

**Magnetic bead extraction from phosphate-buffered saline droplets in
HFE-7500 immiscible oil for mRNA isolation**

Prisha Singhal

Abstract

Droplet microfluidics involve the manipulation of fluids at a microscopic scale to mimic environments in a controlled setting. Genetic material can be extracted within these fluids using Oligo(dT)₂₅ magnetic beads which bind to the poly(A) tail of mRNA molecules. Adding cell lysate to a solution of buffer and magnetic beads allows for the extraction of magnetic beads and mRNA from droplets using an external magnet.

The system to create droplets was designed in a microfluidic device made of polydimethylsiloxane (PDMS), in which opposing flows of immiscible HFE-7500 passed through the flow of a phosphate-buffered saline (PBS) and magnetic bead solution, forming droplets. Both fluids were pushed through the device with air pressure. The droplets then flowed out of the device through tubing connected to a glass capillary. An electromagnet was placed directly underneath whose voltage was manipulated to extract magnetic bead aggregates. A camera captured images of the droplets in the capillary and were analyzed using ImageJ software, determining the droplets' initial size and deformation after the applied magnetic force. Concentrations of 1:3 and 1:5 (magnetic beads to PBS) of the solution were used.

Droplets of 1:3 magnetic bead concentration followed known relationships, where the magnetic force of the aggregate is proportional to the electromagnet voltage and the cube of the droplet size over the square of the distance between the droplet and the bottom of the capillary. However, the extraction of droplets of 1:5 magnetic bead concentration was inconsistent with these relationships and inefficient in comparison to droplets of 1:3 magnetic bead concentration for future mRNA extraction.

Introduction

Research in droplet microfluidics has wide applications in biology and the medical field. The study and manipulation of fluids at a microscopic scale can be used to mimic the environment of cells to analyze them more closely in a controlled setting. Droplets are advantageous as they are closed, encapsulating genetic material which prevents contamination, and can be produced at high levels to expedite analysis[1].

Fluids can be used to extract mRNA by implementing microscopic magnetic beads. Adding cell lysate to phosphate-buffered saline (PBS) and creating a system to facilitate extraction of the mRNA is possible with Oligo(dT)₂₅ beads whose coating targets and binds to the poly(A) tail of mRNA molecules[2]. Applying an external magnet to the system attracts the magnetic bead aggregate, efficiently collecting the mRNA to be analyzed later, such as in a digital polymerase chain reaction (dPCR).

As a proof of principle, Oligo(dT)₂₅ beads were mixed with PBS before forming droplets. The droplets are created in a microfluidic device, used throughout the field and engineered for precise control of the movement of fluids to maximize efficiency in a study. The device is planned and created by the researcher, and facilitates the flow, combination, and interaction of solutions and fluids through microscopic tubing connected to the device. Manufactured plastic NanoPort pieces are a necessity in the microfluidics field to connect elements such as the tubing, device, syringes, and glass capillaries for observing the fluids (Fig. 1).

With these components of the set-up, solutions can be controlled and measured for purposes of extracting mRNA. Methods and materials of greatest efficiency are still being discovered to expand the breadth of applications of the field.

Materials and Methods

Droplets of the PBS and Oligo(dT)₂₅ beads solution are created by passing the fluid through immiscible oil. Both fluids are pushed through tubing using a custom-developed air pressure controller program. The program connects to the air pressure controller and the computer where the pressure is adjusted. The tubings are connected to their respective inlets in the microfluidic device made of polydimethylsiloxane (PDMS) where the droplets are created (Fig. 1, 2)[3].



Figure 1A: The main components of the set-up include the microfluidic device which is viewed through the microscope on the computer. A pressure controller connects the tubing to the air pressure. The capillary is positioned above the electromagnet, viewed through a second, horizontal camera. The current of the electromagnet is controlled by the current supplier located next to the computer.

Figure 1B: Respective tubings for HFE-7500, magnetic bead solution, and produced droplets are connected to the microfluidic device. The other end of the outlet tubing is connected to the glass capillary.

