



MICROFLUIDIC DROPLET GENERATION USING SYRINGE PUMPS AND PRESSURE-BASED FLOW CONTROLLERS

INTRODUCTION

Micrometer droplets and particles are widely used in a broad range of industries. Single and double emulsions consisting of water and oil mixtures are found in cosmetic products¹, for food processing², while PLGA (poly(lactic-co-glycolic acid)) or alginate microparticles are commonly used for drug delivery applications^{3,4}. Standard methods for droplet production include mechanical devices such as high speed blenders, high pressure valve homogenizers, and colloid mills. Droplet breakup typically happens using shear or impact stresses generated by manual/mechanical agitation. Under such conditions, generated stresses are usually not uniform across the system. As a consequence, the generated emulsions are polydisperse in size^{5,6}. This can be a strong limitation in many applications, as the stability of emulsions depends on size. Terjung et al. demonstrated the impact of size on the efficacy of oil-in-water droplets loaded with antimicrobials agents⁷.

MICROFLUIDIC DEVICES FOR CONTROLLED SINGLE EMULSIONS

Droplet production using microfluidic systems was implemented for applications where monodispersity is of high importance. Within micrometer-sized channels, one droplet at a time is generated, allowing for the production of monodisperse droplets. With such level of control, applications that would not have been possible before also emerged, such as digital PCR and single cell encapsulation within droplets. It is also an excellent method for applications that use expensive API (Active Pharmaceutical Ingredient), as it produces less waste. In a typical microfluidic system, the microfluidic chip is connected to one or more flow controllers that inject the fluids within it. It is common knowledge that droplet size is dependent to the flow rates used, and the topic is well detailed in several studies⁸⁻¹⁰. In a flow focusing device, the droplet size increases when increasing the flow rate of the dispersed phase.

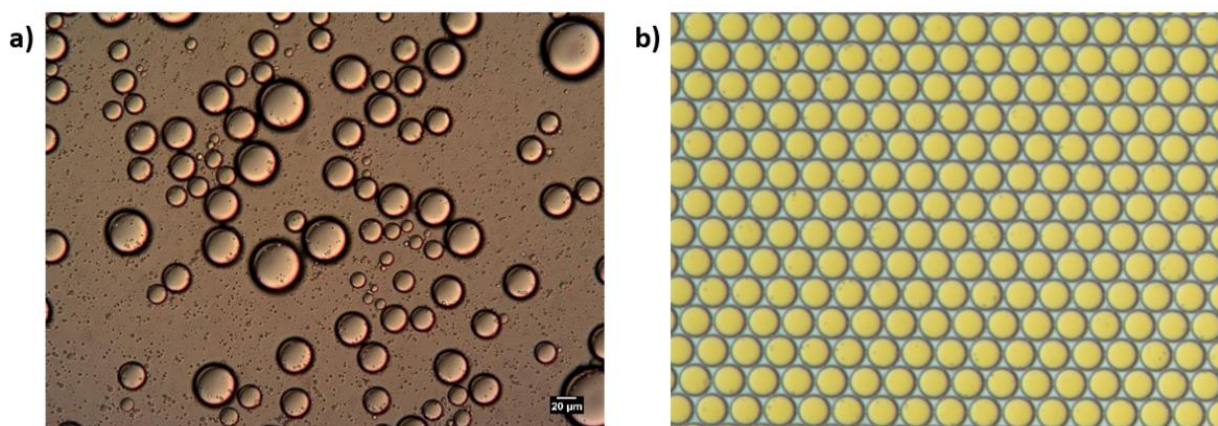


Figure 1: Generation of droplets using a) batch method¹¹ and b) microfluidic method

THE IMPORTANCE OF FLOW RATE FOR MONODISPERSED EMULSIONS

Flow rate stability is critical for having repeatable reactor volume and reproducible results. Syringe pumps are commonly used for generating droplets in microfluidic experiments. Depending on the model in use, syringe pumps show limited flow control. As a consequence, the droplet size, proportional to the flow rate, is affected. The actual flow rate cannot be monitored with such devices. The flow rate value is displayed on the device, but no information on the time required for reaching a set flow rate is given (the time for flow equilibrium may vary depending on the microfluidic setup, and flow can oscillate depending of the instrument). An alternative to syringe pumps are pressure-based flow controllers. These show high-precision flow control, high reaction time, and flow monitoring is possible.

