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Nano-liter size droplet dispenser using electrostatic manipulation technique

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Abstract

A new dispenser made of Teflon that uses electrostatic manipulation technique to dispense nano-liter size droplets based on the electrostatic actuation mechanism has been developed. Two types of dispensers were developed, viz. a single-hole type (Teflon tube) and a multiple-hole dispenser (Teflon block). The single-hole type dispenser successfully dispensed droplets of a solution supplied by a syringe pump on an electrode panel surface; and thus the droplets become available for subsequent manipulation on the electrode panel. Based on the same principle, a compact Teflon block dispenser was designed that can simultaneously dispense five different solutions in a small area (30 mm). The size of dispensed droplets can be controlled by various factors, e.g., flow rate of solution, dispensing height, applied voltage, frequency of voltage pattern. We could dispense the droplets in the range of 55–210 ± 8 nL. This technique can be applied for automation of repetitive pipetting and screening operations in protein crystallization.

Keywords: Droplet dispenser; Electrostatic manipulation; Protein crystallization; Teflon block-dispenser

1. Introduction

Liquid handling with high precision is of paramount importance in few fields; like protein crystallization, drug discovery, etc. The accuracy of assays is largely determined by the fluid volume control of the reagent dosing. New methods of protein crystallization are being searched due to the recent interests of scientists in this field. Many reproducible repetitive dispensing or pipetting operations are necessary to screen conditions suitable for the crystallization of proteins. To ease this laborious and time consuming task, several automatic methods have been introduced. Automatic droplet dispenser by electro-wetting actuation [1] and piezo-electric actuation [2] has been demonstrated in micro-total analysis system (μ-TAS) related works.

On-chip droplet dispensing unit with volume control by electro-wetting actuation and capacitance metering was demonstrated by Ren et al. [1]. Droplets are introduced on the chip through the needle from a pressure source. An electronic system monitors the capacitance between the droplet and a reference electrode. When the desired volume is achieved, a feedback loop shuts off the liquid source.

Electrostatic droplet manipulation is a powerful technique that has received some interests in the recent years, because it has potentially great applications in the filed of μ-TAS [3]. Some advantages, if we compare it with microchannel-based methods, are low dead-volumes, no limitations related to interdependence of channels and no need of micropumps or microvalves [3,4]. Washizu [4] carried out electrostatic actuation in open air; while in our primary work by Taniguchi et al. [3] electrostatic actuation was performed under an inert medium (an oil). In the former case, it is difficult to have precise metering; especially if the volume is less, because evaporation occurs quickly; while in the latter case the oil protects the evaporation.

Cho et al. [5] used two electrode panels, thus they need to introduce the solution in a small on-chip reservoir (2–3 μL)

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before being able to produce droplets. We do not have this limitation. On the other hand, they have more versatility in the droplet control because they used two electrodes. However, our system is more specific for the fabrication of microdroplet array.

Our group has already reported results of experiments carried out to dispense the droplets by using the electrostatic manipulation and syringe needles (Hamilton Co. Revo, Naveda) as a part of sample handling in protein crystallization [6–9]. In those studies we could generate the droplets of 20–700 nL by using various sizes of needles. We wanted to dispense multiple solutions in close vicinity by using the electrostatic actuation technique but it was impossible because putting multiple syringes together required much space. Consequently, we decided to use Teflon tubes instead of syringes.

In the present work, we are reporting two dispensing systems that use electrostatic actuation technique, *viz.* a single-hole and a multiple-hole assembly system. The micro-hole technique provided a good solution to overcome the problem of big size of syringes. The principle and structure of droplet dispenser using electrostatic manipulation is described, and performance of the dispenser is demonstrated. The electrostatic multiple-droplet dispenser can be used in protein crystallization experiments and as a combinatorial chemical device.

2. Fabrication of devices

2.1. Single-hole dispenser (Teflon tube)

Fig. 1 shows the principle and structure of the single-hole type electrostatic droplet dispenser. It consisted of a single microhole (Teflon tube); a motorized syringe system driven by a stepping motor (DRL28PB1-03D, Oriental Motor Co. Japan), an electrode panel, and a controller for these devices. To fabricate this dispenser a micro-hole was machined into a stainless steel block, and a Teflon tube (Cat. #131060, Tokyo Rikakikai Co. Tokyo), having inner dimension of 0.22 mm and outer dimensions of 1.5 mm, was inserted in the microhole to act as a single-hole dispenser. The advantage of this device is that when voltage is applied to the stainless steel block the solution that is in contact with the metallic part and very close to the tip of the tube have a polarity. The Teflon tube was connected with a syringe (500 μL, Hamilton Co. Reno, NV, USA). To dispense droplet it is necessary that the hole as well as its surroundings should be hydrophobic. That is why we selected the Teflon as a material for dispenser tip and that helped in smooth generation of droplets.

