AQ: #1

Generating nanoliter to femtoliter microdroplets with ease

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In this letter, we present a simply constructed and easy-to-use fluidic device that generates arrayed aqueous phase microdroplets in oil of controlled size with volumes ranging from nanoliter to femtoliter without surfactant. This can be applicable with a range of materials, allowing production and storage of monodisperse microdroplets. We illustrate the potential of our methodology in the field of nanoparticle generation © *2011 American Institute of Physics*. [doi:10.1063/1.3560453]

Producing microdroplets,[1] droplets in the micrometer range, is of interest in many fields, including biology, biomedical applications, drug discovery, chemical synthesis, and particle synthesis (for photonics materials, drug delivery). In the literature, different approaches to microdroplet generation are presented, from the classical emulsion-based method or bulk method[2] to the more recent droplet microfluidics.[3] To date, a significant problem encountered when forming droplets via bulk methods, the top-down approach, is the wide microdroplet size distribution that typically results.[4] This is remedied by emulsification at the individual droplet level, the bottom-up approach, based on the ability of microfluidics devices to generate, control, and handle microdroplets. One of the limitations of these approaches is that necessitate the use of surfactant to achieve well-defined structures (droplets) and to avoid droplet coalescence during storage. These surfactants affect chemical composition and fluid interface properties. Another limitation of microfluidics is that it is impossible to produce an ordered pattern for channel sizes below 100 nm, because of wetting properties.[5] Conversely, the classical emulsion-based method can generate droplets in the nanometer range.[6] Microfluidics experiments are often referred to as "high-throughput droplet generation."[1] However, lower-throughput experiments, from generation of a single droplet to dozens of droplets, using nozzles or pipette have also been developed: for instance, levitation of a single droplet,[7] isolated droplets held by a micropipette[8] or nanoscale pipetting.[9] Note that some authors describe a nozzle-free acoustic ejector[10,11] which has limitations due to possible liquid evaporation. All these technologies are efficient but require a complex setup.

In this letter, we present a simply constructed and easy-to-use fluidic device that generates arrayed aqueous phase microdroplets in oil. Up to thousands of microdroplets are generated with volumes ranging from nanoliter to femtoliters, without surfactant. The device enables the entire volume range to be attained in the course of one experiment. All experiments are performed on an 18 mm diameter coverslip treated in a way to obtain an hydrophobic surface to avoid microdroplet spreading and coalescence, which can be thermostatted, under an optical microscope (Zeiss Axio Observer D1). Glass coverslip are spin coated at 4000 rpm for 1 min (SPIN 150, SPS) with 4%-950 K PMMA (All Resist ARP 679.04) annealed 10 min at 170 °C. The coverslip is covered with approximately 100 μl of paraffin oil (Hampton Research HR3–421, refractive index=1.467). The micrometer sized droplets of water solution are generated on the coverslip by a microinjector (Femtojet, Eppendorf) used for the injection of liquids in the volume range from femtoliters to microliters. A home-made micromanipulator consisting of 3 miniature translation stages (piezo electric, MS30 Mechonics) allows displacement of the injector (capillary holder) in X, Y, and Z with a displacement of 18 mm in the three directions by steps of 16 nm. A glass capillary (the micropipette) with an internal diameter of 0.5 μm (Femtotip Eppendorf) is used. The whole setup is shown in Fig. 1. Here the solution is 2.71 M NaCl aqueous solution, half the solubility of NaCl in water at 20 °C.[12] Our first experiments with different solution compositions show the importance of interfacial tension between phases and viscosities of phases for the control of the microdroplet sizes; however, the mechanism of microdroplet generation is independent of the solution composition, as pointed out by Tabeling *et al.*[13] in the case of microfluidics. Note that, here, we succeeded in generating microdroplets with many aqueous phases tested.[14] By controlling the speed displacement of the micropipette (in contact with the surface) and injection pressure we control the droplet size. In Fig. 2(a) we show an array of monodisperse[15] water droplets of mean size 11.8 μm $\pm$ 0.6 μm. When the speed displacement of the micropipette is varied from 1 to 0.1 mm s^{-1}, the droplet size varies from 12 to 31 μm [Fig.

AQ: #2

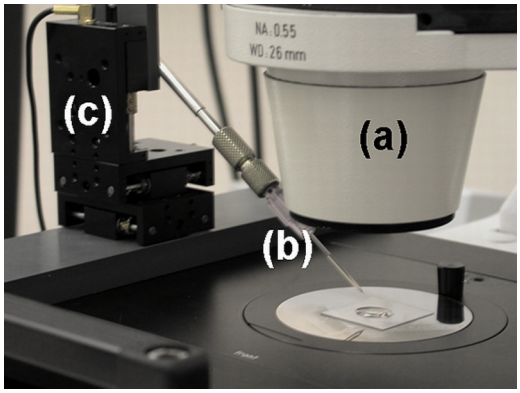

FIG. 1. (Color online) Image of the whole experimental setup, (a) microscope, (b) glass capillary, and (c) XYZ miniature translation stages.