Figure 1C: The side view of the glass capillary shows tubings connected to both ends: the right side connected to the microfluidic device and left side leading to a waste container.

Figure 1D: The top view of the glass capillary shows both tubings bound to the capillary with PDMS. The glass capillary is horizontally positioned directly above the electromagnet using custom 3D-printed pieces.

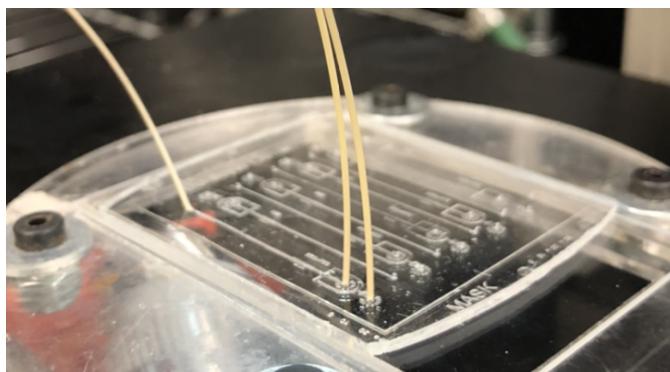


Figure 2: The tubings are connected to their respective inlets. The HFE-7500 tubing is connected in the device to the right of the magnetic bead solution tubing. So HFE-7500 travels around the magnetic bead solution inlet then perpendicularly at opposite sides of the solution's path to form droplets. The droplets and HFE-7500 then travel through the capillary channel through the outlet tubing on the far left.

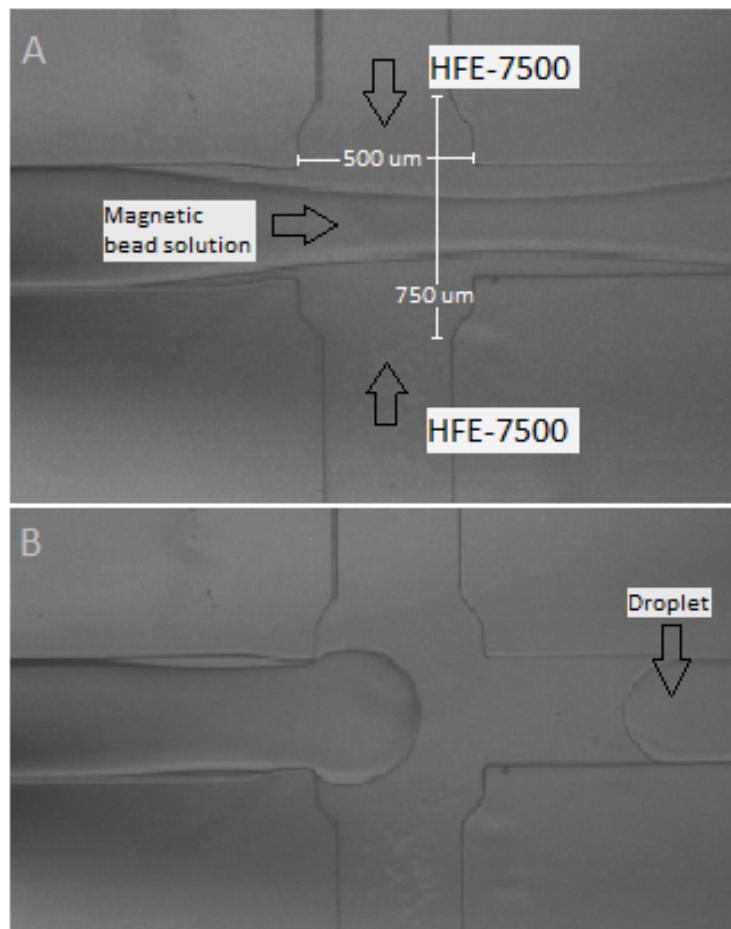


Figure 3A: The largest nozzle on the microfluidic chip is 500 by 750 μm . The magnetic bead solution is pushed through the horizontal path while HFE-7500 is pushed perpendicular to the magnetic bead solution path on opposite sides.