2.2. Multiple-hole dispenser (Teflon block)

Based on the smooth generation of droplets by the single-hole dispenser (Teflon tube), we fabricated a multiple-hole droplet dispenser (Fig. 2) that can dispense multiple solutions in a smaller space. It was fabricated with a Teflon block and five holes were made by drilling (micromachining technique) that provided uniformity in getting the hole size. The diameter of a hole was 130 μm. Fig. 2a shows the setup of the multiple-droplet dispenser. The Teflon block was 55 mm long and 27 mm in width, but the five holes were made in the vicinity of 30 mm (Fig. 2b). Hence, we could solve the constraint of dispensing multiple liquid in close vicinity. To have negative voltage in the multiple-hole dispenser the steel block was replaced with five steel screws.

In our preliminary experiments we tried to use five syringes to dispense five solutions, but due to the size of the syringes it was not possible to dispense five liquids in close vicinity. Although five needles can be assembled close to each other but when voltage will be applied they would affect the performance of each other. Similarly, five Teflon tubes can be combined to dispense five solutions but it would be quite difficult to get droplets of

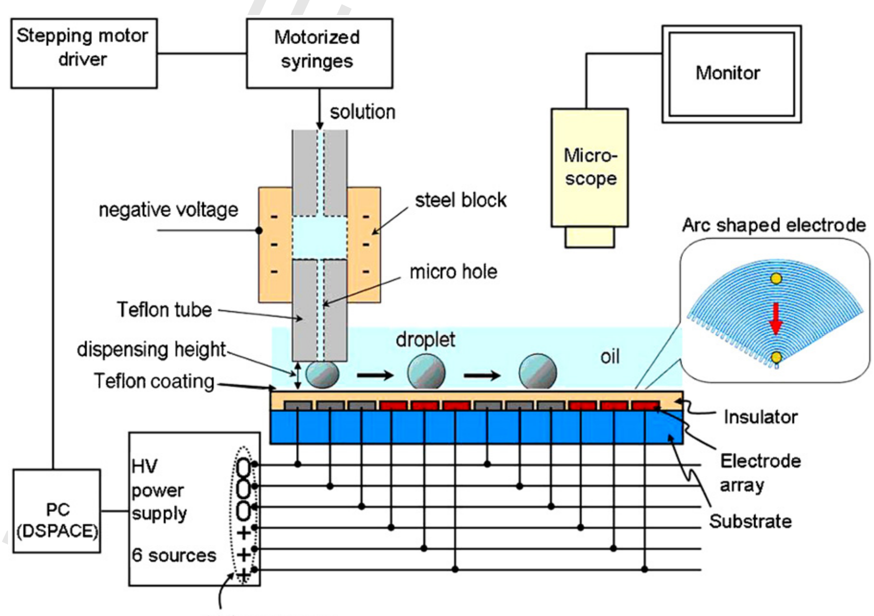

Fig. 1. The principle and structure of the electrostatic droplet dispenser.

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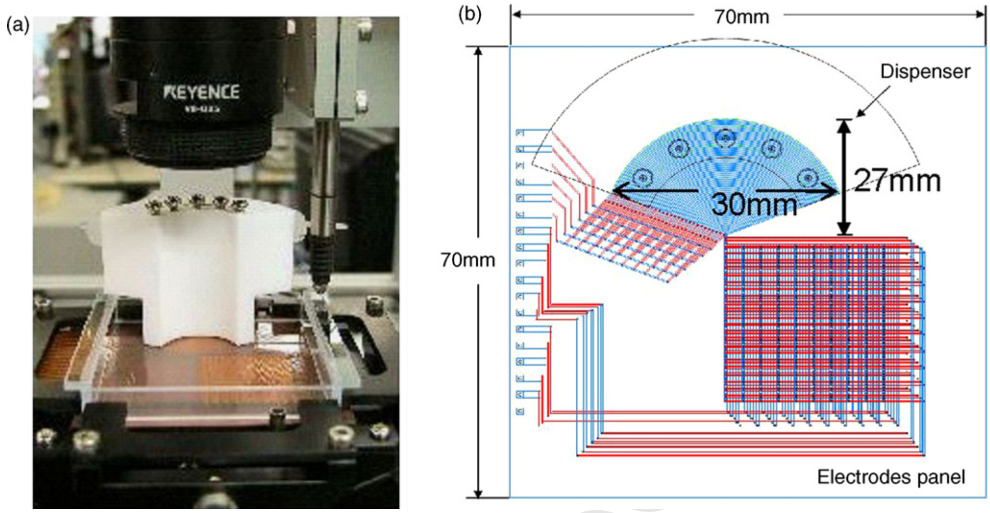

