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A CALIBRATION-FREE, DISPOSABLE, NON-CONTACT REAGENT DOSING CARTRIDGE FOR THE SUB-µL RANGE

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ABSTRACT

We have developed a prototype instrument for the sub-µl range which is calibration-free, i.e. it is able to dispense diverse biochemical reagents without the need for adjustment of dispensing parameters with respect to the different rheological properties (viscosity range: 1.03 to 16.98 mPas, surface tension: 30.49 to 70.83 mN/m). The positive displacement technology is non-contact (i.e. carry-over free per design) and disposable (i.e. no washing procedures are necessary). Aliquots in the sub-µl range are dispensed with a high degree of automation, precision (CV < 2.0% at 1 µl) and accuracy (typical accuracy < 4.0%). A capacitive sensor provides online process control.

KEYWORDS

Non-contact dispensing, calibration-free, low-cost, disposable, positive displacement, online process control, capacitive droplet sensor, liquid handling, cartridge

INTRODUCTION

Dispensing systems for drug discovery or in-vitro diagnostics applications are facing diverse and challenging requirements [1]: target volumes are decreased down to the sub-µl range, a diverse portfolio of reagents exhibits strongly varying rheological properties and potential error sources like cross-contamination must be prevented completely.

To address these challenges, we have developed a non-contact dispensing system which is capable of dispensing liquids with varying rheological properties (viscosity 1.03 to 16.98 mPas and surface tension 30.49 to 70.83 mN/m) without requiring to adjust its dispensing parameters. The dispensing cartridge's design is adapted to common polymer syringes which can be used as disposables rendering elaborate washing procedures obsolete.

DESIGN

Cartridge

The cartridge (70 x 30 x 111 mm), shown in Fig. 1, holds up to three commercial low-cost polymer syringes which function as reservoirs for the reagents. Each syringe reservoir is sealed by a polymer piston. A specially developed low-cost tip comprising a

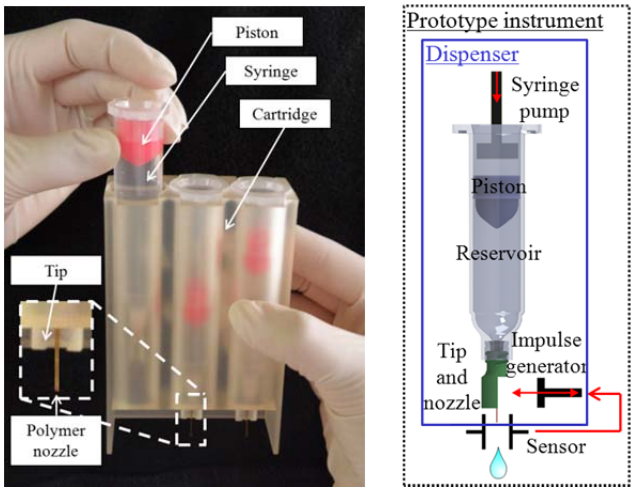

Figure 1: Left: Photograph of the cartridge containing three 10 ml syringes connected to a polymer nozzle each. Right: Sketch depicting the functional elements of one dispensing channel.

polymer nozzle (length 18 mm, diameter 500 µm) is connected to the outlet of the syringe by a standard Luer Lock thread. All components feature an injection-moldable design to allow for low-cost mass fabrication. The tip and the cartridge are currently fabricated by 3D printing (material "Visijet EX 200") to realize the prototype instrument.

Prototype instrument

The main components of the prototype instrument (239.5 x 282.5 x 442.5 mm) are a syringe pump, a piezoelectric impulse generator and a capacitive sensor (see Fig. 1, right sketch).

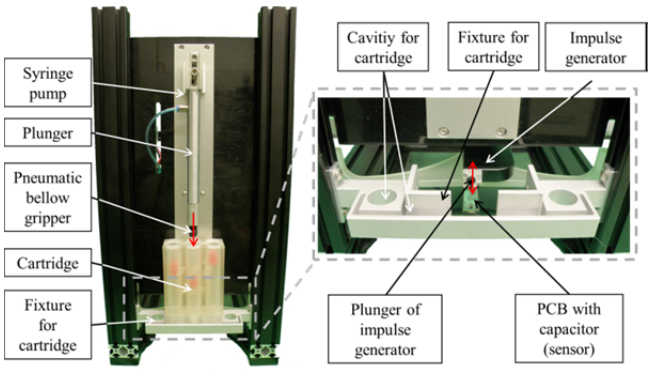

Figure 2: Left: prototype instrument with the inserted cartridge. Right: Close-up of the fixture for the cartridge, the impulse generator and the sensor.

