Video Article

Phase Diagram Characterization Using Magnetic Beads as Liquid Carriers

Nicholas Blumenschein[1], Daewoo Han[1], Andrew J. Steckl[1]

[1]Department of Electrical Engineering and Computing Systems, University of Cincinnati

Correspondence to: Andrew J. Steckl at a.steckl@uc.edu

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Abstract

Magnetic beads with ~1.9 µm average diameter were used to transport microliter volumes of liquids between contiguous liquid segments with a tube for the purpose of investigating phase change of those liquid segments. The magnetic beads were externally controlled using a magnet, allowing for the beads to bridge the air valve between the adjacent liquid segments. A hydrophobic coating was applied to the inner surface of the tube to enhance the separation between two liquid segments. The applied magnetic field formed an aggregate cluster of magnetic beads, capturing a certain liquid amount within the cluster that is referred to as carry-over volume. A fluorescent dye was added to one liquid segment, followed by a series of liquid transfers, which then changed the fluorescence intensity in the neighboring liquid segment. Based on the numerical analysis of the measured fluorescence intensity change, the carry-over volume per mass of magnetic beads has been found to be ~2 to 3 µl/mg. This small amount of liquid allowed for the use of comparatively small liquid segments of a couple hundred microliters, enhancing the feasibility of the device for a lab-in-tube approach. This technique of applying small compositional variation in a liquid volume was applied to analyzing the binary phase diagram between water and the surfactant C12E5 (pentaethylene glycol monododecyl ether), leading to quicker analysis with smaller sample volumes than conventional methods.

Video Link

The video component of this article can be found at http://www.jove.com/video/52957/

Introduction

Magnetic beads (MBs) on the order of 1 micrometer in diameter have been used[1,2] quite often in microfluidic-based applications, particularly for biomedical devices. In these devices, MBs have offered capabilities such as cell and nucleic acid separation, contrast agents, and drug delivery, to name a few. The combination of external (magnetic field) control and droplet-based microfluidics has enabled[3] control of immunoassays using small volumes (<100 nl). MBs have also shown promise when used for liquid handling[4]. This approach uses the MBs to transport biomolecules between liquid segments within a tube separated by an air valve. This method is not as powerful as other more complex lab-on-chip devices seen in the past, but it is much simpler and does offer the capability of handling microliter-sized volumes of liquid. A similar approach has recently been reported[5] by Haselton's group and applied to biomedical assays.

One of the most important aspect of this device is the liquid segment separation offered by the surface-tension-controlled air valve. Microliter volumes of liquid attached to MBs are transported through this air gap between liquid segments using an externally applied magnetic field. Microparticle MBs (from ~0.4-7 µm in diameter with an average of 1.9 µm) under the effect of the external magnetic field create a micro-porous cluster that traps liquid within. The strength of this liquid entrapment is sufficient to withstand the forces of surface tension when transporting the MBs from one reservoir to the next. Typically, this effect is undesirable, as most approaches only want transport of specific molecules (such as biomarkers) contained within the liquids[6]. However, as can be seen in our work, this effect can be utilized to become a positive aspect of the device.

We have utilized this 'lab-in-tube' approach, shown schematically in **Figure 1**, for analyzing phase diagrams in binary materials systems. The surfactant C12E5 has been chosen as the main focus of characterization, as it is widely used in industrial applications such as pharmaceuticals, food products, cosmetics, etc. In particular, the H_2O/C12E5 binary system was investigated because it provides a rich set of phases to explore. We have focused on one specific aspect of this chemical mixture, namely the transitions to liquid crystalline phases under certain concentrations[7-9]. This transition is readily observed in our device by incorporating polarizers in the optical microscopy studies in order to highlight phase boundaries.

Being able to map phase diagrams is a very important area of study in order to understand the kinetics involved with phase transition[10]. The ability to precisely determine the interaction of surfactants with solvents and other components is crucial due to their complexity and many distinct phases[11]. Many other techniques have previously been used to characterize phase change. The conventional approach involves making many samples, each consisting of different concentrations and allowing them to equilibrate, which requires lengthy processing times and high quantity of sample volumes. Then, samples are typically analyzed by optical methods such as diffusive interfacial transport (DIT), which offers

high-resolution of such surfactant compositions[12,13]. Similar to the method we have utilized, the DIT method uses polarized light to image distinct phase boundaries.

