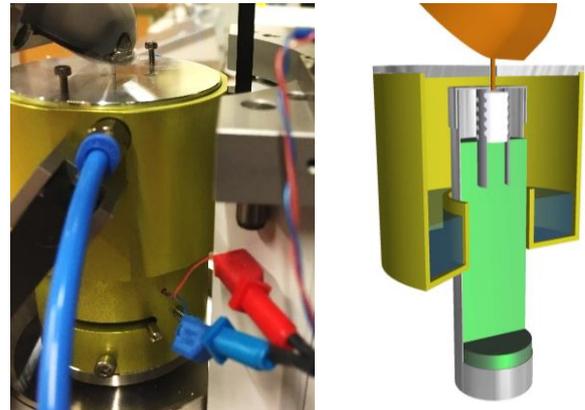
Figure 3: The patch pump is fixed above the weighing zone in order that the tubing is placed on the glass filter in the beaker, which is under the cover.

Another setup was also constructed for the patch pump where the tubing is immersed into water with an oil layer on top to prevent evaporation (Figure 4). This technique is also widely used to collect the water on the balance [3].
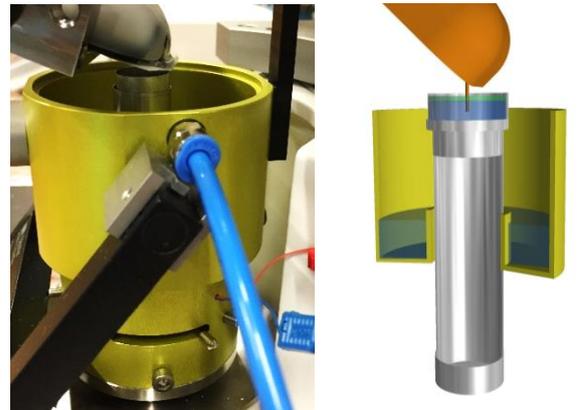
Figure 4: The patch pump is fixed above the weighing zone in order that the tubing is immersed into water in the open beaker. To prevent evaporation an oil layer is added on top of the water.

4. Results for the tethered pump

The flow rates are first determined by the standard flow rate determination method and the deviations are calculated according to the preset flow rate at the tethered pump. The deviations are shown in Figure 5 (black circles) and Table 1. The deviations are between 0 % and 1.3 %, which are within the specified accuracy of ± 5 %.
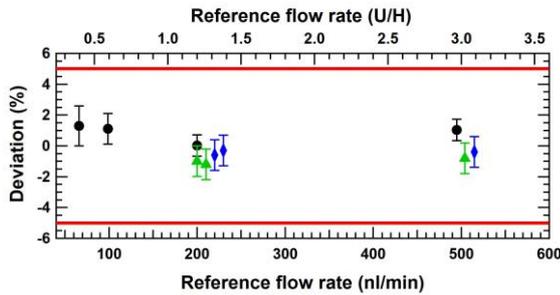
Figure 5: Deviations of tethered pump with respect to the standard flow rate determination method (black circles) and the delivered volume per unit time by using the peak of the histogram (green triangles) or the maximum of the Gaussian fit (blue diamonds). Calibration results are well within the specifications of the manufacturer for the accuracy of 5 % (red solid line). The data at 200 nL/min and 500 nL/min are slightly shifted in flow rate for better illustration.

Table 1: Tethered pump standard method

| Water T	Q_{REF}	Selected flow rate (U100)		Dev	U
(°C)	(nL/min)	(nL/min)	(U/H)	(%)	(%)
22.2	494.9	500.0	3.0	+1.0	0.7
22.2	200.0	200.0	1.2	+0.0	0.7
22.2	98.9	100.0	0.6	+1.1	1.0
22.2	65.8	66.7	0.4	+1.3	1.5
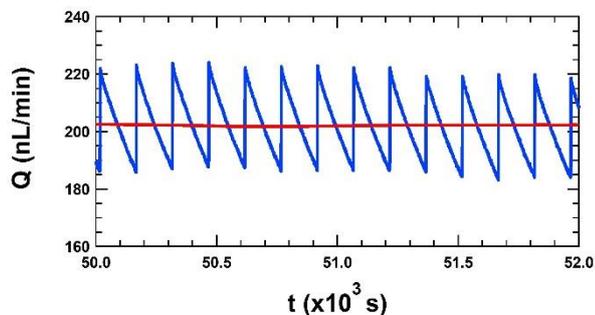
Figure 6: Instantaneous flow rate measured by a thermal mass flow meter (blue line). The average taken over 3600 s is close to 200 nL/min (red line).