Table 1: Comparison of droplet generation using a syringe pump and the Flow EZ

	Microfluidic syringe pump	Flow EZ (Pressure-based flow control)
Long term generation of stable droplets	Medium	Excellent
Time to reach stable droplet size	High (40 – 300 s)	Very low (< 6 s)
Flow rate control	Yes	Requires flow sensor (and calibration for non-aqueous phases)
Droplet size range*	Limited by min and max flow rate specific to the syringe	Wide

*For a microfluidic system with fixed material and microfluidic channel dimensions

We compare the production of water-in-oil emulsions using microfluidic syringe pumps, and Fluigent pressure-based flow controllers. Droplet size stability and the time required to reach several droplet diameters are determined for the two instrument.

MATERIALS AND METHODS

MATERIALS

Reagents

Dispersed phase: Pure water filtered with 0,22 μm syringe filters.

Continuous phase: dSurf 2% diluted in 3M™ Novec™ 7500 filtered with 0,22 μm syringe filters.

Microfluidic system

Flow control

Two types of flow controllers were used during the experiments. Standard syringes pumps, and Fluigent pressure-based flow controllers (two Flow EZ 345 mbar).

Flow sensor

The Flow Unit is a flow sensor that allows real time flow rate measurement. One Flow Unit S and one Flow Unit M are used here to monitor and control the flow rates of the dispersed and continuous phases during the run.

Microfluidic device

The microfluidic chip used for droplet generation is the Fluigent EZ-Drop. The chip is made of PDMS and features three droplet generator designs.



DROPLET STABILITY ANALYSIS OVER TIME

Figure 2 a) shows the setup for droplet generation using pressure-based flow controllers. A 15 mL reservoir containing distilled water and a 1,5 mL reservoir containing 2% dSurf are connected to the two inlets of the microfluidic chip via 1/32 in. PEEK tubing of 254 μm inner diameter. The tubing passes through flow units allowing flow rate measurement and control. The tubing length from the reservoir to the microfluidic chip is about 80 cm. Figure 2 b) shows the setup for droplet generation using syringe pumps. Two syringe pumps of 15 mL containing distilled water and 5 mL dSurf are connected to the microfluidic chip. Identical tubing is used, and also pass through flow units for flow rate measurements. Water is injected in the inner channel and dSurf is injected in the surrounding channel of the microfluidic device, and droplets are generated. Visualization of the chip channels is performed using an optical microscope. In this experiment, constant flow rates of water and oil of respectively 2,0 $\mu\text{L}/\text{min}$ and 1,5 $\mu\text{L}/\text{min}$ are used. Flow rates are recorded every 0,1 s for 5 min. A picture of the channels with the generated droplets is taken every 10 seconds. The droplet diameters are next measured using the software ImageJ for all picture. Flow and droplet measurements are performed two minutes after ordering the selected flow rate for ensuring each device to reach the targeted flow rates. The average droplet diameter and corresponding standard deviation are calculated.