Protocol

1. Preparation of One-Time Use Materials in Device

1. Preparation of tube
    1. Cut tubing into 15 cm segments. Tubing has 1.6 mm inner diameter and 3.2 mm outer diameter.
    2. Hang tube segments vertically using tape. Place paper towel underneath tubes to collect the excess fluoropolymer solution.
    3. Inject 100 µl of fluoropolymer solution into top opening of each tube segment using syringe, such that it will come into contact with entire circumference on the inner-wall.
    4. Allow tube segments to hang in place for 1 hr to remove excess amount of fluoropolymer solution.
    5. Clean out any fluoropolymer solution from bottom side of tube that didn't drip out. Remove tubing from hanging position and dispose of paper towels.
    6. Place tube segments into oven at 100 °C for 1 hr to anneal the fluoropolymer coating layer.
    7. Remove tube segments from oven. Use tweezers, as tube segments will be hot.

2. Preparation of diluted magnetic bead solution
    1. Calculate magnetic bead concentration needed to achieve the desired carry-over volume, as determined by the relationship between carry-over volume and MB mass shown in **Figure 2**.
    Note: The original MB solution has 1 g of MBs in 50 ml of solution. Considering a test chamber volume of 20 µl, dilute original MB solution with distilled water to a ratio of 6:4 (MB solution:water) to obtain a carry-over volume of ~0.4 µl. Adjust dilution ratio when different carry-over volume is desired.
    2. Place a 20 ml sample vial onto a micro-balance. Zero the balance.
    3. Agitate the magnetic bead solution container, then withdraw 0.6 ml using a micro-pipette.
    4. Dispense pipetted solution into the sample vial on balance.
    5. Dispense 0.4 ml of distilled water into the sample vial.

3. Fluorescent dye liquid preparation
    1. Dissolve 2 wt.% of dye into DI water by vortexing the solution for 1 min.

2. Preparation of Experimental Setup for Fluorescence Experiments

1. Preparation of tubing device.
    1. Insert a female Luer-lock connector onto one end of the tubing.
    2. Place the tube into a Luer-lock syringe that has a 3 ml volume and 0.1 ml graduation.
    3. Place the syringe into the syringe pump and set the feed rate at 2 ml/hr.
    4. For accurate insertion of liquids into the tubing, use the syringe pump to withdraw the solution containing the magnetic beads and fluorescent dye.
    5. Insert 20 µl of magnetic bead solution into tube using syringe pump withdrawal. This liquid segment is referred to as the test chamber (test chamber volume can vary depending on the experiment). Vortex the container with magnetic bead solution for 1 min and then agitate by hand during the withdrawal cycle to form uniform MB dispersions.
    6. After test chamber liquid insertion is concluded, withdraw 6 µl of air into the tube. This volume of air will later form a valve in between the two liquid segments.
    7. After air gap insertion is completed, begin withdrawal 180 µl of liquid with fluorescent dye. This liquid segment is referred to as the reservoir. Reservoir volume can vary depending on the experiment. Larger reservoir volume is beneficial to minimize the change of dye concentration.
    8. Place a second female Luer-lock connector onto the other end of the tube.
    9. Remove the tube device from the syringe.
    10. Place Luer-lock caps on both ends of the device.

2. Optics setup for fluorescence experiments
    1. Turn on all components connected to the inverted microscope.
    2. Turn on the computer and open the microscope imaging software.

3. Experimental Procedure for Fluorescence Experiments

1. Take initial fluorescence intensity measurement of test chamber and reservoir using the inverted microscope. When analyzing the fluorescence of the sample, ensure that the focus is in the center position (in both x and y directions) of the liquid segment within the tube. Record measurements in data spreadsheet.
2. Place device over top of cube magnet such that the magnetic beads all segregate to one area in the test chamber. Transfer the beads to the reservoir by moving the device over top of the magnet (~ 10 sec). The 1 inch neodymium cube magnet is grade N48 with a pull force of 45.6 kg.