To illustrate the delivery of 500 nL per unit time, we show in Figure 6 the flow rate at 200 nL/min measured by a thermal mass flow meter. The instantaneous delivered volume increases the pressure at the outlet of the tethered pump, but the pressure is decreasing along the tubing due to the impedance of the tubing. This is smoothening the flow rate and a stepwise increment of the flow rate at the delivery of the volume followed by a slowly decrease of the flow rate is observed. The same feature is observed at a flow rate of 200 nL/min in the data of the balance shown Figure 7. As the volume is delivered the increment in weight is clearly visible, which is flattening out before the next delivery. The instantaneous flow rate determined by the gravimetric method at 200 nL/min shown in Figure 8 reflects this

behaviour as the strong increase of the flow rate is always followed by a decrease.
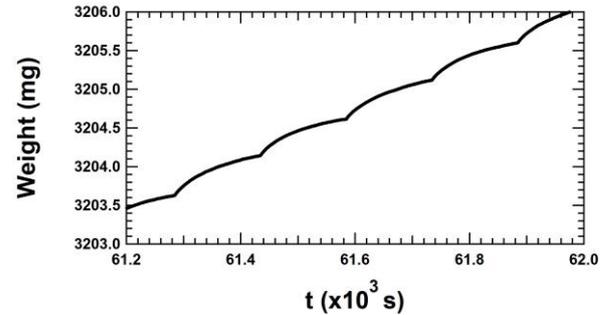
Figure 7: Zoom in of the increase of weighing data. It is a smooth increase of weight in steps and not a sharp step function.
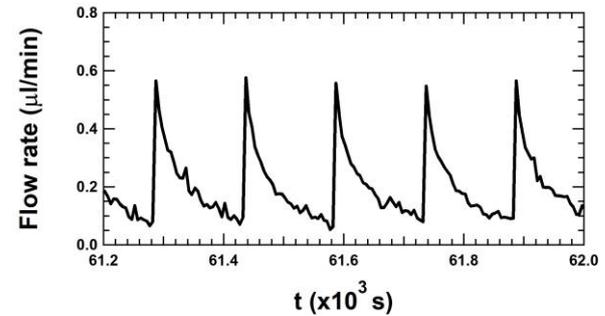
Figure 8: Instantaneous flow rate at 200 nL/min obtained by the weight increase shown in .Figure 7. The fit window is 5 s for the determination of the flow rate by means of the linear least square fit.
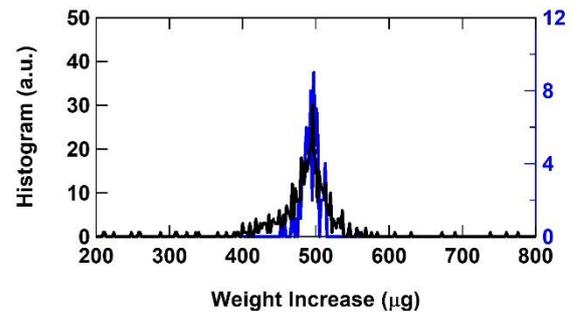
Figure 9: Histogram of the weight increments of all the data of the measurement (black line) and of a selected period being 75 ks – 85 ks (blue line) at 200 nL/min. The second distribution is slightly narrower than the first one. The data of the histogram are raw data without corrections.

Therefore, it is more appropriate to determine the increment of volume per unit time. The histogram of the weight increments per unit time of all data and a selected period is shown in Figure 9. The peak of the histogram for all data resp. a selected period is at 496 µg resp. 497 µg. Performing a Gaussian fit on the histograms leads to a maximum at 492.6 µg resp 494.1 µg for all data resp. a selected period. Converting

the mass into volume by applying the water density of 997.7 kg/m^3 and adding the evaporated amount of water being 7.75 nL in 2.5 min we obtain 504.9 nL resp. 505.9 nL in 2.5 min starting from the peak values and 501.5 nL resp. 503.0 nL starting from the maximum value of the Gaussian fit. These different values differ only by 0.9 %. Expressing this in flow rates we have obtained the flow rates and deviations represented in Table 2, Table 3 and Figure 5 (green triangles and blue diamonds). The deviations obtained are between - 0.3 % and - 1.2 % for both flow rates. The same delivered volume is expected for all the flow rates as only the time interval is changing. Also, the capillary force is not constant any more at the outlet needle with the strong variations in flow rate, but it can be assumed that for each cycle of the delivery of volume the changes in the capillary forces are similar. This contribution to the uncertainty has not yet been investigated und several contributions due to the flow rate determination have to be re-evaluated. A rather empiric estimation of the total uncertainty is 1.0 %.