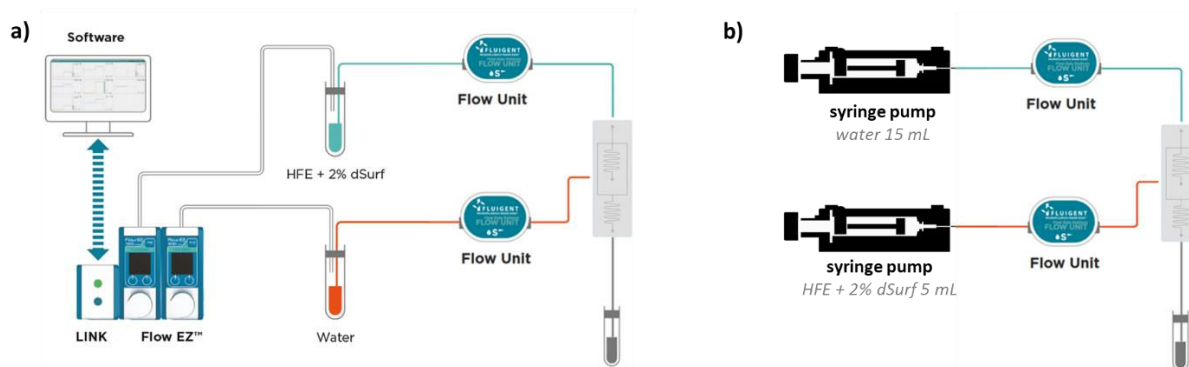


Figure 2: Scheme of droplet generation using the Flow EZ and syringe pumps

ANALYSIS OF TIME REQUIRED TO REACH DIFFERENT DROPLET DIAMETERS

To determine the response time for reaching a specific droplet diameter, we use the same setup from the previous part, "Droplet stability analysis over time". Here, the water flow rate is varied over time to modify the diameter of the generated droplets. The HFE flow rate is kept constant at 1,5 $\mu\text{L}/\text{min}$, and the water flow rate is changed over time in the following order: 1 $\mu\text{L}/\text{min}$ - 4 $\mu\text{L}/\text{min}$ - 1 $\mu\text{L}/\text{min}$ - 2 $\mu\text{L}/\text{min}$. The time to move from one droplet diameter to another (thus from one flow rate value to another) is determined by measuring the time required to reach a flow rate after ordering it, with a tolerance error of $\pm 0,1 \mu\text{L}/\text{min}$. This experiment is repeated three times for each instrument. The average time and standard deviation are subsequently calculated. If a flow rate is not reached in the time limit of two minutes, the flow rate is switched to its next value.

RESULTS & DISCUSSION

DROPLET STABILITY

To determine the droplet stability over time with the different instruments, droplets are generated within a microfluidic chip, working with constant water and oil flow rates of respectively 2,0 $\mu\text{L}/\text{min}$ and 1,5 $\mu\text{L}/\text{min}$. Figure 3 shows the flow rate of water and oil as a function of time for standard syringe pumps, and the Flow-EZ, the pressure-based flow controller from Fluigent. The average flow rates, standard deviations, and coefficients of variation were calculated and are displayed in table 1. The water and HFE flow rate variations using the Flow EZ are lower than when using standard syringe, confirming the enhanced flow stability over time of pressure-based flow controllers.

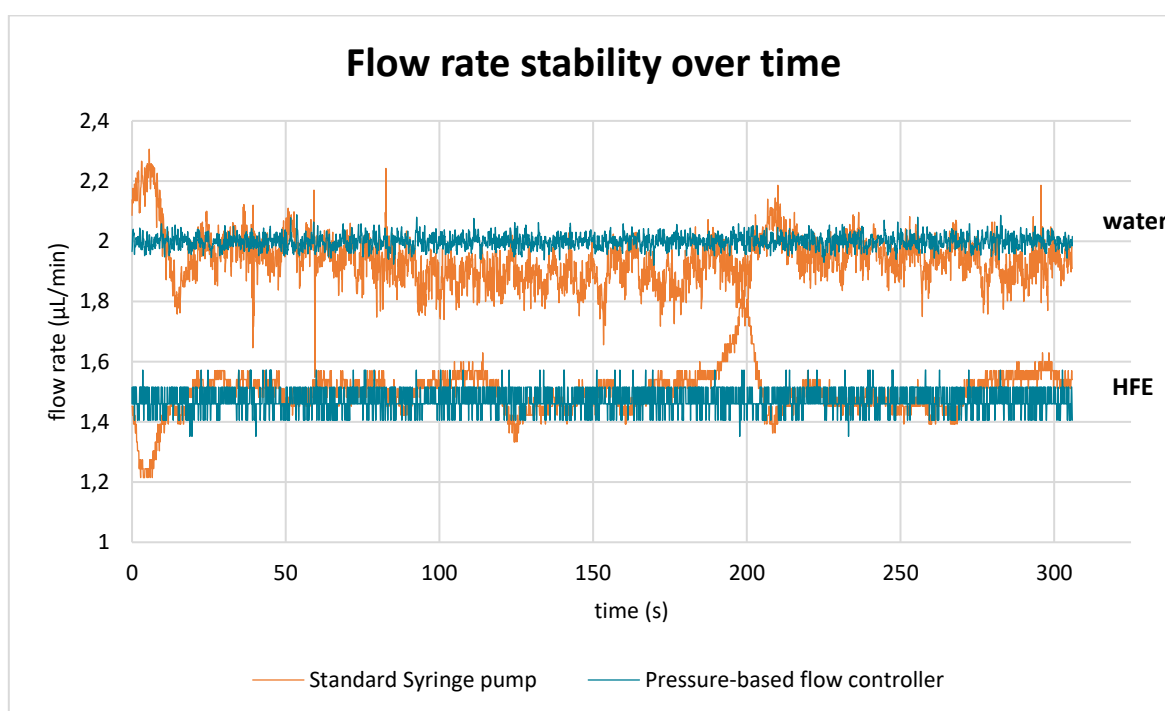


Figure 3: Flow rate stability of water and HFE over time, using the Flow EZ and syringe pumps