3. Once magnetic bead cluster is transferred through the air gap and into the reservoir, agitate the magnetic beads by placing the device over top of the magnet and rotating to release the liquid being trapped within the cluster. Continue agitation of the magnetic beads until homogenization of the reservoir has been completed (~ 30-45 sec).
4. Place device over top of magnet such that the magnetic beads in the reservoir all segregate to one area. Transfer the magnetic bead cluster back to the test chamber.
5. Once the cluster reaches the test chamber, agitate the magnetic beads by placing the device over top of the magnet and rotating to release the trapped fluorescent liquid within. Continue agitation of the magnetic beads until homogenization of the test chamber has been completed (~ 30-45 sec).
6. Take fluorescence intensity measurements of both the test chamber and reservoir using the inverted microscope. Record measurements in data spreadsheet.
7. Steps 3.2-3.6 are repeated until both liquid segments converge to similar fluorescence intensities (~ 100 cycles).

4. Numerical Analysis of Fluorescent Data

1. With the fluorescent intensity data stored into a spreadsheet, perform numerical analysis using MATLAB.
2. Derive equations to calculate a theoretical value of fluorescence intensity in both the reservoir and test chamber. Incorporate the following equations into a MATLAB script file:
where I is the fluorescence intensity (A.U.), V is volume (µl), *n* is the number of transfers, R is the reservoir, T is the test chamber, and C is carry-over.
3. Using MATLAB, generate plots and analyze to determine the carry-over volume for all experiments. Use this data to produce **Figure 2**.

5. Preparation of Experimental Setup for Surfactant Experiments

1. Preparation of tubing device.
    1. Insert a female Luer-lock onto one end of the tubing.
    2. Place the tube into a Luer-lock syringe.
    3. Place the syringe into the syringe pump and set the feed rate at 2 ml/hr.
    4. For accurate insertion of liquids into the tubing, use the syringe pump to withdraw the solution containing the magnetic beads and surfactant.
    5. Insert 20 µl magnetic bead solution into tube using syringe pump withdrawal. This liquid segment is referred to as the test chamber (test chamber volume can vary depending on the experiment). Agitate the container with magnetic bead solution by hand during the withdrawal cycle to form uniform MB dispersions.
    6. After test chamber liquid insertion is concluded, withdraw 6 µl of air into the tube. This volume of air will later be referred to as the air gap.
    7. After air gap insertion is completed, begin withdrawal 180 µl of pure C12E5 surfactant. This will later be referred to as the reservoir.
2. Optics setup for surfactant experiments.
    1. Move syringe pump with tubing device such that the test chamber with magnetic beads is in focus with the stereo microscope.
    2. Place a sheet of polarizer film on top of an LED light source. Slide the LED light source underneath the tube attached to the syringe pump.
    3. Attach another polarizer film to the lens of the stereo microscope using tape. Be sure that the two polarizer films have a 90 degree offset from each other.
    4. Mount a CCD (charge coupled device) camera to the stereo microscope. Connect the camera to the computer and open up the imaging software.

6. Experimental Procedure for Surfactant Experiments

1. Place the cube magnet next to the test chamber while the magnet is mounted onto a stand.
2. Once the magnetic beads form a cluster, begin pumping liquids in the tube at the feed rate of 2 ml/hr such that the magnetic bead cluster is moved from the test chamber, across the air gap, and into the surfactant reservoir chamber.
3. Once the magnetic bead cluster reaches the midpoint of the reservoir chamber, stop the pumping on the syringe pump.
4. Move the cube magnet away from the tube, allowing for magnetic beads to separate and reduce the diffusion time of the liquid trapped in the magnetic bead cluster.
5. Watch the computer screen to observe the H_2O/C12E5 mixture cycle through different phases.
6. Once the diffusion and phase change of the liquid is completed, place the magnet back to its former destination by the reservoir so the magnetic beads form into a cluster.
7. Using the syringe pump, withdraw the liquids such that the magnetic bead cluster is transferred from the surfactant reservoir, across the air gap, and back into the H_2O test chamber.
8. Once the magnetic bead cluster reaches the midpoint of the test chamber, stop the pumping on the syringe pump.
9. Move the cube magnet away from the tube. This will allow magnetic beads to separate and will help reduce the diffusion time of the liquid trapped in the magnetic bead cluster.
10. Watch the computer screen to observe the H_2O/C12E5 mixture cycle through different phases.
11. Once the diffusion and phase change of the liquid is completed, place the magnet back to its former destination by the test chamber so the magnetic beads form into a cluster.
12. Repeat steps 6.2-6.11 until the test chamber displays a phase change.