Fig. 2. Setup of multiple-hole dispenser (Teflon block): (a) multiple-droplet dispenser and an electrode panel and (b) schematic representation showing dimensions of electrode panel and Teflon block.

uniform size because many factors are responsible for precision, e.g., dispensing height (i.e., gap between the electrode panel and the dispensing tip). Hence, we decided to fabricate a Teflon block to overcome these problems and to have smooth generation of microdroplets (Fig. 2).

2.3. Electrode panel

An electrode panel (70 mm × 70 mm) was fabricated from copper electrode with glass epoxy substrate for dispensing and actuation of droplets (Fig. 2b). An array of arc-shaped electrodes was made having the pitch and width of 300 μm and 200 μm, respectively. Every six lines of the electrode array were connected together and thus a voltage pattern of six phases could be supplied and reproduced along the electrode. The voltage was applied by a high-voltage power supply (Spec80162A, Kikusui Electronics Co. Japan) and the voltage pattern was moved along the array of the electrodes. The voltage pattern used was three positive electrodes followed by three grounded electrodes (+++000) (Fig. 1). The voltage pattern sequence and number of phase can be changed but this pattern was found to be a good compromise [3]. The voltage was controlled by using the software Control Desk (dSPACE GmbH, Germany).

2.4. Experimental setup

The solution was supplied by pressing the syringe with a stepping motor. If the moving voltage pattern was concurrently applied to the arc-shaped electrodes, the droplets were generated directly on the surface, and transferred to the center of the arc-shaped electrodes by the electrostatic force produced by the moving-voltage pattern. The stability of droplet generation was improved by applying negative voltage (~ -1 V) to the steel block; and because the solution was in contact with the steel block, it would also be negatively charged.

In the case of multiple-hole dispenser, the droplets were dispensed by using five motorized syringes that were connected by Teflon tubes to the Teflon block. The Teflon block was placed over the arc-shaped electrodes (Fig. 2a). The generated droplets were merged in the center of the arc-shaped electrodes. The merged droplets were transferred to another set of electrodes to form a microdroplet array. The droplets were synchronized by changing the flow rate of the solution and the frequency of the voltage pattern.

3. Results and discussion

3.1. Droplet formation and transportation

Initially, pure water was dispensed on the arc-shaped electrodes by the single-hole dispenser (Teflon tube) having 220 μm diameter. When a voltage pattern of six phases was applied to the electrode array and its frequency was 0.5 Hz, the droplets were generated at intervals of every six lines of the electrode array.

The droplet size can be controlled by various factors; such as the flow rate of solution, the dispensing height, the voltage applied to the electrode array, and the frequency of voltage pattern. These factors were controlled by using our own developed software.

Generated interval of droplets was changed according to frequency of voltage pattern and flow rate. Fig. 3 shows dispensed droplets of pure water. Generated droplets proceed to the center of the arc-shaped electrodes and are merged at the center of the arc-shaped electrodes.

A pure water droplet of 113 nL was obtained by using this method compared with a 1 μL droplet dispensed manually with a micropipette. The conditions used to get 113 nL droplet were dispensing height of 0.5 mm; voltage, 200 V; frequency, 0.5 Hz; and flow rate, 0.07 mL/h. The smaller size of droplets can be generated by changing the various factors.