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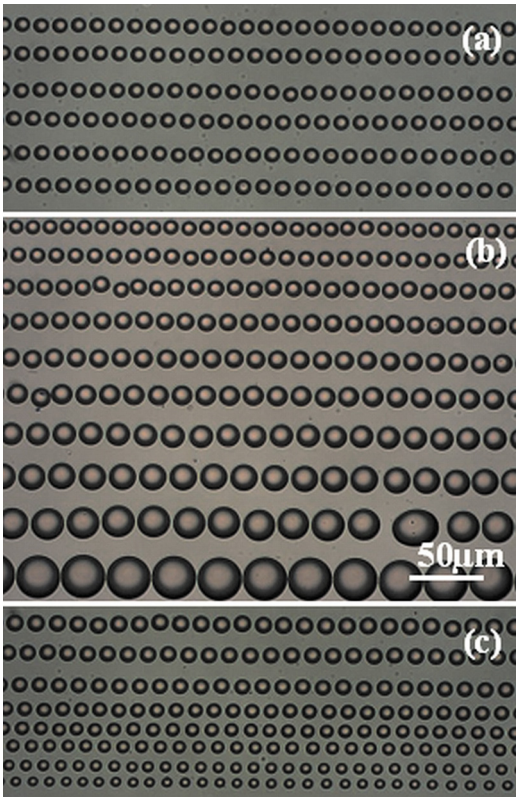

FIG. 2. (Color online) (a) Image of droplets generated with P=5000 Pa and v=1 mm s^{-1}, (b) Image of droplets generated with P=4000 Pa and v varying from 0.1 (first line) to 1 mm s^{-1} (last line) by 0.1 mm s^{-1} step, and (c) Image of droplets generated with v=1 mm s^{-1} and P varying from 5000 Pa (first line) to 1500 Pa (last line) by 500 Pa step. All images are at the same magnification: 1 pixel=0.5625 μm. (enhanced online) [URL: http://dx.doi.org/10.1063/1.3560453.1]

2(b), see video]. Furthermore, when the injection pressure is varied from 1500 to 6000 Pa, the droplet size varies from 8.5 to 14 μm [Fig. 2(c)]. Mean diameter variations as a function of distance between droplets, speed displacement, and injection pressure are plotted in Fig. 3.

We analyze the micropipette velocity v influences on experimental results in the frame of a simple model. The distance d between droplets is as follows:

$$d = v \times t, \tag{1}$$

where t the time interval between successive droplet formations. The volume V of the droplet is as follows:

$$V = \eta \times t, \tag{2}$$

where η is the flow rate from the micropipette. From Eqs. (1) and (2) we deduce that

$$V = \frac{\eta \times d}{v}. \tag{3}$$

Assuming a spherical shape for the droplet with a diameter D, thus

$$V = \frac{\pi}{6} \times D^3 \tag{4}$$

leads to the following:

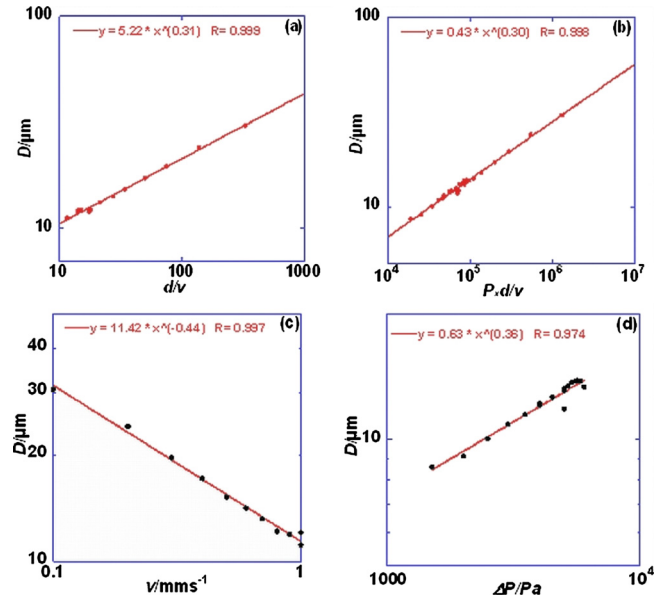

FIG. 3. (Color online) Droplet diameter vs (a) distance between droplets over speed displacements at ΔP=4000 Pa, (b) distance between droplets with ΔP varying between 1500 and 6000 Pa, (a) and (b) v varying between 0.1 and 1 mm s^{-1}, (c) speed displacements at injection pressure of 5000 Pa and, (d) injection pressures at speed displacement of 1 mm s^{-1}.

$$D = \left(\frac{6 \times \eta}{\pi}\right)^{1/3} \times \left(\frac{d}{v}\right)^{1/3} \tag{5a}$$

in a first approximation, ignoring dynamic factors, we assume proportionality between flow rate and injection pressure ΔP

$$D = A \times \left(\frac{\Delta P \times d}{v}\right)^{1/3}, \tag{5b}$$

where A is a constant. Figure 3(a), a plot of D vs d/v at ΔP=4000 Pa, gives $D \propto (d/v)^{0.31}$ and Fig. 3(b), a plot of D vs $\Delta P \times d/v$, gives $D \propto (\Delta P \times d/v)^{0.30}$ showing a very good experimental agreement with Eqs. (5a) and (5b).