Representative Results

Using the Lab-in-Tube approach for transporting µl-volume amounts of liquid with magnetic beads along with MATLAB for numerical analysis, average liquid carry-over volumes, as a function of magnetic bead mass, were found **(Figure 2**). Higher mass of magnetic beads provides higher carry-over volume in the rate of 2-3 µl/mg. The experimental setup (**Figure 1**) was used to observe phase change within the H_2O/C12E5 binary system. Since the H_2O/C12E5 system is well-known and has many distinct phases, which can be seen in **Figure 3B**, it served as an appropriate point of reference to further characterize our device. The dashed line in **Figure 3B** shows the nominal temperature that experiments were performed at of ~ 20 °C. Reactions were carefully observed from short times, such as 0 to 90 sec seen in **Figure 3C**, to longer times, such as 1.5 to 25 min seen in **Figure 4**. The L_1 to L_α phase change was used to verify carry-over volume in the H_2O/C12E5 binary system. Short term observation shows phase transitions into various liquid crystalline phases when the carried water is transferred into the C12E5 surfactant chamber. However, this phase change can be temporal as diffusion continues to reach a homogenous state in the liquid chamber. Eventually, the multiple transfers will lead to a permanent phase change as shown in **Figure 5B**. Even though a hydrophobic coating was applied to the inner-wall of the tube, one concern of our device was variation in carry-over volume due to liquid sticking to the inner-wall of the tube as larger volumes were pumped back-and-forth. One way to disprove this concern was to remove the magnetic beads from the device and carry out the same experiments exactly as if the magnetic beads were still in place. This would eliminate the carry-over volume, allowing us to observe any effects on chemical composition originating from this undesired liquid transfer. A comparison of phase change seen when the magnetic beads are in place (**Figure 5 A, B**) versus when they're removed from the system (**Figure 5 C, D**) was made. Fortunately, this concern was found to be insignificant when compared to the carry-over volume.

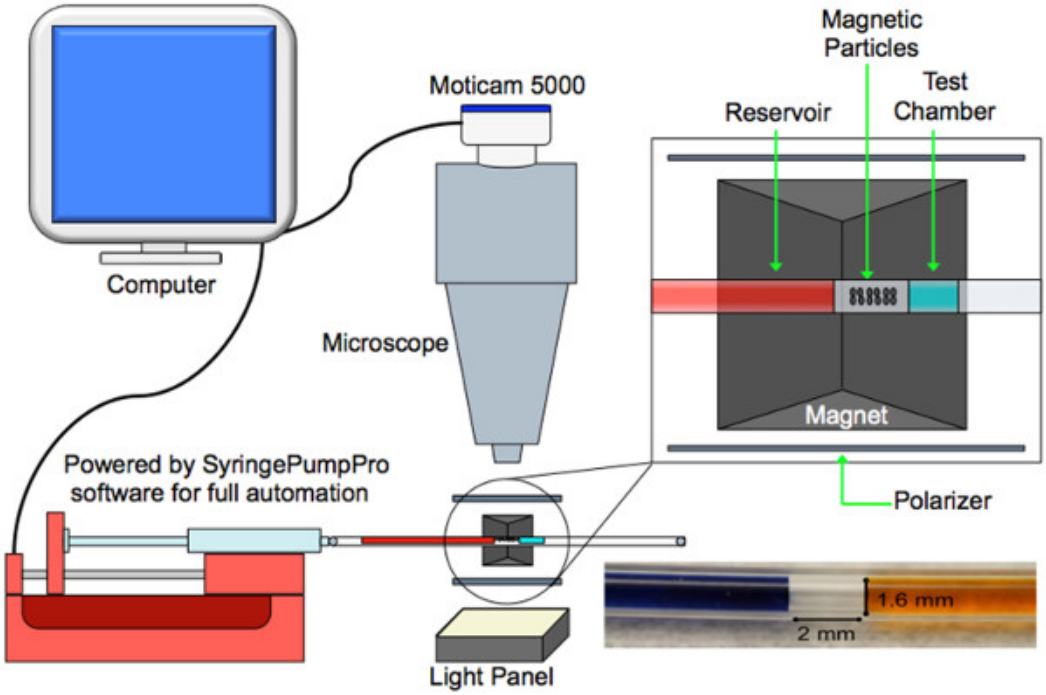

Figure 1. Schematic diagram of experimental setup and photo of tube used showing two liquid segments separated by an air valve. Reprinted (adapted) with permission from Blumenschein, N., Han, D., Caggioni, M., Steckl, A. Magnetic Particles as Liquid Carriers in the Microfluidic Lab-in-Tube Approach To Detect Phase Change. ACS Applied Materials & Interfaces. 6 (11), 8066-8072, doi: 10.1021/am502845p (2014). Copyright 2014 American Chemical Society. Please click here to view a larger version of this figure.