3.2. Effect of the droplet charge

For the droplet dispensing and the actuation by the electrostatic manipulation technique charging of the droplet is very

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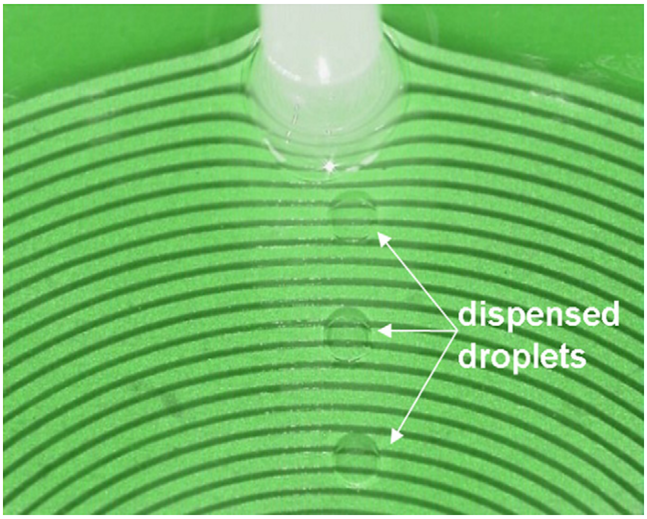

Fig. 3. Droplet dispensing on an arc-shaped electrode panel: experimental conditions were dispensing height = 0.5 mm, voltage = 200 V, frequency = 0.5 Hz, and flow rate = 0.07 mL/h.

crucial. In order to charge the droplets the steel block was connected to a negative voltage of −1 V. We observed that the negatively-charged droplets were more smoothly generated. Uncharged droplets were difficult to be generated from the Teflon tube and to be actuated on the electrode panel. However, the droplets charged with negative voltage were dispensed well. The threshold voltage for electrostatic charging of droplet was about 120 V. A droplet could be generated only when voltage over this threshold value was applied to the electrodes.

3.3. Variation of the droplet size with various factors

There are many factors that effect droplet formation and actuation process; such as flow rate of solution, dispensing height, applied voltage, frequency of voltage pattern, diameter of the hole, thickness of the insulator, viscosity of the oil, pitch and width of the electrodes, state of Teflon coating, and so forth. In this manuscript we will talk about the effect of flow rate, dispensing height, applied voltage, and frequency of voltage pattern.

By having slower flow rate, smaller dispensing height, higher applied voltage and frequency of voltage pattern, more smaller-sized droplets could be generated. However, if flow rate and dispensing height were kept too small the droplet formation process became unstable. On the other hand, if flow rate and dispensing height were kept too large the size of the droplets became too big or droplet could not be generated at all. In this case, the higher voltage had to be applied to the electrodes in order to generate the droplet. However, if voltage of higher than 220 V was applied for a long duration, the electrode array could be short-circuited. Table 1 shows the ranges of the factors to control the droplet size.

Fig. 4 shows the distribution of droplet volume and standard deviation with flow rate and dispensing height. Under the above conditions, the droplet of 58–212 nL could be generated and the standard deviation of the droplet volumes was ±8.8 nL

Table 1
The factors to control the droplet size[a]

Factors	Range
Flow rate (mL/h)	0.03–0.4
Dispensing height (mm)	0.3–0.6
Applied voltage (V)	120–220
Frequency of voltage pattern (Hz)	0.3–2.5

[a] In this case, pitch and width of electrodes were 300 μm and 200 μm, respectively.

(CV = 7.2%). If the diameter of dispensing hole is made further smaller, the droplet size will also be smaller. The droplet size did not vary linearly with the flow rate. However, in general as the flow rate became fast the droplet size increased. Similarly, when the dispensing height was increased the droplet size also increased. The droplet size at the dispensing height of 0.3 mm could not be measured by us because the flow rate over 0.15 mL/h was too fast and droplet formation was very unstable to measure. The standard deviation of droplet volume increased if flow rate was kept too slow or too fast (Fig. 4). For example, at dispensing height of 0.5 mm and flow rate of 0.03 mL/h and 0.4 mL/h, the standard deviation of the droplet volume was ±16.8 nL and ±14.5 nL, respectively. The control of dispensing height and flow rate is necessary for diminishing variation of droplet size. In this study, variation of droplet size was the minimum (S.D. = ±4.3 nL, CV = 3.4%) at dispensing height of 0.5 mm and the range of the flow rate was 0.15–0.25 mL/h. In that case, the droplet production rate was 22–33 droplets/min.

Fig. 5 shows that droplet production rate increased when flow rate was fast and dispensing height was low. Appropriate droplet production rate can be chosen considering droplet volume and its variation.