We take the analysis further by introducing the mechanism of drop formation through the Rayleigh–Plateau instability as follows:[16] the flow generated by the microinjector passes through the exit orifice and breaks up to form drops, assuming a cylindrical stream (a liquid jet breaks because the surface energy of a liquid sphere is smaller than that of a cylinder, while having the same volume). If we assume that the fluid flowing out of the micropipette is a cylinder of radius r and length d (d is also the distance between droplets), the instability occurs for the following:

$$\frac{r}{d} = B, \tag{6}$$

where B is a constant (critical ratio in the Raleigh–Plateau instability). V the volume of fluid flowing out of the pipette during time t [Eq. (2)], is equal to the volume of the cylinder at t, thus

$$V = \eta \times t = v \times t \times \pi \times r^2. \tag{7}$$

Combining Eqs. (6), (7), and (1) gives the following:

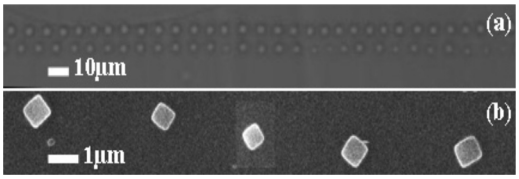

FIG. 4. (a) Array of droplets of NaCl solutions generated through the layer of liquid oil. The size of droplets is 3 μm corresponding to an effective volume of 14 fl, and (b) SEM images of single crystals generated.

$$D = (6 \times B^2)^{1/3} \times d \tag{8}$$

showing the linear dependence of D with d. Combining Eqs. (6) and (7) gives the following:

$$d = \frac{1}{B}\sqrt{\frac{\eta}{\pi \times v}} = v \times t. \tag{9}$$

Combining Eqs. (8) and (9) gives the following:

$$D = \left(\frac{6}{B \times \pi \times \sqrt{\pi}}\right)^{1/3} \times \sqrt{\frac{\eta}{v}} = \boldsymbol{C} \times \sqrt{\frac{\Delta P}{v}}, \tag{10}$$

where in a first approximation, ignoring dynamic factors, we assume proportionality between flow rate and injection pressure ΔP via a constant C. Experimental results presented in Figs. 3(c) and 3(d) give $D \propto \Delta P^{0.36}/v^{0.44}$ in good agreement with this model.

While such an analysis roughly describes for the relationship of D and d with injection pressure ΔP and velocity v, there may be variations in the relationship, for various reasons as follows:

(1) The fluid wets the substrate which changes the surface energy balance. We observed this when the substrate was changed.
(2) The fluid injected by the micropipette requires a threshold value of ΔP, in function of its diameter.
(3) The quantity of fluid injected by the micropipette is dependent on the particular geometry of its apex. Wall thickness seems to influence the droplet size, for instance.

Figure 2 show droplets of diameters ranging from 32 to 8.5 μm corresponding to volume ranging from 321 to 15 pl (assuming a spherical shape for the droplet). Further research should determine the three-dimensional shape of the droplets; for instance, some authors have proposed a hemisphere for microdroplets of 5 μm deposited on a glass coverslip.[9] Note that generating droplets of hundreds of microns, in the nanoliter range, is easy with this setup. Conversely, in the example presented below droplets of 3 μm are generated, thus the femtoliter range is also attainable.

Finally, we illustrate the potential of our methodology in the field of particle generation, where small volume systems offer promising properties.[17,18] Figure 4(a) presents an array of monodisperse droplets of 3 μm containing NaCl 2.71M solution. Droplets slowly evaporate until supersaturation is established. A high supersaturation level is reached and a single nucleation event occurs, always yielding one single crystal of monodisperse size ($740 \times 740 \times 370$ nm^3) per droplet [Fig. 4(b)], heights of crystals were measured by AFM. However, applications fields are not restricted to this field, as pointed out in the introduction.

In conclusion, we have developed a technique using a commercial microinjector coupled with an X, Y, and Z micromanipulator which is applicable to a range of materials and allows the production and storage of monodisperse microdroplets of aqueous phase in oil and without surfactant, maintaining control over size. Moreover, because isolated aqueous microdroplets are generated by micropipette, they can be manipulated individually by micropipette. Finally, this technology can be implemented in standard laboratory environments.

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[14] Saturated $AgNO_3$, KCl, KNO_3, $CaSO_4 \cdot 2H_2O$, Na_2SO_4, glycine and sucrose solutions, and undersaturated proteins (bovine pancreatic trypsin inhibitor and lysozyme) in NaCl solutions.
[15] All the droplets are the same size because $2\sigma \approx 1$ pixel with the standard deviation $\sigma = 0.280$ μm and 1 pixel=0.5625 μm.
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