Comparison of step and flow-focusing emulsification methods for water-in-oil monodisperse drops in microfluidic chips

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Abstract. Emulsions are widely used in various disciplines such as food, chemistry, and pharmaceuticals, due to their efficient encapsulation properties. Recently, the emulsion was emerged as a unique tool for fabrication of multi-complex microparticles, which used as drug delivery agents, as effective manipulators for sorting proteins, DNA, and etc. The high productivity of stable monodisperse emulsions formation is of great importance for these aims. Here, the step and the flow-focusing methods for water-in-oil drops formation in microfluidic chips were compared as a tool for generating well-defined and monodispersed drops using mineral oil as a continuous phase. As a result, drops diameter compares with aperture (nozzle) dimensions for the flow-focusing generator and their diameter is much higher for the step design. Monodispersion and productivity for the step-chip strongly depend on phase densities difference. Flow-focusing method is more suitable for fabrication of monodispersed microparticles and step method for rough technologies such as investigating in food industry or cosmetics.

1. Introduction
Emulsions have long been utilized in various fields, such as food industry, cosmetics, pharmaceuticals, etc. [1,2]. Recently, the emulsions were emerged as a unique tool for fabrication of multi-complex microparticles, which used as drug delivery agents, effective manipulators for sorting proteins, DNA, and etc. [3]. To extend the opportunities of emulsions for microparticles generation, the high productivity of stable monodisperse emulsions generation is required. High monodispersity of emulsions can increase signal-to-noise ratio and improve predictability and standardization of assays [4]. There are classical bulk methods, such as colloid mills, mixers and sonicators, which give high-throughput emulsion generation but have lack control over droplet size and monodispersity.

Recently microfluidics technologies were introduced for droplet formation. These technologies are known as droplet microfluidics. Such emulsifier systems are usually based on T-junctions, co-flow or flow focusing generators, which enable to precisely control droplets size and achieve qualitative monodispersity. But these generators are significantly slower than the traditional emulsification techniques [5]. To overcome low productivity of microfluidic emulsifier systems scaling-up can be used [6].
Besides, in the work of E. Stolovicki, R. Ziblat and D. A. Weitz [4] it was proposed to use parallel step emulsifier devises without shear stress and efficient nozzle clearance during droplet formation for high-throughput droplet generation. Their emulsifier worked well for a wide range of drops, sizing from 30 to 1000 μm (from 14 pl to 520 nl) with coefficient of variation (CV) ≤5.2% at production rates of 0.03 and 10 L per hour and achieved by 400 and 120 parallelized nozzles respectively. They used the high purity water (Milli-Q, USA) as a disperse phase and the fluorocarbon oil HFE 7500 (Novel Engineered Fluid, 3M, USA) as a continuous phase. The construction of the step-emulsification chip which allows self-cleaning of nozzles (step place) should provide both high-throughput and well-define droplet sizes [4]. The microfluidic step-emulsifier operates with two immiscible liquids. The first liquid (dispersed phase) flow through nozzles of the device into a deep and wide reservoir (tank) with the second liquid (continuous phase). Under certain conditions, the confined stream of the disperse phase breaks into small monodisperse droplets at the step [7]. Efficient removal of the drops from the nozzle outlet is imperative to achieve high production rates while maintaining low CV. The self-cleaning method of steps is based on droplet buoyancy in continuous phase. Drops do not stay near the step exit, because they either fall under the action of gravity or float up [4].

In this work, we compared the step and the flow-focusing methods for water-in-oil drops formation in microfluidic devices. A mineral oil was used as a continuous phase due to its low cost compared with fluorocarbon oil. The flow focusing design was used due to its operating principle which is based on the fact that the continuous phase flowing through two side channels meets the dispersed phase at a channels’ intersection, where the dispersed phase is squeezed by the continuous phase and breaks up into droplets. It allows avoiding a contact of forming droplets with channels’ walls and helps to prevent potential negative effects on sample’s components of the dispersed phase [8].

2. Materials and methods

The PDMS microchips were fabricated by standard soft lithography method [9]. In case of the flow-focusing design, to cover a PDMS replica, an oxygen plasma bonding with a glass slide was used. To cover a PDMS replica for the step emulsifier, an oxygen plasma bonding with a PDMS film was used. To create hydrophobization coating on microchannels walls, commercially available anti-rain agent Turtle Wax (the USA) was used. The mineral oil (330779 light, Sigma-Aldrich) with the 3.5% surfactant Abil EM 180 was used as a continuous medium. The oil density was 0.84 g/cm³. The surfactant is needed to prevent the droplets coalescence and provide droplets stability. Deionized water was used as a disperse phase. In the flow-focusing droplet generator microchannels height was 40 μm, output channel width was 200 μm and aperture width was 15 μm (figure 1a). The step microchip had 256 nozzles with dimensions 45x15 μm² (figure 1d).

In case of the flow focusing device, two syringe pumps PHD 2000 (Harvard Apparatus) with 100 µl and 500 µl Hamilton–Microliter Series Gastight syringes for dispersed and continuous phases respectively were used. In contrast, to making an emulsion in the step generator, only one dispersed phase was pumped by the one syringe pump PHD 2000. During droplet generation in the step microchip, the dispersed phase flows into the opened reservoir with volume ~10 ml of the continuous medium (figure 1d). The tank was made like a sandwich by an oxygen plasma bonding of two glass slides 5x10 cm² and 5 mm PDMS layer between slides.