Table 1: Water and HFE average flow rate, STD and CV using Flow EZ and Syringe pump

	Water		HFE	
	syringe pump	Flow EZ	syringe pump	Flow EZ
Average flow rate	1,939 $\mu\text{L}/\text{min}$	1,999 $\mu\text{L}/\text{min}$	1,499 $\mu\text{L}/\text{min}$	1,472 $\mu\text{L}/\text{min}$
Standard Deviation	0,080 $\mu\text{L}/\text{min}$	0,022 $\mu\text{L}/\text{min}$	0,069 $\mu\text{L}/\text{min}$	0,038 $\mu\text{L}/\text{min}$
CV (Coefficient of variation)	4,14%	1,10%	4,59%	2,56%

Figure 4 shows droplet generation within the microfluidic device, working with the constant flow rates mentioned above. To determine the effect of flow rate on droplet stability, a picture of the channels was taken every 10 seconds, and the average diameter over 5 minutes was calculated for each device.

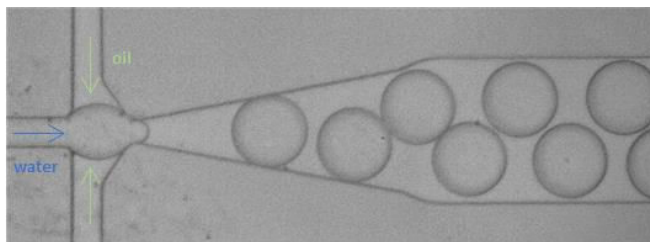


Figure 4: Droplet generation in the microfluidic chip channels

Table 2 shows the average diameter, standard deviation and coefficient of variation of the syringe pump, and the Flow EZ. We observe that the coefficient of variation is lower when using the Flow EZ. This is in good agreement with the results obtained regarding the flow rate stability and confirms that the more precise the flow rate, the more monodisperse the droplets are. Interestingly, the droplet diameters obtained are not similar when using similar flow rates. In fact, the average diameter obtained using standard syringe pumps are approximately 95 μm , while the average droplet diameter obtained using the Flow EZ is 101,4 μm . This trend will be confirmed in the next part when droplet will be generated using several flow rate couples. This can be due to external parameters that can influence droplet size. For instance, if the temperature is not constant, the viscosity will vary and this can affect the droplet diameter. Also, as the microfluidic chip is made of PDMS, the pressures exerted on it by the syringe pump and the Flow EZ can differ. These pressures can possibly inflate the chip channels, and thus affecting the droplet size.

Table 2: Water and HFE average flow rate, STD and CV using Flow EZ and Syringe pump

	Average diameter	Standard deviation	Coefficient of variation (CV)
Syringe pump	94,82 μm	1,65 μm	1,74%
Flow EZ	101,4 μm	0,36 μm	0,36%

To better compare between the different instruments, we use normalized values. This can be done by dividing the droplet diameters calculated at specific times by the average droplet diameter. Figure 5 shows the normalized diameter variation over time. We can observe that droplets are more stable over time using the Flow EZ, compared to the standard syringe pump.