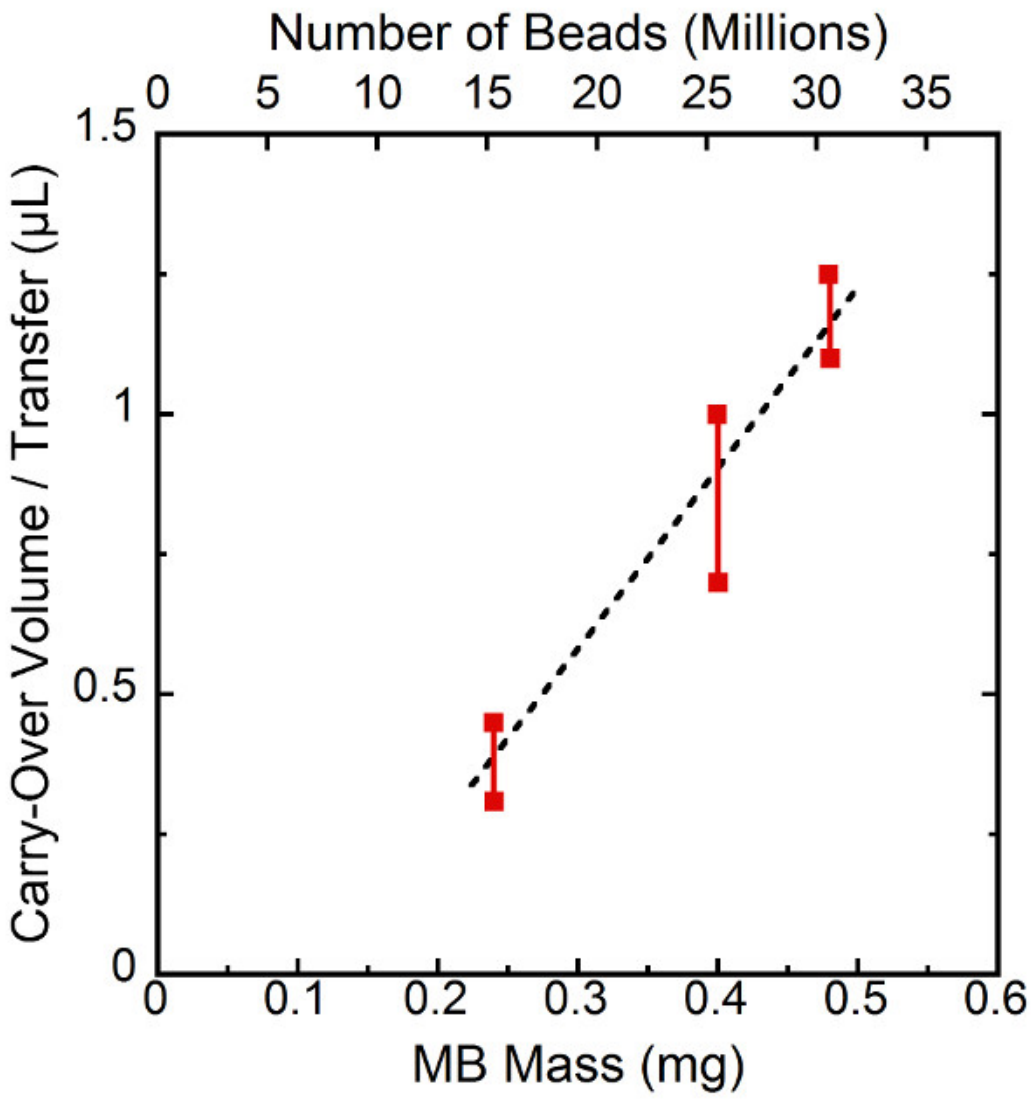

Figure 2. Average liquid carry-over volume per transfer vs. magnetic bead mass. Numerical analysis of plot using MATLAB. Reprinted (adapted) with permission from Blumenschein, N., Han, D., Caggioni, M., Steckl, A. Magnetic Particles as Liquid Carriers in the Microfluidic Lab-in-Tube Approach To Detect Phase Change. ACS Applied Materials & Interfaces. 6 (11), 8066-8072, doi: 10.1021/am502845p (2014). Copyright 2014 American Chemical Society. Please click here to view a larger version of this figure.