Changes in the droplet volume according to applied voltage and frequency of the voltage pattern are shown in Fig. 6. Droplet size was affected by applied voltage (Fig. 6a) and frequency of voltage pattern (Fig. 6b). If only voltage is increased, a larger

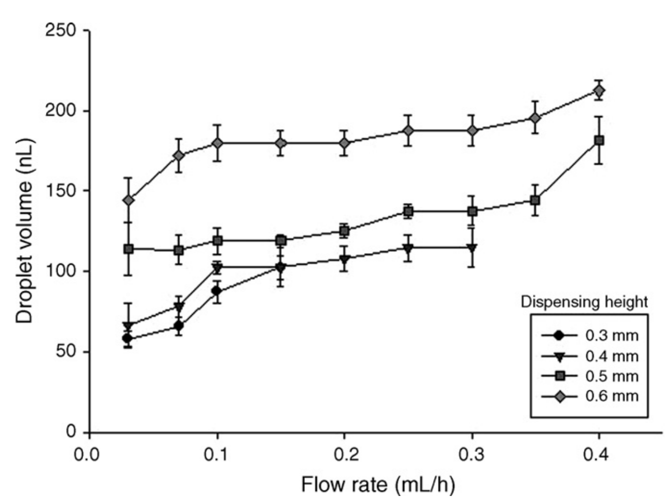

Fig. 4. Distribution of the droplet volume with flow rate and dispensing height. Experimental conditions: the pitch and width of electrode = 300 μm and 200 μm, respectively; the diameter of the dispensing hole = 220 μm; applied voltage = 200 V, and the frequency of the voltage pattern = 1.5 Hz.

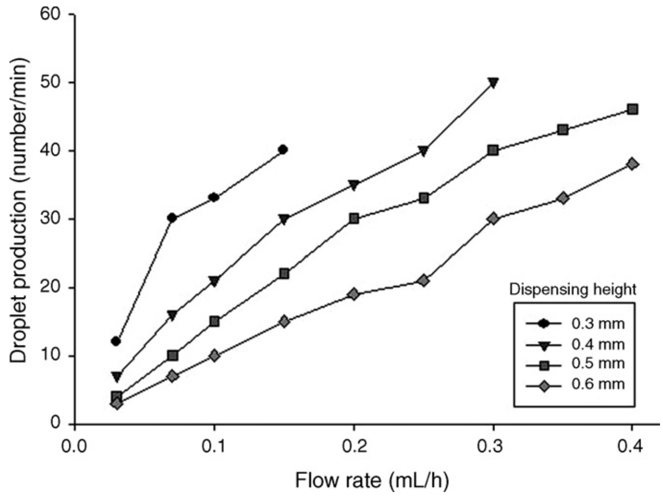

Fig. 5. Droplet production rate with flow rate and dispensing height. The pitch and width of electrode was 300 μm and 200 μm, respectively, the diameter of the dispensing hole = 220 μm, applied voltage = 200 V, and the frequency of the voltage pattern = 1.5 Hz.

droplet is formed. The droplet size is, however, controlled by frequency of voltage pattern and applied voltage. As applied voltage and frequency of voltage pattern increased droplet size decreased. The large difference between the first and the second point in Fig. 6a is due to the fact that at 120 V a droplet is produced every two frequency pattern (thus each droplet is separated by a distance of 12 pitches); whereas from 140 V, one droplet is produced at each frequency pattern (distance of 6 pitches). Further research is needed to understand the phenomenon of this variation. It is probably due to a trade between the electrostatic force and the capillary force. The electrostatic force needs to overcome the capillary force to separate the droplet from the hole. The capillary force does not change with the voltage while the electrostatic force increases with voltage, thus at larger voltage the capillary force overcomes faster and smaller-size droplets are generated.

Viscosity of the oil had influence on the voltage for droplet dispensing and actuation. Silicon oil of various viscosities has been tested for the droplet dispensing and results are shown in Fig. 7. Each point represents the minimum voltage value that can stably dispense the droplet at that frequency. With the increase in the viscosity of oil the voltage needed for droplet dispensing increased. The lower the viscosity of oil, the better was the dispensing performances of droplets.