For driving the pistons of the syringes a neMESYS® syringe pump from Cetoni GmbH, Germany is used. The specified positioning accuracy of 70 nm corresponds to a minimum change of volume of e.g. 13 pl if 10 ml Optimum® syringes from Nordson EFD, Germany are used as shown in Fig. 1. In this configuration the maximum flow rate of liquid is 1.19 ml/s.

In order to prevent the displaced liquid to remain attached to the nozzle by surface tension, an impulse generator is applied for liquid release. Therefore, a modified PipeJet™ P9 dispenser from BioFluidix GmbH, Germany [2] is used to introduce mechanical impulses into the liquid by hitting the polymer nozzle with a small piston close to the nozzle.

Both actuators together, the syringe pump and the impulse generator, form the dispenser or actuation mechanism of the prototype instrument as depicted in Fig. 1. Prior to the dispensing process, the cartridge is placed manually into the prototype system and connected automatically with the dispenser: The syringe pump grips the piston with a pneumatic bellow gripper and the impulse generator is engaged with the polymer nozzle by the movement of a pneumatic linear stage.

In order to monitor the liquid meniscus at the nozzle, a capacitive sensor, developed by Ernst et al. [3] is applied. It uses a standard PCB through connection (via) with a diameter of 3 mm as a sensing capacitor. The polymer nozzle is positioned concentrically inside this capacitor when the cartridge is placed into the prototype instrument. To prevent the contamination of the sensor by dispensed droplets, the capacitor surrounds the nozzle approximately 2 mm above the nozzle's orifice. The sensor is used to detect the presence of a pending droplet at the nozzle orifice.

WORKING PRINCIPLE

To perform a dispensing process, the syringe pump positively displaces the target volume by moving the piston into the syringe. Since this cannot be accomplished with high velocity to attain a large Weber number required for liquid break-off, part of the liquid forms a pending droplet at the orifice. After a short idle time (e.g. 0.5 s), waiting for the droplet to fully develop, the impulse generator hits the polymer nozzle close to the orifice to provide sufficient momentum to the pending droplet to detach it.

The capacitive sensor at the nozzle checks whether the droplet tear-off was successful and therefore provides a non-contact, online process control. If the droplet tear-off failed, the parameters of the impulse generator are re-adjusted automatically to provide more energy and re-triggered subsequently.

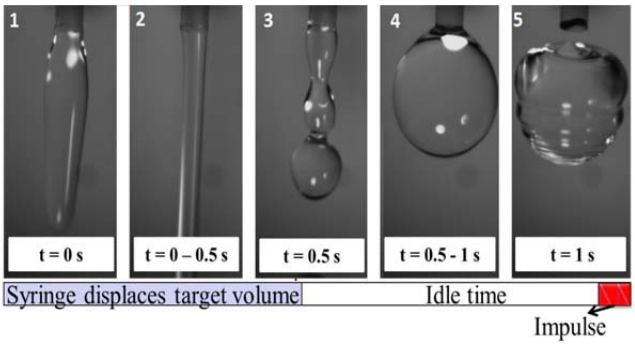

Figure 3. Phases of the dispensing process. The syringe pump positively displaces the target volume (picture 1-3 and blue time beam at the bottom). After a waiting time of 0.5 s (4) a pending droplet forms at the orifice and is knocked off (5) by the impulse generator (red beam).

The total dispensing process, shown in Fig. 3, typically takes 1 to 2 s depending on the target volume and the rheological properties of the liquid. The dispenser and the sensor are not contaminated by the liquid during the dispensing process.

EXPERIMENTS

In order to evaluate the performance of the dispenser and the capacitive sensor, each sub-system is characterized individually in the following section.

Dispenser

The basic function of the dispenser has been characterized for a target volume range of 0.5 to 100 µl with a simplified early stage set-up (see Fig. 4) not yet featuring the capacitive sensor and the automatic engagement. Standard Combitip Plus® 2.5 ml syringes from Eppendorf, Germany (for target volumes < 5 µl) and Plastibrand® 25 ml from Brand, Germany (for target volumes ≥ 5 µl) were utilized.

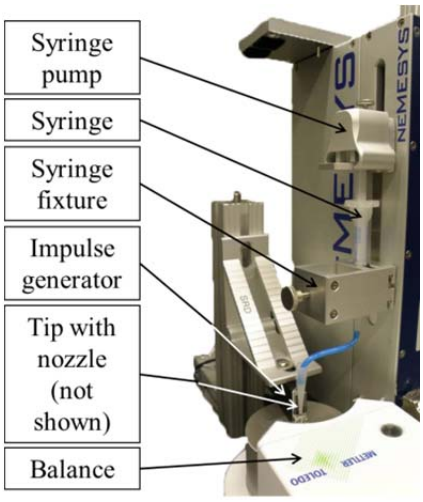

Figure 4. Experimental set-up of the dispenser with the syringe pump and the impulse generator.