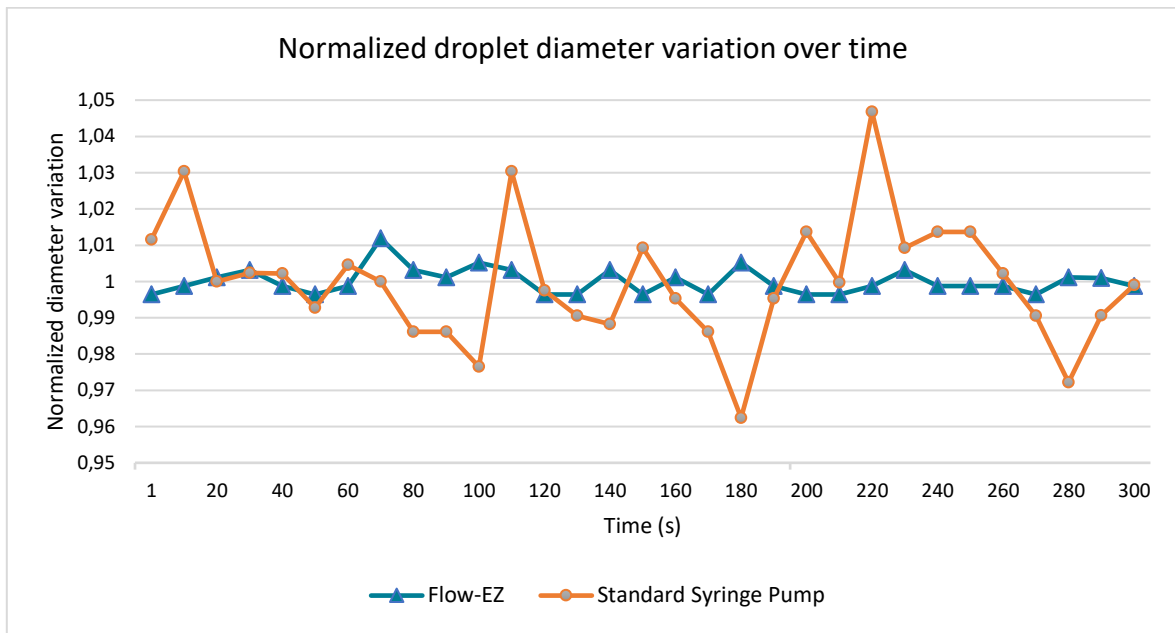


Figure 5: Normalized droplet diameter as a function of time using the Flow EZ and syringe pump

DIAMETER CHANGE

We demonstrated the importance of a stable flow rate to generate highly monodisperse droplets. It can also of interest to be able to quickly move from one droplet size to another. We can determine the response time for reaching a specific droplet diameter. The water flow rate is varied, while the oil flow rate is kept constant at 1,5 $\mu\text{L}/\text{min}$. The set flow rates were the following: 1 $\mu\text{L}/\text{min}$ - 4 $\mu\text{L}/\text{min}$ - 1 $\mu\text{L}/\text{min}$ - 2 $\mu\text{L}/\text{min}$. Figure 6 shows the results obtained using the Flow EZ. We can observe that the set flow rates are reached in a few seconds. After reaching a set flow rate, we can observe the droplet size that results. Droplets of 89 μm , 101 μm and about 110 μm diameter are obtained using respectively 1 $\mu\text{L}/\text{min}$, 2 $\mu\text{L}/\text{min}$, and 4 $\mu\text{L}/\text{min}$ when using the Flow EZ.