Table 2: Tethered pump with the method of the volume delivered per unit time. Taking the peak of the histogram.

Data	Q$_{REF}$	Selected flow rate (U100)		Dev	U
	(nL/min)	(nL/min)	(U/H)	(%)	(%)
all	504.3	500.0	3.0	-0.8	1.0
all	202.0	200.0	1.2	-1.0	1.0
75-85 ks	202.4	200.0	1.2	-1.2	1.0

Table 3: Tethered pump with the method of the volume delivered per unit time. Taking the maximum of the Gaussian fit of the histogram.

| Data	Q$_{REF}$	Selected flow rate (U100)		Dev	U
	(nL/min)	(nL/min)	(U/H)	(%)	(%)
all	502.2	500.0	3.0	-0.4	1.0
all	200.6	200.0	1.2	-0.3	1.0
75-85 ks	201.2	200.0	1.2	-0.6	1.0

The largest differences between the two flow rate determination techniques are 1.2 % resp 1.8 % for the flow rate of 200 nL/min resp. 500 nL/min. The results obtained with the standard flow rate determination and the delivered volume per unit time are with the assumption of the uncertainty for the volume delivered per unit time for the first case consistent. This example highlights also that for this type of delivered volume per unit time the analysis method has to be adapted. The standard flow rate determination might not be the best method for this case. A possibility to investigate additional contributions to the uncertainty of this technique can be investigated with the METAS piston provers [2]. A stepwise delivery of a small volume by the METAS piston provers at a given time interval can simulate the stepwise extraction of volumes by insulin pumps. Then, all the effects can be studied, as the generated constant volume per unit time is traceable.

With such investigations a more realistic uncertainty could be obtained.

5. Results for the patch pump

For the patch pump, we had the possibility to apply two different water collection techniques. The first one is the standard method, where the water is flowing through a glass filter and collected in the beaker [1] (see Figure 3). The second one is the collection of the water with the immersed needle in water with a layer of oil on top of it [3] (see Figure 4).

The flow rates are first determined by the standard flow rate determination method and the deviations are calculated according to the preset flow rate at the patch pump. The deviations for both water collection techniques are shown in Figure 10 and Table 4 and Table 5.
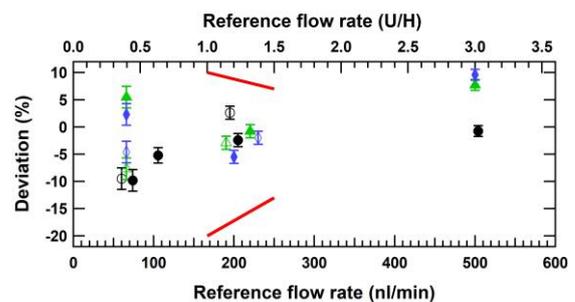
Figure 10: Deviations of the patch pump with respect to the standard flow rate determination method. Two water collection techniques are compared: the standard water collection technique (full black circles) and the water collection technique with the immersed needle in water covered by an oil layer (open black circles). The red lines are the accuracies reported in the reference [4]. Volume increment per unit time for the standard collection technique (Peak - full green triangles; Gaussian - full blue dismonds) and the collection technique with immersed needle (Peak - open green triangles; Gaussian - open blue dismonds). See text for more details.

Table 4: Results of the patch pump with the standard flow rate determination method and the standard water collection technique

| Water T	Q$_{REF}$	Selected flow rate (U100)		Dev	U
(°C)	(nL/min)	(nL/min)	(U/H)	(%)	(%)
22.2	503.8	500.0	3.0	-0.8	1.0
22.2	204.9	200.0	1.2	-2.4	1.2
22.2	105.5	100.0	0.6	-5.2	1.4
22.2	74.0	66.7	0.4	-9.8	2.0

Table 5: Results of the patch pump with the standard flow rate determination method and the water collection technique with immersed tubing in water with oil cover.

Water T	Q$_{REF}$	Selected flow rate (U100)		Dev	U
(°C)	(nL/min)	(nL/min)	(U/H)	(%)	(%)
22.2	194.9	200.0	1.2	+2.6	1.2
22.2	73.7	66.7	0.4	-9.5	2.0

The results at 66.7 nL/min are consistent with both techniques. However, the deviations at 200 nL/min are not consistent with a difference of 5 %. The reasons for this discrepancy are not yet known.