Figure 3B: HFE-7500 pushes through the magnetic bead solution and forms a droplet.

A solution of HFE-7500 oil with 2% EA surfactant was pushed through the tubing into the microfluidic device simultaneously with the magnetic bead solution towards a nozzle, where the HFE-7500 solution approaches the magnetic bead solution from two sides (Fig. 3).

Then droplets were formed and traveled through a collection channel in the device. The droplets then travel through the outlet tubing in the HFE-7500 solution into a glass capillary. This was connected to the tubing with PDMS. The droplets in the capillary were viewed by a second camera. The droplets and oil then travel out of the capillary through tubing connected to a waste container (Fig. 1).

Before pushing water droplets through the glass capillary, however, a trichlorosilane (TCS) treatment was prepared. Because of the hydrophilic property of glass, PBS droplets would be deformed in shape while passing through the capillary. Pushing a solution of TCS and HFE-7500 through the glass would allow stability of the droplets as TCS wets both hydrophilic and hydrophobic surfaces. The surface treatment comprised 950 microliters of HFE-7500 and 50 microliters of TCS: 5% by volume solution. TCS is a volatile substance and reacts to moisture in air, forming a white precipitate. So to extract the TCS from its container, argon gas was released into the container to displace air as argon is inert and heavier than air, and does not contain moisture. With a micropipette, 50 microliters of TCS were then obtained with minimal exposure to air. Then the treatment was applied to the capillary using tubing, NanoPort pieces, and a glass syringe by pushing TCS solution through the capillary.

The droplets were viewed through a microscope as they were formed in the device. A box light was placed on a 3D-printed attached above the microscope to view the device. The camera was then viewed through a program named Multivision, which allows for the view of several cameras connected to one computer.

The capillary was positioned horizontally on a 3D-printed set-up, upholding the capillary level close to that of the device to minimize the length of the outlet tubing from the device to the capillary. The capillary was viewed through a Sony XCVD-70 camera on a custom-developed Labview program called Multivision. A fiber optic illuminator was placed next to the capillary

opposite to the camera. A sliding mechanism was created for the camera, allowing for focus on the capillary.

Once the set-up was finalized, magnetic beads were added to the aqueous solution in a 1 to 3 ratio (Oligo(dT)₂₅ to PBS). An electromagnet was placed directly underneath the capillary to apply a controlled magnetic force to the magnetic beads within the droplets. A power supply was used to set the voltage of the electromagnet as droplets were viewed through the capillary.

The images were collected with the Sony camera in sequence using the Multivision software. Individual images were then analyzed using ImageJ, a program for image analysis, to extract parameters relevant to droplets' size and deformation. The pixel sizes of aforementioned values were used to calculate the size of droplets.

Results and Discussion

After collecting images of the droplets in the capillary, the number of pixels of the width of the capillary was measured with ImageJ to be 191. The inner width of the capillary, excluding the glass walls, was known to be 1000 um; therefore each pixel represented 5.2356 um, applicable to all images taken of the droplets since the positioning of the camera relative to the capillary was constant.