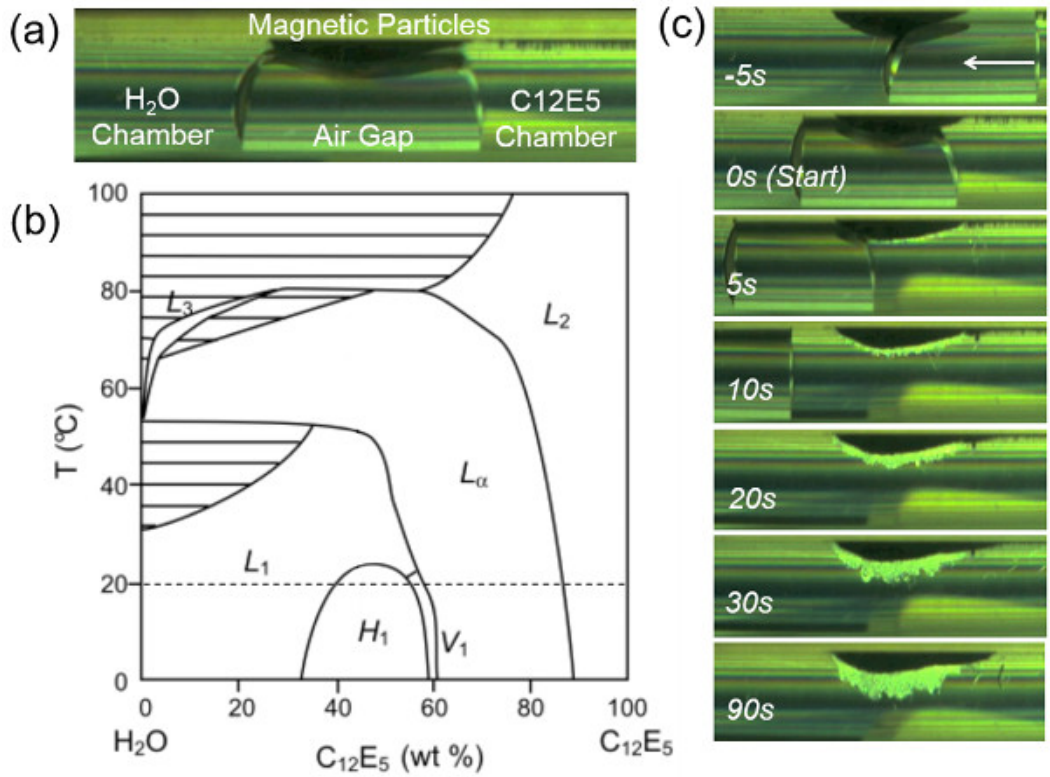

Figure 3. (**A**) Lab-in-tube experimental setup. (**B**) Phase change plot of the H_2O/C12E5 binary system. (**C**) Observed phase change of H_2O/C12E5 from 0 to 90 sec. Reprinted (adapted) with permission from Blumenschein, N., Han, D., Caggioni, M., Steckl, A. Magnetic Particles as Liquid Carriers in the Microfluidic Lab-in-Tube Approach To Detect Phase Change. ACS Applied Materials & Interfaces. 6 (11), 8066-8072, doi: 10.1021/am502845p (2014). Copyright 2014 American Chemical Society. Please click here to view a larger version of this figure.