For the standard water collection technique, the tubing of the patch pump is not as stiff as the standard outlet needle used and therefore the capillary force between the tubing and the glass filter might not be as stable as expected. As the tubing is only 12 mm long, the delivered volume creates a strong step increase on the balance, as shown in Figure 11 (black curve) at flow rate 66.7 nL/min with a volume of 500 nL every 7.5 min. The strong peaks at each step reflect the strong variations of the capillary force.
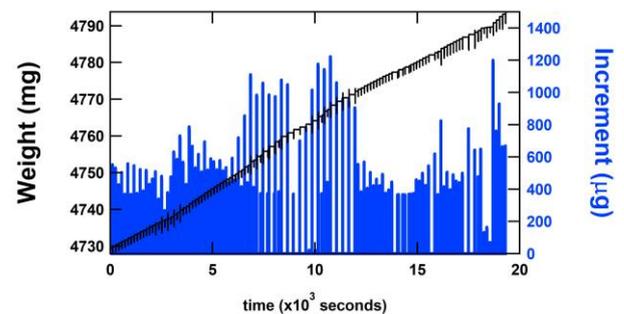
For the water collection technique with the immersed needle, the buoyancy correction force due to the immersed needle has not been determined for this method. It is not clear if a stick-slip effect influences the weighing values at each delivery of the volume. The typical increments of weight are shown in Figure 11 (blue curve). It is surprising to see that after each delivered volume the weight is still slightly increasing during the time before the next delivery of volume. We would expect that the strong increase in weight at the delivery of the volume flatten out over this period or would slightly decrease due to a small evaporation contribution. However, the increase in weight is not expected and further investigations are needed.
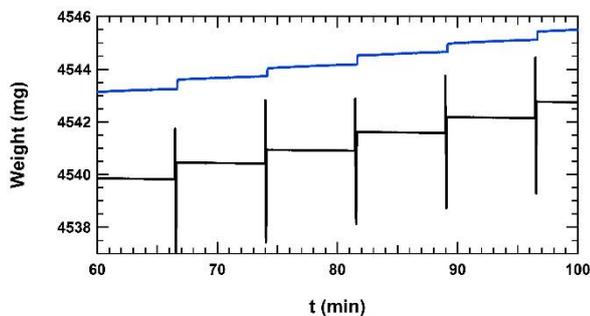

Figure 11: The increase of weight of the two water collection techniques at 66.7 nL/min (500 nL every 7.5 min) are presented: the standard water collection technique (black line) and the water collection technique with the immersed needle in water covered by an oil layer (blue line).

For both water collection technique the increment of volume per unit time is investigated at 200 nL/min. Figure 12 resp. Figure 13 shows the weighing data and the step increments of weight for the standard water collection technique resp. the water collection technique with the immersed needle in water covered by an oil layer. We clearly see in Figure 12 the changes in the capillary force (peaks downwards) when the volume is delivered and water flows on the glass filter. The histograms of the weight increment per unit time of both techniques are shown in Figure 14. The distributions are very broad, but we observe 3 distinct peaks for both

water collection techniques. This leads to the assumption that the delivered volume is not always constant and possibly some stick-slip effects inside the pumps lead to delivered volumes of the order of 400 nL, 500 nL and 600 nL. We identify the main peak and the maximum of the Gaussian fit to estimate the delivered volume per unit time, reported in Table 6 and Table 7. When the distribution is very broad and shows 3 distinct peaks, the Gaussian fit is not very representative. Nevertheless, it is a first attempt for the analysis. The slight differences of the histograms indicate also that the water collection technique and the data analysis for these patch pumps have to be improved and further investigations are needed.


Figure 12: Weighing data (black line) and the step increment of weight for the standard water collection technique (blue line) at 200 nL/min.
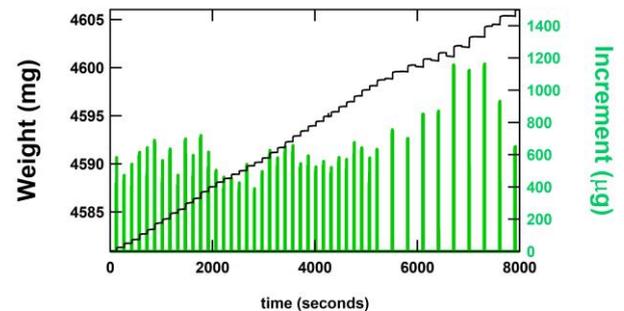
Figure 13: Weighing data (black line) and the step increment of weight for the water collection technique with the immersed needle in water covered by an oil layer (green line) at 200 nL/min.