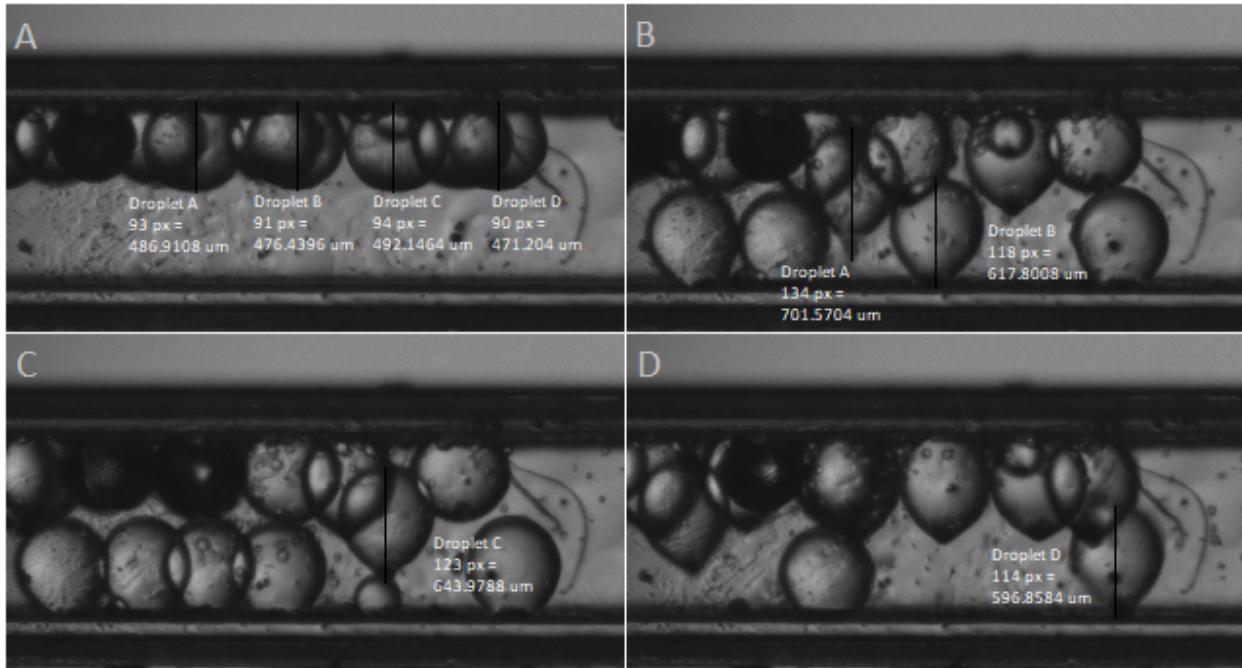


Figure 4A: Four droplets (arbitrarily labeled A, B, C, and D) are suspended at the top of the glass capillary when the electromagnet voltage equals 0V. Their measured diameters are written below each respective droplet.

Figure 4B: The magnetic bead aggregate of Droplet A is extracted as Droplet B is attracted to the bottom of the capillary. The length of Droplet A's vertical stretch is measured, and written, just before its magnetic bead aggregate is extracted. Droplet B's measured deformed length is written below the droplet.

Figure 4C: The magnetic bead aggregate of Droplet C is extracted, and the droplet deformation is measured directly before the extraction.

Figure 4D: Droplet D is attracted to the bottom of the capillary. Its deformed length is measured and written next to the droplet.

The number of pixels of the diameter of each droplet was measured using a line tool on ImageJ, then multiplied by 5.2356 um using the identity 1 px = 5.2356 um (Fig. 4A). All diameters of the droplets were calculated this way. Then the deformation of the droplets was measured. For droplets with the magnetic bead aggregate extracted, the droplet was measured the

instant before the aggregate was extracted (Fig. 4B, 4C). If the droplet was attracted to the bottom of the capillary towards the electromagnet, then the deformation of the droplet was measured once the droplet reached the bottom of the capillary (Fig. 4B, 4D).

These measurements were collected along with the electromagnet voltage of each respective droplet, as well as the behavior of the droplet and that of its magnetic bead aggregate (Fig. 5, 6, 7, 8).

Figure 5:

Electromagnet Voltage As a Function of Initial Droplet Diameter

1:3 Magnetic Bead Concentration

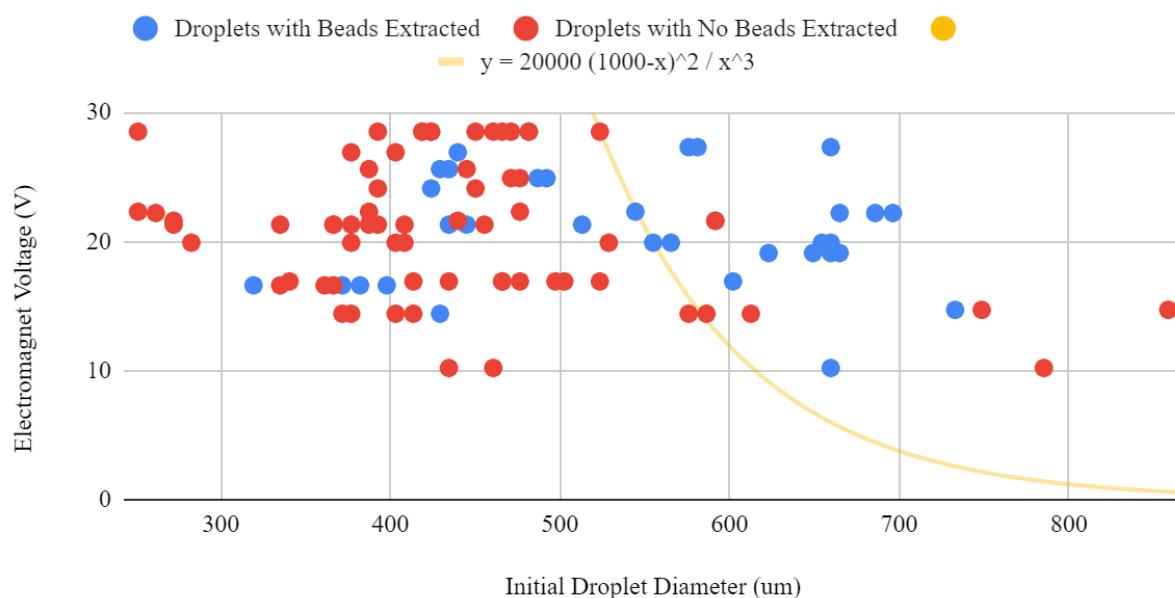


Figure 6:

Electromagnet Voltage As a Function of Initial Droplet Diameter

1:5 Magnetic Bead Concentration

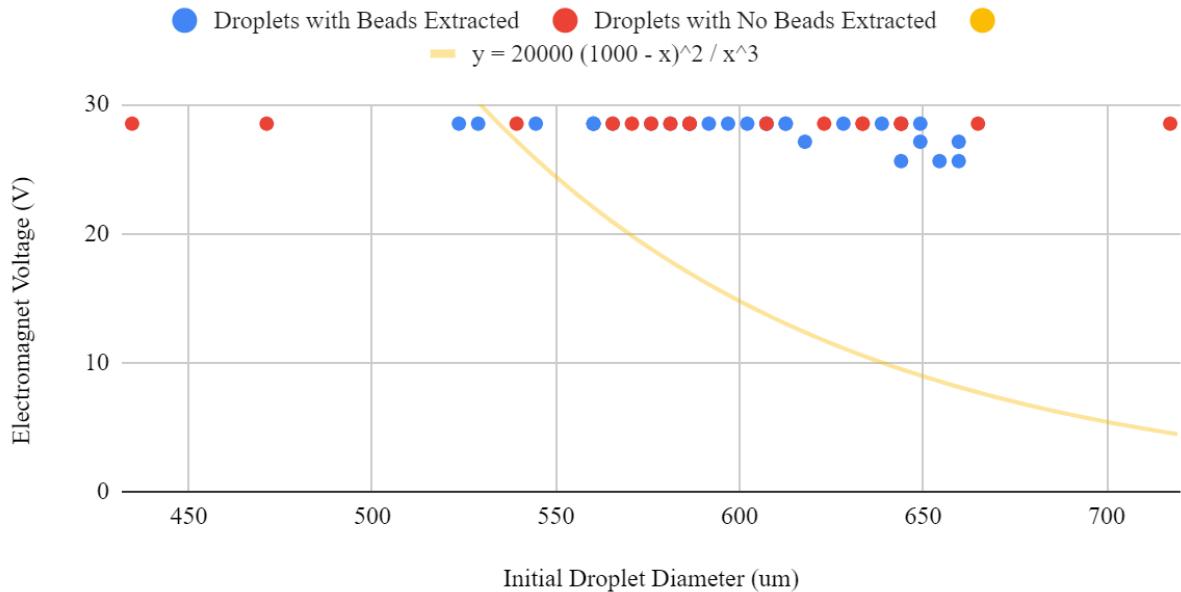


Figure 7:

Initial Droplet Diameter and Deformed Droplet Length

1:3 Magnetic Bead Solution Concentration

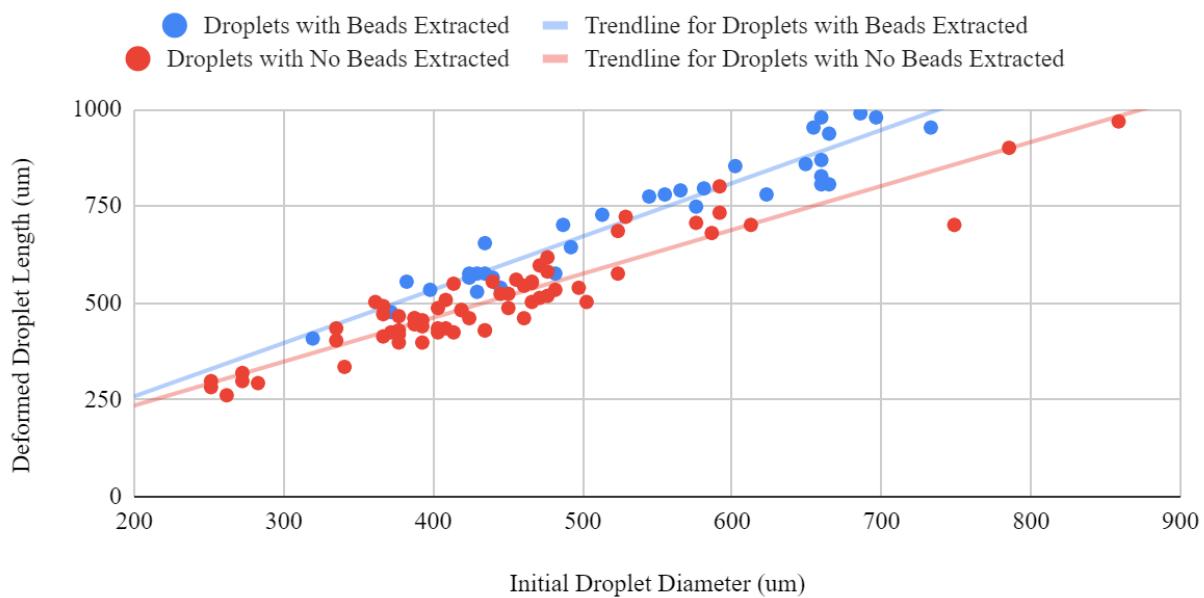


Figure 8:

Initial Droplet Diameter and Deformed Droplet Length

1:5 Magnetic Bead Solution Concentration

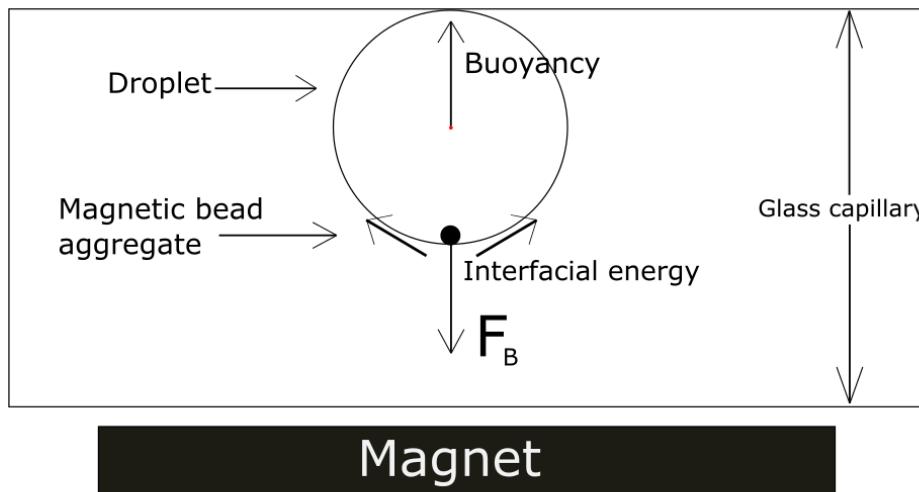
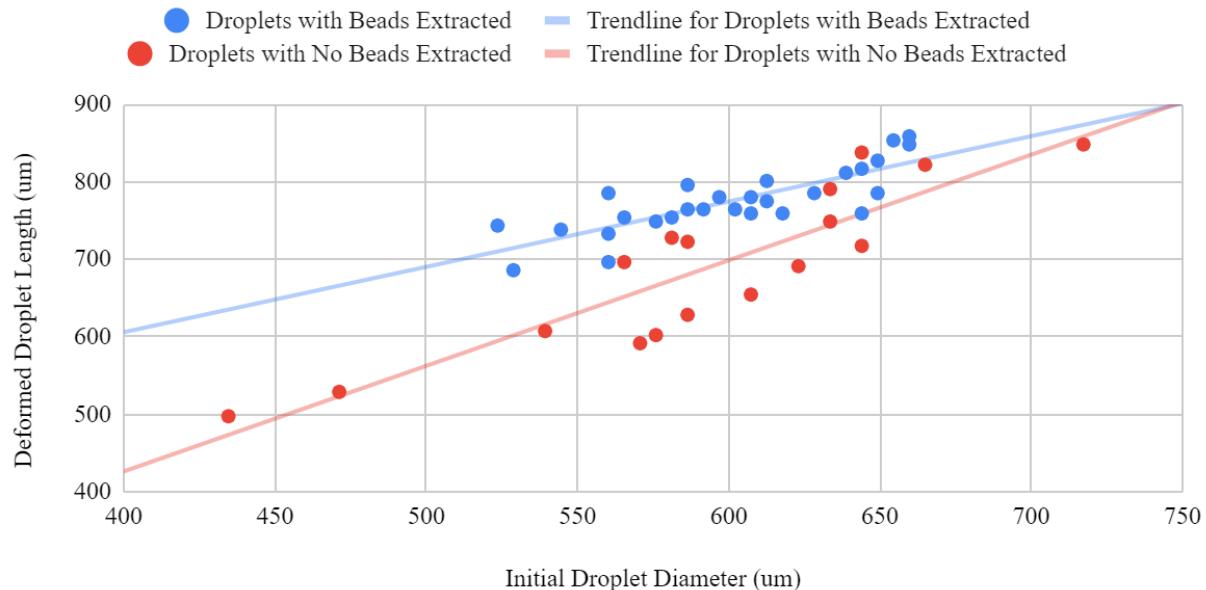


Figure 9: A droplet with greater buoyancy than the magnetic force of the aggregate will remain at the top of the capillary. There is also interfacial energy between the droplet (of a saline solution) and the HFE-7500 oil solution.

Theoretical Background

Smaller droplets have greater buoyancy than magnetic force since they contain fewer magnetic beads, so they remain at the top of the capillary (Fig. 9). Larger droplets, containing more magnetic beads, have a greater magnetic force than buoyancy and will be attracted to the bottom of the capillary towards the electromagnet. Additionally, the magnetic bead aggregate in

larger droplets is closer to the electromagnet than that of smaller droplets since the larger droplets have a greater diameter. So larger droplets are attracted to the bottom of the capillary due to its closer proximity to the electromagnet. Therefore, the behavior of a droplet and its magnetic bead extraction is largely dependent on its size as the magnetic force of the aggregate and the distance to the electromagnet is determined by that.