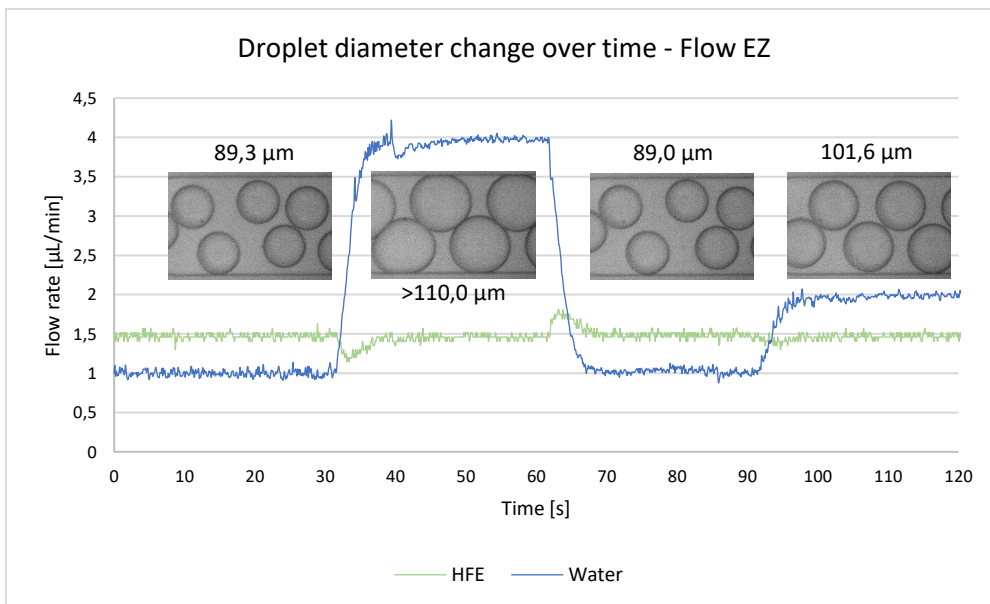
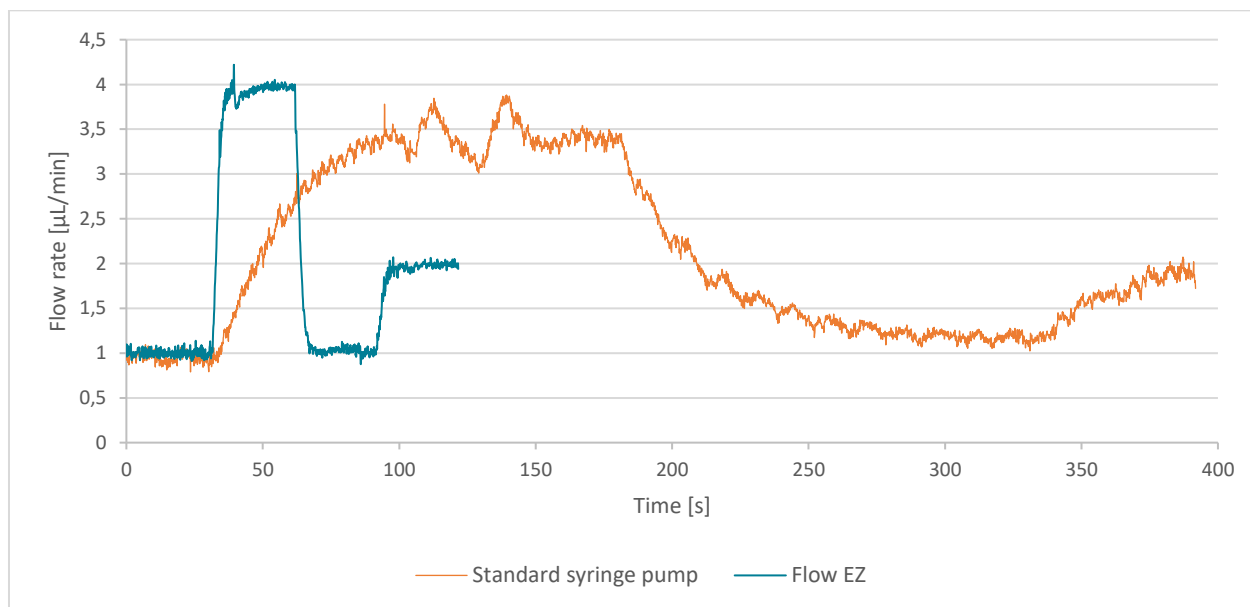


Figure 6: Droplet diameter change as a function of time using the Flow EZ. The water flow rates is varied over time, while the HFE flow rate was kept constant

Figure 7 shows the water flow rate as a function of time using the syringe pump, and the Flow EZ, and the table below shows the time required to move from one flow rate to the other after starting the system. We observe that the maximum time required to switch to another flow rate using the Flow EZ and the syringe pump are respectively 6 s and 93 s. Thus, the Flow EZ is the instrument with the highest response time. This allows to rapidly change from one droplet size to another.



	1 → 4 µL/min	4 → 1 µL/min	1 → 2 µL/min
FEZ 345 mbar	5,9 +/- 0,6 s	5,7 +/- 0,4 s	4,4 +/- 0.6 s
Syringe pump	not reached within time limit (> 2 min)	93,0 +/- 7,3 s	39,9 +/- 3,7 s

Figure 7: Determination of time required to reach one water flow rate to another using the Flow EZ and syringe pumps.