The same techniques were applied for the analysis of the data at 500 nL/min and 66 nl/min. All the results are presented in Figure 10, Table 6 for the "peak technique" (green triangles) and Table 7 for the "Gaussian fit technique" (blue diamonds) with the full symbols for the standard water collection technique and the open symbols for the water collection technique with the immersed needle. All the differences in the results indicate that the water collection technique and the data analysis for these patch pumps have to be improved and further investigations are needed to avoid systematic

measurement errors and determine a realistic measurement uncertainty.
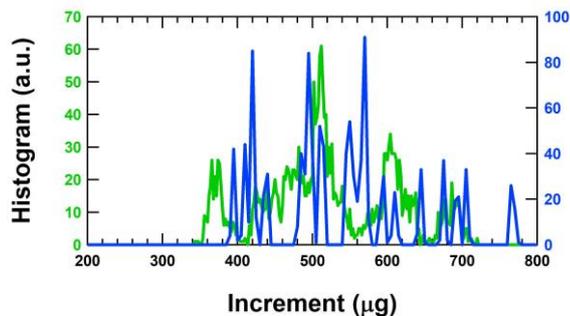
Figure 14: Histograms of the step increments of weight for the standard water collection technique (blue line) and for the water collection technique with the immersed needle in water covered by an oil layer (green line) at 200 nL/min.

Table 6: Patch pump with the method of the volume delivered per unit time. Taking the peak of the histogram.

Method & Data	Q_{REF}	Selected flow rate (U100)		Dev	U
	(nL/min)	(nL/min)	(U/H)	(%)	(%)
Standard 1-12 ks	464.2	500.0	3.0	+7.7	1.0
Immersed needle 1-5 ks	206.0	200.0	1.2	-2.9	1.2
Standard 3-7 ks	201.6	200.0	1.2	-0.8	1.2
Immersed needle 47-54 ks	72.2	66.7	0.4	-7.7	2.0
Standard 1-70 ks	63.2	66.7	0.4	+5.5	2.0

Table 7: Patch pump with the method of the volume delivered per unit time. Taking the maximum of the Gaussian fit of the histogram.

Data	Q_{REF}	Selected flow rate (U100)		Dev	U
	(nL/min)	(nL/min)	(U/H)	(%)	(%)
Standard 1-12 ks	456.1	500.0	3.0	+9.6	1.0
Immersed needle 1-5 ks	204.0	200.0	1.2	-2.0	1.2
Standard 3-7 ks	211.6	200.0	1.2	-5.5	1.2
Immersed needle 47-54 ks	69.9	66.7	0.4	-4.6	2.0
Standard 1-70 ks	65.2	66.7	0.4	+2.3	2.0

6. Conclusion

The METAS Microflow facility has been upgraded to perform measurements with insulin pumps delivering a volume of 500 nl at a given time interval of several minutes. A tethered pump and a patch pump have been characterized for flow rates from 70 nL/min to 500 nL/min. The standard flow rate determination due to the continuous collection of water on the balance and the determination of the delivered volume per unit time were applied to the weighing data.

For the tethered pump, the results were consistent or close to consistency with a difference of 1.8 % with measurement uncertainties of 1 %. For all the flow rates, the deviations were between -1.2 % and +1.3 %. This is expected as on average the same amount of volume is delivered, but only the time interval between two deliveries is changing.

For the patch pump, the setup was slightly more complicated to realise. Using the standard flow rate determination, we obtained similar results for the two water collection techniques. The results at 66.7 nL/min were consistent, while at 200 nL/min the difference is 5 %. The reason for this is yet unknown and further investigations are needed. The second technique determining the delivered volume per unit time shows a large discrepancy for the patch pump. Furthermore, it is not clear, why the standard flow rate determination shows larger negative deviations for lower flow rates. It seems that the stepwise weight increment and other effects occurring due to this non-continuous increase influence the reference flow rate determined with the standard flow rate determination. Further investigations are needed to clarify this issue.

Overall, the upgrade of the METAS Microflow facility enables the determination of constant volume increments at different time intervals. Several issues have to be investigated and the analysis of the weighing data has to be improved.

References

[1] H. Bissig, M. Tschannen and M. de Huu, "Micro-flow facility for traceability in steady and pulsating flow", *Flow Meas. and Instrum.*, Vol. 44, 34-42, 2015.

[2] H. Bissig, M. Tschannen and M. de Huu, "Recent Innovations in the field of traceable calibration of liquid milli-flow rates with liquids other than water", in *Flomeko Proc.*, 2016.

[3] H. Bissig, H.T. Petter, P. Lucas et al., "Primary standards for measuring flow rates from 100 nl/min to 1 ml/min – gravimetric principle", *Biomed Eng – Biomed Tech*, Vol. 60(4), 301-316, 2015.

[4] Zisser H. "Siphon Effects on Continuous Subcutaneous Insulin Infusion Pump Delivery Performance", *J Diabetes Sci Technol,* Vol 4(1):98-103, 2010.