The water-in-oil drops formation in the flow-focusing chips was studied by optical microscope Leica DM4000 B LED (Leica Microsystems). Formation of droplets was recorded on video by the LEICA camera. MatLab script was used to recognize drops on video frames and obtain the dependences of droplet diameter and productivity for the flow-focusing method. In case of the step emulsifier, the digital USB microscope Prima Expert was used. To analyze drop diameter, also the MatLab script was used. The productivity for the step method was estimated as the water flow rate divided by the average diameter.
3. Results and discussion

The drop formation regime (jetting, dripping) depends on capillary number, which describes a ratio of the forces of surface tension and viscous [10]. The dripping mode was in both types of droplet generators.

The flow-focusing microfluidic droplet generator with the aperture \(a=15\) µm allowed to form small volume droplets in the range of diameters from 20 to 70 µm (figure 2a). These diameters correspond to volumes in the range from 4 to 180 pl. The data indicates that droplet diameter depends only on the ratio between disperse and continuous phases flow rates, not by their values. An emulsion is monodisperse at any stable regime, the CV is \(\leq 1\)% To estimate the CV, probability as function of the drop diameter data was approximated by a Gaussian (figure 2b). Moreover, the droplet production rate was analyzed. The productivity is strongly depends on both flow rate values and their ratio. The single flow-focusing generator provided the productivity up to 200 Hz.

In case of the step emulsifier, droplet diameter (figure 2c) was in the range from 350 to 750 µm (from 20 to 220 nl) and had up to 22% CV, so it was a quite polydisperse emulsion. To find the CV, also a probability as function of the drop diameter data was approximated by a Gaussian (figure 2d). The maximum productivity was up to 1800 Hz. Note that the 1800 Hz production corresponds to 256
nozzles of the step microchip, consequently, on average a single nozzle have only about 7 Hz. It is according to formation of the large drop volume (from 20 to 220 nl). When droplets are generated in the step emulsifier their detachment from the nozzle occurred under the action of gravity due to differences of phase densities. So, a velocity of drops removing from the nozzle outlet is strongly depended on phases densities. The greater the difference between densities, the faster a drop disengaging from the nozzle. In the work [4], where the step method was investigated, the fluorocarbon oil [4] was used, which density is 1.6 g/cm$^3$, water droplets floated upward and the difference was 0.6 g/cm$^3$. In our case of mineral oil its density is 0.84 g/cm$^3$, so water drops fell down and the density difference was only 0.16 g/cm$^3$. Perhaps it is the reason for forming drops from 30 to 1000 µm with the CV $\leq$5.2% and the single nozzle productivity from 1500 to 36 Hz, respectively, in case of fluorocarbon oil [4].

![Flow-focusing generator](image1)

![Step generator](image2)

**Figure 2.** Droplet diameter as a function of ratio between water and oil flow rate (a) and probability as function of drop diameter (b) for the flow-focusing microfluidic droplet generator with the aperture $a=15$µm. The average (black squares) and standard deviation (error bars) of drop diameter from water flow rate (c) and probability as function of drop diameter (d) for the step generator with 15 µm nozzle width.

4. Conclusions
To sum up, the comparison of step and flow-focusing emulsification methods for water-in-oil monodisperse drops in microfluidic chips was done using the mineral oil as a continuous phase. The flow-focusing emulsifier have such advantages: monodispersity ($\leq$1% CV), low consumption of continuous phase, getting small drops to 0.5 pl. Also, the single flow-focusing generator gives up to
200 Hz production rate. In contrast, the step emulsifier is convenient method for simple generating of large volumes of emulsion with drops in the range from 20 to 220 nl. The step generator with 256 nozzles give up to 1800 Hz production (or 7 Hz per single nozzle), but lack monodispersity (≤22% CV). Moreover, the production of step method depends on phases densities that makes the system selective. So if the fluorocarbon oil is used as a continuous phase, it is possible to form drops from 14 pl to 520 nl with the CV ≤5.2% at the single nozzle production from 1500 to 36 Hz, respectively.

Note that drop diameter compared with aperture (nozzle) dimensions for the flow-focusing generator and is much higher in case of the step design for the mineral oil as a continuous phase.

The flow-focusing generator can be used for single analysis of cells and molecules, for droplet PCR, for the microparticles creation because of monodispersity. To solve drops mass production problem, scaling up and using several parallel flow-focusing generator is required [6]. It is more reliable and gives stable monodisperse emulsions. Moreover, to form drops in the flow-focusing generator, the big volume like 10ml step tank is not needed. The step microchip is more suited for rough technologies such as investigating in food industry or cosmetics.

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References
[1] Nozdriukhin D V, Belousov K I, Filatov N A, Bukatin A S 2017 IOP Conf. Series: Journal of Physics: Conf. Series 917 042015
[2] Kim S-H, Weitz D A 2011 Angew. Chem. 123 8890-8893
[3] Choi A, Seo K D, Kim D W, Kim B C and Kim D C 2017 Lab Chip 17 591-613
[4] Stolovicki E, Ziblat R and Weitz D A 2018 Lab Chip 18 132-138
[5] Ofner A, Moore D G, Rühs P A, Schwendimann P, Eggersdorfer M, Amstad E, Weitz D A, Studart A R 2016 Macromol. Chem. Phys. 218 1600472
[6] Mulligan M K, Rothstein J P 2012 Microfluidics and Nanofluidics 13 65-73
[7] Charkaborty I, Ricouvier J, Yazhgr P, Tabeling P, Leshansky A M 2017 Lab Chip 17 3609-3620
[8] Filatov N A, Belousov K I, Bukatin A S, Kukhtevich I V and Evstrapov A A 2016 J. Phys.: Conf. Ser. 741 012052
[9] Bukatin A S, Mukhin I S, Malyshev E I, Kukhtevich I V, Evstrapov A A and Dubina M V 2016 Tech. Phys. 61 1566-71
[10] Cubaud T and Mason T G 2008 Phys. Fluids 20 053302