We also observe that the syringe pump did not reach the set flow rate of 4 µL/min in less than 2 min. In fact, the flow rate reaches a plateau at around 3,5 µL/min and cannot manage to go above in the recorded time. We investigated this behavior by repeating the experiment with the standard syringe pump but this time with no time limit. Figure 8 shows the oil and water flow rate as a function of time using the syringe pump. We observe the same behavior than with figure 7, but in about 6 min, the flow rate stabilizes at 4 µL/min. This can be explained by the fact that the droplets produced at such flow rates are relatively large, and start touching each other and the channel walls of the chip. This increases the hydraulic resistance within the chip, and as a consequence it could take longer time for the syringe pump to properly increase the pressure and reach 4 µL/min. However, the pressures used here are relatively low as it required 230 mbar to reach the targeted flow rate when using the Flow EZ.

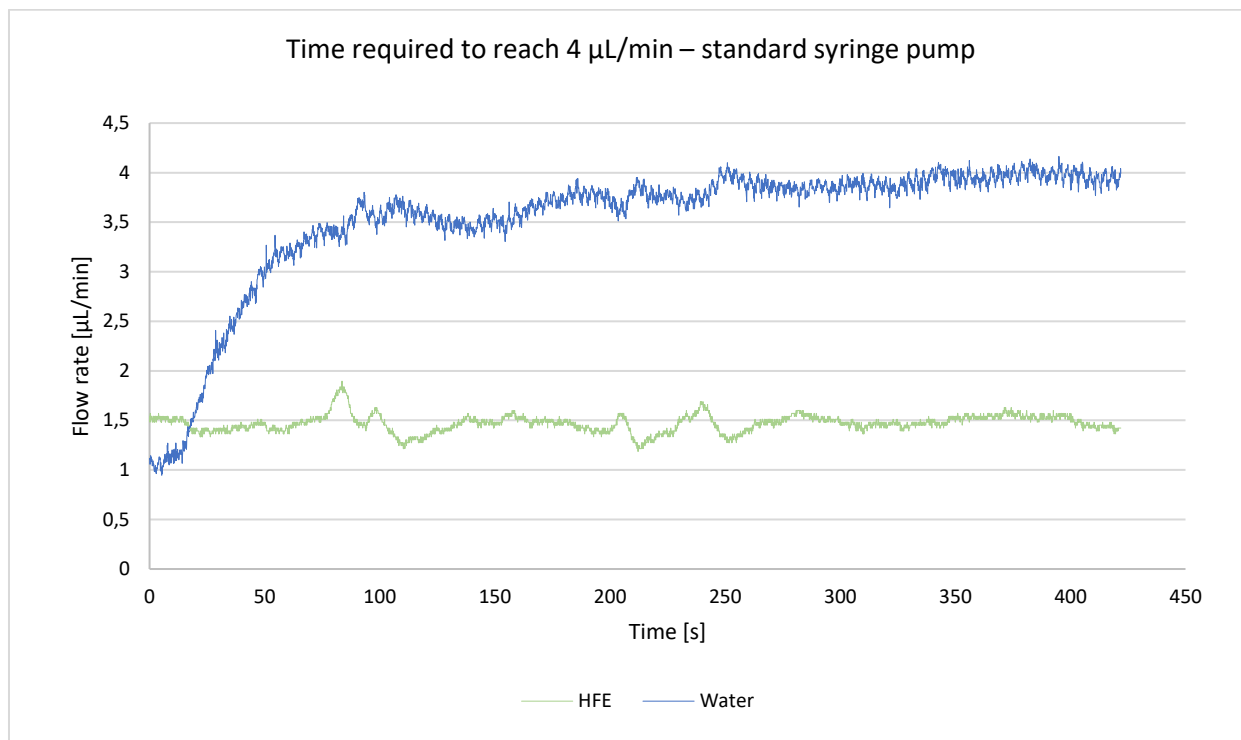


Figure 8: Droplet diameter change as a function of time using the Flow EZ. The HFE flow rate is kept constant while the water flow rates varies over time

CONCLUSION

We compared the generation of micrometer droplets using standard syringe pumps, and the Flow EZ, a Fluigent pressure-based flow controller. More stable flow rates were observed when generating droplets using the Flow EZ, consequently leading to more monodisperse droplets compared to syringe pumps, and confirming the relationship between flow rate and droplet size. The response time between the different devices was also determined. The higher response-time was observed when using the Flow EZ, allowing for responsive control over droplet size during an experiment.

Micrometer size droplets and particles are widely used in a broad range of industries. Fluigent provides complete, cost-effective, solutions for the production of monodisperse droplets. They allow for control of droplet size and frequency by adjusting flow parameters.

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