This is supported by the known relationships, where ϕ is the initial diameter of the droplet, V is the electromagnet voltage, L is the width of the capillary, and F is the magnetic force:

$$\text{Number of beads} \propto \phi^3$$

$$F \propto 1 / (L - \phi)^2$$

$$F \propto \text{number of beads} \times 1 / (L - \phi)^2 \times V$$

⋮

$$F \propto \phi^3 / (L - \phi)^2 \times V$$

A larger droplet (larger ϕ value) with the same electromagnet voltage as a smaller droplet (smaller ϕ value) will have a greater magnetic force on its magnetic bead aggregate. Similarly, droplets of the same size with different electromagnet voltages will have contrasting magnetic forces on the magnetic bead aggregate. However, the aggregate is also facing breakage from the water-oil interface between the droplet and HFE-7500, a barrier of surface tension (Fig. 9)[4]. But the value of the interfacial energy is low relative to the other forces on the droplet due to the stability of the droplets from the 2% EA surfactant in the oil whose hydrophobic/hydrophilic-polarized molecules stabilize the water-oil surface. When the magnetic force is greater than the interfacial energy, the magnetic bead aggregate is extracted, and a larger proportion of large droplets to small droplets will have their aggregates extracted (Fig. 9).

Analysis of Graphs

A higher electromagnet voltage on the same sized droplet will cause the extraction of the magnetic bead aggregate. The electromagnet voltage as a function of the initial size of the droplet was calculated using the relationship of forces. Assuming that force is a constant C :

$$C = V \times \phi^3 / (L - \phi)^2$$

L is known to be 1000 um. This function can be manipulated to solve for V as a function of the size of the droplet, with constant C :

$$V = C \times (1000 - \phi)^2 / \phi^3$$

This function was plotted on the graphs for both droplets of 1:3 and 1:5 magnetic bead concentration (Fig. 5, 6). Φ is x on the graphs, and C was adjusted for the graphs to suit the data points of magnetic bead extraction. The electromagnet voltage as a function of the size of the droplets of both 1:3 and 1:5 magnetic bead concentration was:

$$y = 20000 (1000-x)^2 / x^3$$

where y is the electromagnet voltage, and x is the initial droplet diameter (Fig. 5, 6).

This function generally followed the trend of the droplets of 1:3 magnetic bead concentration whose magnetic bead aggregate was extracted. These data points in blue were negatively correlated in an exponential form, like the function plotted on the graph. The red points, representing the droplets whose magnetic bead aggregate was not extracted, were more scattered and did not follow the trend of the function as much as the droplets whose magnetic bead aggregate was extracted (Fig. 5)

However, the electromagnet voltage as a function of the initial size of the droplets was inconsistent for the droplets of 1:5 magnetic bead solution concentration (Fig. 6). In fact, most droplets whose aggregates were extracted underwent a smaller electromagnet voltage than same-sized droplets whose aggregates were not extracted, rather than a larger electromagnet voltage in accordance with the droplets of 1:3 magnetic bead solution concentration and the aforementioned proportions between magnetic force, droplet size, and electromagnet voltage.

Additionally, droplets of 1:5 magnetic bead solution concentration whose magnetic bead aggregate was extracted were generally more deformed before extraction relative to droplets of the same concentration whose magnetic bead aggregate was not extracted than droplets of 1:3 concentration whose magnetic bead aggregate was extracted (Fig. 7, 8). The greater separation of the trendlines in Figure 8 than those of Figure 7 imply the drawn out extraction of aggregates of droplets of 1:5 concentration, as greater deformation is generally caused by a longer time of deformation. This inefficiency of extraction is unfavorable. Quicker deformation before aggregate extraction, like that of the 1:3 concentration droplets, is preferred to make the process of mRNA extraction more efficient (Fig. 7).

Overall, the magnetic bead aggregate extraction of droplets of 1:3 magnetic bead concentration were more consistent and efficient than that of droplets of 1:5 magnetic bead concentration. The droplets of 1:3 magnetic bead concentration generally follow the known relationships between forces, droplet size, and electromagnet voltage, so these parameters can be predicted for successful aggregate extraction when applied to collect mRNA to make the process even more efficient. However, this technology is still being explored to achieve efficient extraction of smaller droplets, as well as to expand the throughput of magnetic bead extraction. Rather than extracting from a handful of static droplets at a time, researchers in the field are aiming to extract aggregates from many droplets instantaneously, and even ones that are not static, to increase the efficiency of collecting mRNA from cell lysate, facilitating the process of genetic analysis[1].

Works Cited

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