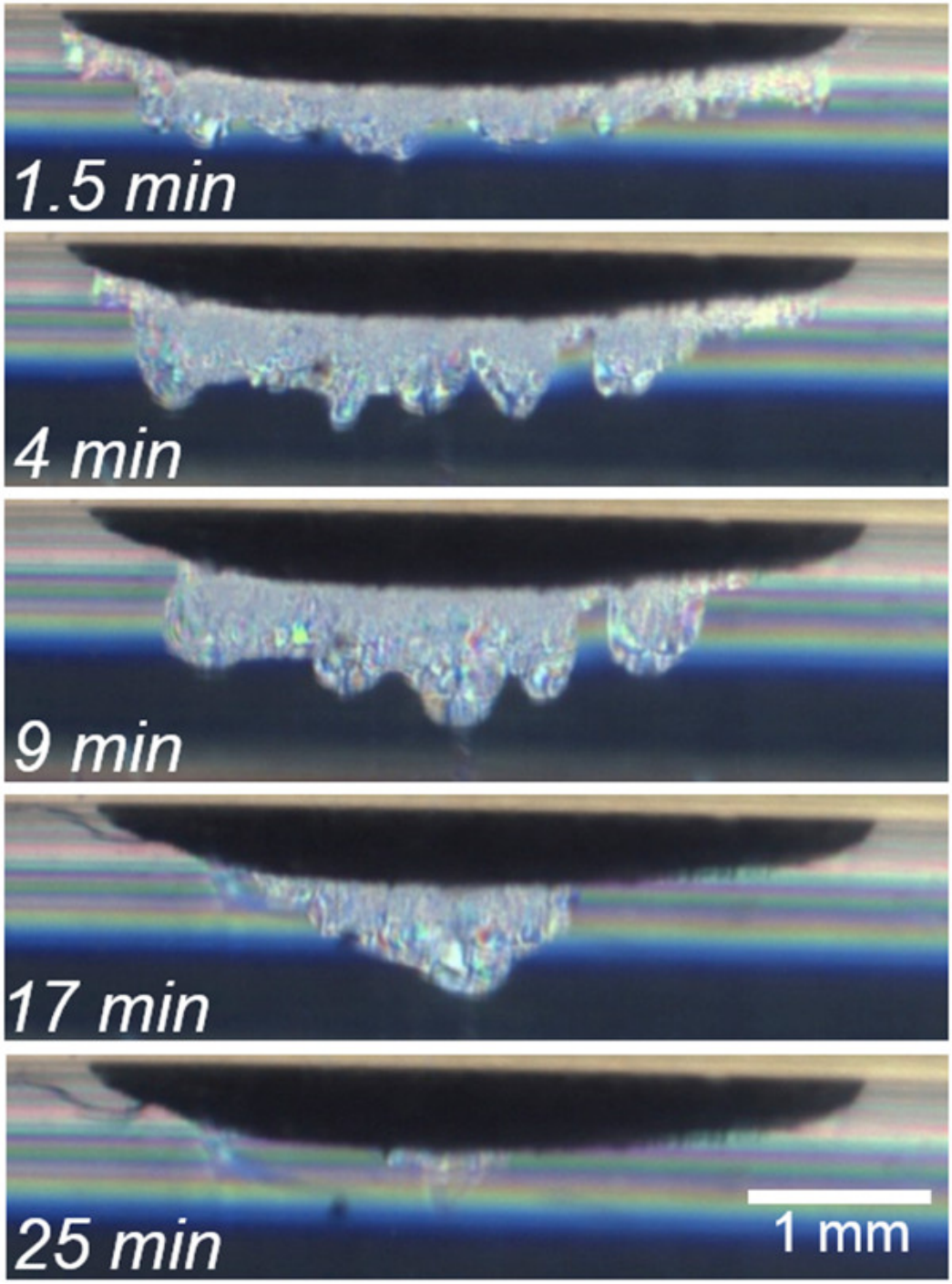

Figure 4. H_2O/C12E5 phase change over period of 1.5 to 25 min. Reprinted (adapted) with permission from Blumenschein, N., Han, D., Caggioni, M., Steckl, A. Magnetic Particles as Liquid Carriers in the Microfluidic Lab-in-Tube Approach To Detect Phase Change. ACS Applied Materials & Interfaces. 6 (11), 8066-8072, doi: 10.1021/am502845p (2014). Copyright 2014 American Chemical Society. Please click here to view a larger version of this figure.