The dispensed volume was measured gravimetrically using the measurement method described by Liang et al. [4]. For each volume 24 individual measurements were averaged and the corresponding precision (i.e. coefficient of variation (CV)) and accuracy (i.e. relative deviation from the target volume (Acc)) were evaluated as described in more detail in [5].

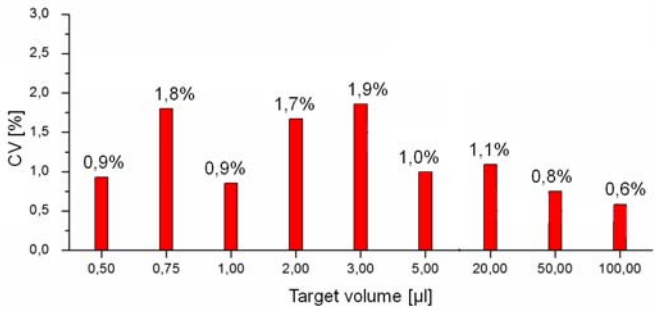

Figure 5. The coefficient of variation (CV) for target volumes of 0.50 µl to 100.00 µl for water.

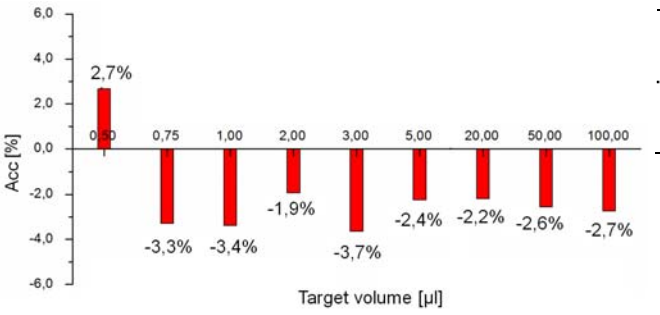

Figure 6. The accuracy (i.e. the relative deviation from the target volume) for target volumes of 0.50 µl to 100.00 µl for water.

Fig. 5 and 6 show the dispensing performance for water in the volume range of 0.5 to 100 µl. The CV ranged between 0.8% at 50 µl to 1.9% at 3 µl. The CV at 0.5 µl was 0.9% Overall, the dispensing performance with water was very precise. The volumetric accuracy was better than ±4.0% for the entire volume range, indicating also a very accurate dispensing performance.

To evaluate the dispensing performance for liquids with different viscosities and surface tension than water, exemplary target volumes of 1 and 25 µl were characterized with the test liquids presented in [6] and displayed in Fig. 7. The actuation parameters of syringe and impulse generator were not changed for any liquid, i.e. no calibration was performed. The dispensing performance was as precise for the test liquids as for water, with a CV below 2.0% for all liquids. The accuracy ranged in-between +0.6% at 25 µl for liquid *B* to -8.8% at 1 µl for liquid *A*.

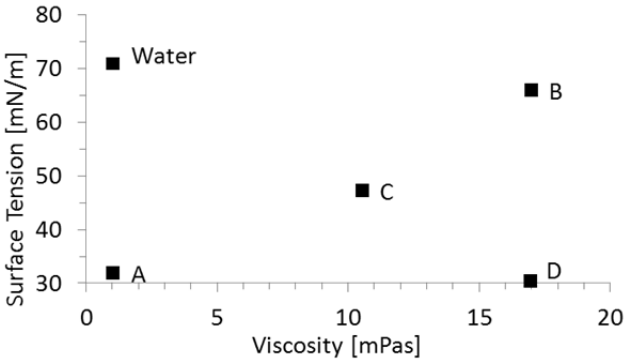

Figure 7. Rheological properties of the tested liquids at 20° C. The test liquids, proposed by Losleben et al. [6], cover the typical range of viscosities (1.03 to 16.98 mPas) and surface tensions (30.49 to 70.83 mN/m) of reagents used in in-vitro diagnostics.

Table 1: The coefficient of variation (CV) and the accuracy (Acc) obtained with the test liquids.