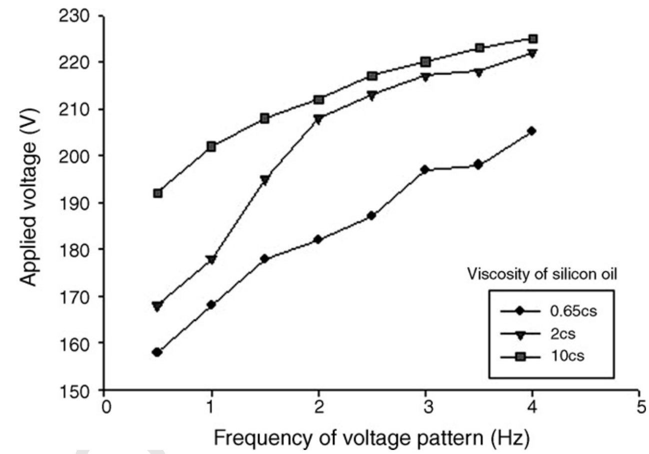

Fig. 7. Voltage and frequency of voltage pattern for droplet dispensing with silicon oil of various viscosities: experimental conditions were dispensing height = 0.5 mm, and flow rate = 0.07 mL/h.

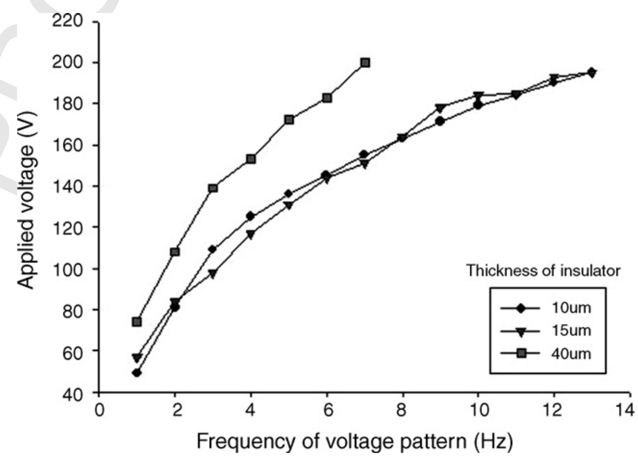

Fig. 8. Voltage and frequency of voltage pattern for droplet actuation with thickness of insulator: experimental conditions were dispensing height = 0.5 mm and flow rate = 0.07 mL/h.

Fig. 8 shows the actuation voltage of droplet with thickness of insulator. The voltage versus frequency of voltage pattern curve was recorded. The performance of 15 μm shows the smaller actuation voltage as compared with 40 μm. Comparing with the

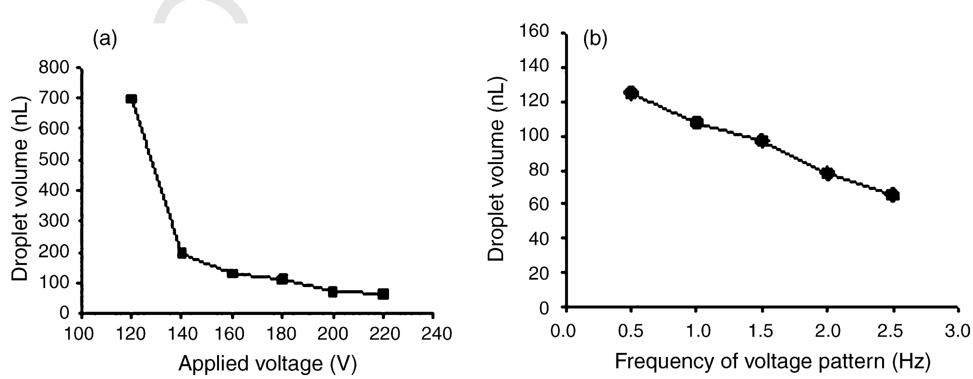

Fig. 6. Changes in the droplet volume with (a) applied voltage: experimental conditions were dispensing height = 0.5 mm, frequency = 1.5 Hz, and flow rate = 0.07 mL/h; and (b) frequency of voltage pattern: experimental conditions were dispensing height = 0.5 mm, voltage = 200 V, and flow rate = 0.07 mL/h.