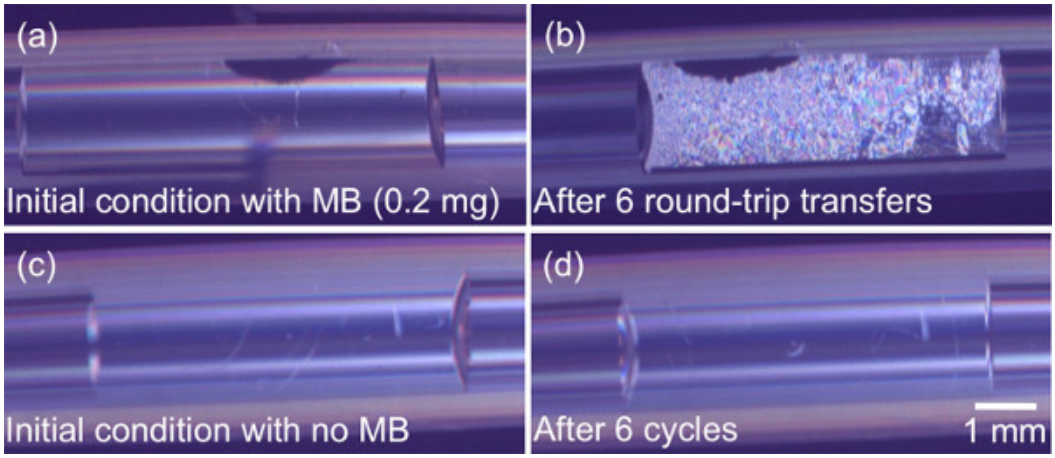

Figure 5. Two devices prepared with a test chamber initial concentration of 1:1 H_2O/C12E5 and reservoir containing pure C12E5. Using ~0.2 mg beads from initial condition (A) to 6 transfers (B), the sample transitions from L1 to Lα phase. In the absence of MBs, no phase change is seen (**C, D**). Experiment was performed at 25 °C. Reprinted (adapted) with permission from Blumenschein, N., Han, D., Caggioni, M., Steckl, A. Magnetic Particles as Liquid Carriers in the Microfluidic Lab-in-Tube Approach To Detect Phase Change. ACS Applied Materials & Interfaces. 6 (11), 8066-8072, doi: 10.1021/am502845p (2014). Copyright 2014 American Chemical Society. Please click here to view a larger version of this figure.

Discussion

In most common techniques for phase diagram investigation, multiple samples with different compositions and ratios need to be prepared and have to reach thermodynamic equilibrium which causes a lengthy process and a significant amount of material. Some challenges can be resolved by DIT (diffusive interfacial transport) method using flat capillary and the infrared analysis method, but none of them can resolve all challenges with low cost investment.

The feasibility of using magnetic beads as liquid carriers in this microfluidic “lab-in-tube” approach was demonstrated for the use of detecting phase change between adjacent liquid segments. This method allows for precise composition change, which can be predetermined using the shown numerical analysis technique. The ability to view live changes in a water-surfactant system while making miniscule alterations to the chemical make-up proved to be a valuable asset in this device. Current techniques used in industry for analyzing phase change have some undesirable aspects associated. Cost is always a concern, and having the ability to use such small volumes of expensive chemicals like C12E5 during experimentation is certainly an advantage. Likewise, when reducing sample size, the wait time for the diffusion process to take place is reduced significantly. The H_2O/C12E5 system is fairly complex and can take a long time to settle into a specific phase when its composition is altered. These lengthy diffusion times may appear to be undesirable, but when comparing it to diffusion times of methods practiced in industry, it is quickly seen as a progressive step in analyzing composition of intricate systems.

When analyzing phase change of a binary system, or any number of mixed chemicals, it is crucial to have adequate precision in the method being used. Much time was spent finding a relationship between the carry-over volume and magnetic bead mass. A few different variables, such as magnetic bead cluster porosity, test chamber volume versus reservoir volume, and magnetic bead cluster mass, were studied, allowing us to amalgamate different sets of data and create a model. During this process, the big takeaway was the obtained linear relationship between carry-over volume and magnetic bead cluster mass. We found the carry-over volume to be ~2 to 3 µl/mg of beads. Of course, this relationship doesn’t correlate with the test chamber and reservoir volumes, allowing for more complex experimentation methods. Meaning, since the carry-over volume acts almost as a constant depending on magnetic bead mass, the liquid volumes in the system can be predetermined to create desired step changes in the composition of the two liquids. This can come in handy when the user wants to see composition fluctuations anywhere from 0.25% to 10%.

The protocol provides high feasibility for exploring phase diagram with small sample quantity and fine resolution on the composition. However, current protocol still requires several minutes for single transfer, leading to days for complete phase diagram investigation. This limitation can be overcome either by using thinner tube diameter or mechanical actuation induced by external magnetic field variation.

Disclosures

The authors have no competing financial interests.

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