		Water	*A*	*B*	*C*	*D*
CV	1 µl	*0.9%*	*1.9%*	*1.8%*	*1.8%*	*1.3%*
Acc	1 µl	*-3.4%*	*-8.8%*	*-1.0%*	*0.3%*	*-1.3%*
CV	25 µl	*2.1%*	*3.7%*	*0.7%*	*3.4%*	*0.7%*
Acc	25 µl	*+0.5%*	*-0.9%*	*+0.6%*	*-1.2%*	*-0.3%*

Capacitive sensor

Fig. 8 shows two sensor readouts of five successive dispenses of 1 µl for water and test liquid *B*. The rising edge (at e.g. *t1, t3,* and *t5*) depicts the point of time when the syringe pump starts to pump the target volume (corresponding to picture 1 of Fig. 3). The upper level of the sensor signal (e.g. S1 and S3) indicates the pending droplet corresponding to picture 4 in Fig. 3. When the impulse generator is triggered at e.g. *t2, t4,* and *t6* the droplet is detached (see also picture 5 in Fig. 3) and the sensor signal drops suddenly to the lower level. The dispensing time for each dispense was constantly $\Delta t = 1.4$ s.

The difference between the two sensor signal levels for a 1 µl droplet was $S1 - S2 \approx 0.29$ V for water or $S3 - S4 \approx 0.17$ V for fluid *B*. As expected from theory, the sensor signal is different for liquids with different dielectric constants. This clearly indicates that the droplet tear-off can be detected and verified in real-time by the capacitive sensor for different liquids. The signal peak of 1.9 V at *t4* for water can be explained by the piezoelectric actuation of the impulse generator that can induce spikes into the signal. These are detected only rarely due to the low sampling frequency of 0.1 kHz and the short pulse duration of the impulse generator.

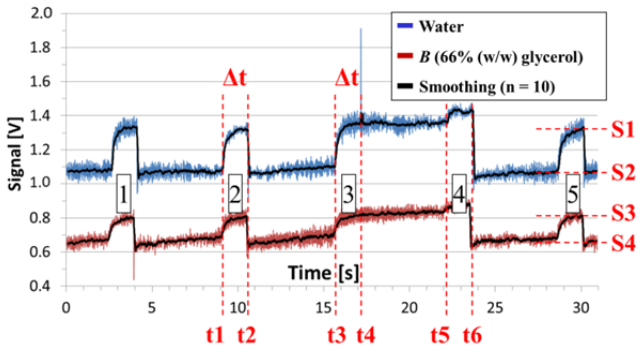

Figure 8. Sensor readout (sample rate 0.1 kHz) of the capacitive sensor of five dispenses of 1 µl for water and fluid B. For dispense #3 a failure of the droplet tear-off was detected.

In order to challenge the droplet tear-off detection, the impulse generator's parameters were manipulated to prevent a successful droplet tear-off for dispense #3 at *t4*. The obvious absence of the signal drop at *t4* indicates a still pending droplet. At *t5* a relatively small increase can be seen when the pending droplet's volume was increased from 1 to 2 µl by the following actuation of the syringe pump. The subsequent impulse at *t5* successfully removed the droplet finally.

Thus, a feedback loop is easily implemented which automatically increases the impulse generator's stroke and re-issues a trigger if the droplet tear-off was not successful in the first attempt. The effect of such feedback loop on the dispensing performance will be characterized by future experiments.

CONCLUSION

The presented prototype of a novel positive displacement technology enables calibration-free dispensing of liquid aliquots in the sub-µl range. All contaminated parts are made of polymers and are therefore fully disposable rendering elaborate washing procedures obsolete. Due to the non-contact dispensing process the liquid handling is carry-over free per design.

The dispensing performance was very precise (CV < 2.0% at 1 µl) and accurate (accuracy -8.8% to +0.3% at 1µl) for test fluids with widely varying rheological properties (viscosity range: 1.03 to 16.98 mPas, surface tension: 30.49 to 70.83 mN/m), corresponding to typical values found for reagents used for in-vitro diagnostic applications. In addition to the good performance, the dispenser is calibration-free, i.e. the only input parameter the system is requiring is the target volume to be delivered independent of the respective liquid to be dispensed.

The capacitive sensor at the nozzle orifice enables an online process control for positive dispensing confirmation and additional safety. A failure of the droplet tear-off is detectable by the sensor and an adjustment of the impulse generator parameters can be realized by feedback loop to correct for such incidences.

With the ability to hold up to three individual dispensing channels, each hosting a different liquid, the disposable cartridge is capable of performing most assays used within in-vitro diagnostic applications. Thus, the presented technology could be used for safe, low-cost and automated reagent handling in in-vitro diagnostic automation systems or for similar industrial applications.

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