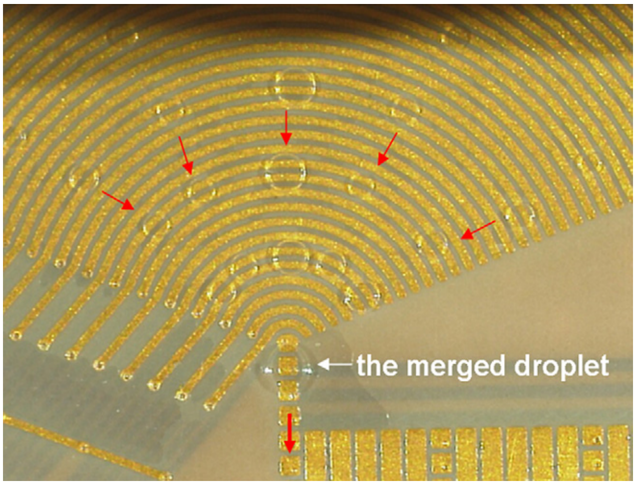

Fig. 9. Pure water droplets dispensed by the Teflon block (multiple dispenser).

dispensing voltage in Fig. 7, voltage needed in actuating droplets on the electrodes was much less than that in dispensing droplets and the usable frequency range was broad.

For the droplet dispensing, if the thickness of insulator was too thick (>40 μm), the droplet could not be generated. For smooth movement of droplet the surface of the insulator should be hydrophobic. Therefore, the insulator was coated by a hydrophobic material, i.e. Teflon AF. If the surface of the insulator is not hydrophobic a droplet could not be generated.

The pitch of the electrodes influenced the droplet size (data not shown). Smaller size of electrodes generated the smaller size of droplets. However, if pitch of the electrodes was too small compared with the applied voltage, electrodes become shorted. If the applied voltage is in the range of 120–220 V the pitch of electrodes should be >250 μm. We observed that the short circuit usually occurred near the through hole because at these points the electric lines were closer to each other. The problem of short circuiting may be overcome by giving due importance to the insulating strength of the Teflon coating.

3.4. Multiple dispensing in small space

Fig. 9 and the movie show the pure water droplets dispensed by the multiple-hole dispenser (Teflon block). We could make the droplets at the range of 15–140 nL.

Both of the dispensing systems developed by our group can be used for setting up protein crystallization experiments. To assess the influence of voltage on protein crystallization our team member Hirano et al. [10,11] set up protein crystallization experiments by using chicken egg-white lysozyme (CEWL) and thaumatin and reported satisfactory results. The newly-developed multiple dispenser (Teflon block) has been made a part of the high throughput protein crystallization system (HTPCS) fabricated by our team and tested for protein crystallization [8]. One of the solutions was CEWL, while the other solutions can be precipitants, buffers, etc. (Fig. 10). The electric-field induced protein crystallization has also been recently reported by Al-Haq et al. [12].

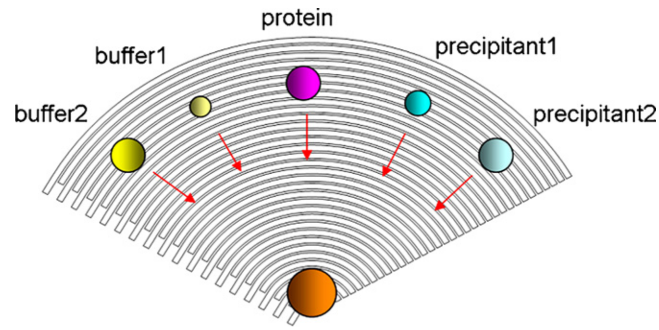

Fig. 10. A schematic representation for dispensing different solutions for protein crystallization.

4. Conclusions and future work

In this work, we have demonstrated two droplet dispensers, i.e. a single-hole dispenser (Teflon tube) and a multiple-hole dispenser (Teflon block). The single-hole system had the inner diameter of 220 μm and the nano-liter size droplets were generated (range, 58–212 nL). The droplet size could be controlled by changing the various factors, e.g., the applied voltage, frequency, the height between hole and the electrode, viscosity of oil, etc. The variation in droplet size could be reduced by selecting the proper value for the each factor. The second system was the compact multiple-droplet dispenser (Teflon block) and its electrodes assembly that could dispense and actuate various solutions in a reduced space. It can be used for protein crystallization experiments, where variation of droplet size is related to the accuracy of the concentration of the solutions. Therefore, it is important to keep uniformity in size of the dispensed droplets. In future, we plan to realize the on-chip high-throughput screening system for protein crystallization by using this multiple-droplet dispenser technology.

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Biographies

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Eric Lebrasseur was born in France, in 1971. He received the BS degree in mathematics, the MS degree in physics, and the PhD degree in physics from the Université Lyon I, France, in 1991, 1993, and 1999, respectively. His research interests include microelectromechanical systems (MEMS) design, fabrication and development.

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