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TITLE:

Methane Hydrate Crystallization on Sessile Water Droplets

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KEYWORDS:

Methane, hydrate, droplet, high-pressure, additives, inhibitors, stability boundaries

SUMMARY:

We describe a method to form gas hydrate on sessile water droplets to study the effects of various inhibitors, promoters, and substrates on the hydrate crystal morphology.

ABSTRACT:

This paper describes a method to form methane hydrate shells on water droplets. In addition, it provides blueprints for a pressure cell rated to 10 MPa working pressure, containing a stage for sessile droplets, a sapphire window for visualization, and temperature and pressure transducers. A pressure pump connected to a methane gas cylinder is used to pressurize the cell to 5 MPa. The cooling system is a 10 gallon (37.85 L) tank containing a 50% ethanol solution cooled via ethylene glycol through copper coils. This setup enables the observation of the temperature change associated with hydrate formation and dissociation during cooling and depressurization, respectively, as well as visualization and photography of the morphologic changes of the droplet. With this method, rapid hydrate shell formation was observed at ~-6 °C to -9 °C. During depressurization, a 0.2 °C to 0.5 °C temperature drop was observed at the pressure/temperature (P/T) stability curve due to exothermic hydrate dissociation, confirmed by visual observation of melting at the start of the temperature drop. The “memory effect” was observed after repressurizing to 5 MPa from 2 MPa. This experimental design allows the monitoring of pressure, temperature, and morphology of the droplet over time, making this a suitable method for testing various additives and substrates on hydrate morphology.

INTRODUCTION:

Gas hydrates are cages of hydrogen-bonded water molecules that trap guest gas molecules *via* van der Waals interactions. Methane hydrates form under high-pressure and low-temperature conditions, which occur in nature in the subsurface sediment along continental margins, under Arctic permafrost, and on other planetary bodies in the solar system[1]. Gas hydrates store several thousand gigatons of carbon, with important implications for climate and energy[2]. Gas hydrates can also be hazardous in the natural gas industry because conditions favorable for hydrates occur in gas pipelines, which can clog the pipes leading to fatal explosions and oil spills[3].

Due to the difficulty of studying gas hydrates *in situ*, laboratory experiments are often employed to characterize hydrate properties and the influence of inhibitors and substrates[4]. These laboratory experiments are performed by growing gas hydrate at elevated pressure in cells of various shapes and sizes. Efforts to prevent gas hydrate formation in gas pipelines have led to the discovery of several chemical and biological gas hydrate inhibitors, including antifreeze proteins (AFPs), surfactants, amino acids, and polyvinylpyrrolidone (PVP)[5,6]. To determine the effects of these compounds on gas hydrate properties, these experiments have employed diverse vessel designs, including autoclaves, crystallizers, stirred reactors, and rocking cells, which support volumes from 0.2 to 10^6 cubic centimeters[4].

The droplet method used here and in previous studies[7-12] involves forming a gas hydrate film on a sessile droplet of water inside a pressure cell. These vessels are made of stainless steel and sapphire to accommodate pressures up to 10–20 MPa. The cell is connected to a methane gas cylinder. Two of these studies used the droplet method to test AFPs as gas hydrate inhibitors compared to commercial kinetic hydrate inhibitors (KHIs), such as PVP[7,11]. Bruusgard et al.[7] focused on the morphologic influence of inhibitors and found that droplets containing Type I AFPs have a smoother, glassy surface than the dendritic droplet surface without inhibitors at high driving forces.

Udegbunam et al.[11] used a method developed to assess KHIs in a previous study[10], which allows for the analysis of morphology/growth mechanisms, the hydrate-liquid-vapor equilibrium temperature/pressure, and kinetics as a function of temperature. Jung et al. studied CH_4-CO_2 replacement by flooding the cell with CO_2 after forming a CH_4 hydrate shell[8]. Chen et al. observed Ostwald ripening as the hydrate shell forms[9]. Espinoza et al. studied CO_2 hydrate shells on various mineral substrates[12]. The droplet method is a relatively simple and cheap method to determine the morphologic effect of various compounds and substrates on gas hydrates and requires small amounts of additives due to the small volume. This paper describes a method for forming such hydrate shells on a droplet of water using a stainless-steel cell with a sapphire window for visualization, rated up to 10 MPa working pressure.

PROTOCOL:

1. Design, validate, and machine the pressure cell.

1.1. Design the cell to allow direct visualization of hydrate formation from a water droplet. Ensure that the cell has a main chamber with a see-through sapphire window and four ports for

fluid/gas inlet, outlet, light, and wires, respectively (**Figure 1)**. Create the final design in engineering design software (**Supplemental Figure S1**).

1.2. To check that the pressure cell is safe under working high pressure, conduct a finite element analysis using simulation software.

1.2.1. Input the full-size pressure cell model from the engineering design software into the simulation software.

1.2.2. Assign a Young's modulus of 400 GPa and a Poisson's ratio of 0.29 to the sapphire window.

1.2.3. For all stainless-steel parts, assign stainless steel 316 with a Young's modulus of 190 GPa and Poisson's ratio of 0.27.

1.2.4. In a step-by-step manner, apply 0 to 1, 2, 3, 4 5, 6, 7, 8, 9, and 10 MPa air pressure to the inside of the cell (**Supplemental Video S1** and **Supplemental Video S2**). Treat each loading step as a static problem by ignoring the time-dependent terms in the governing equations and consider only elastic deformation during pressurization.

1.2.5. Use the direct linear equation solver in simulation software to calculate the stress distribution and the deformation of the cell under various pressure conditions (**Supplemental Table S1** and **Supplemental Table S2**).

1.3. Once the pressure cell design is verified to be safe, have all parts machined based on the engineering design software blueprint.

2. Assemble the pressure cell (Figure 1).

2.1. Screw the four National Pipe Tapered (NPT) threads into the respective ports on the pressure cell with plumber's tape.

2.2. Assemble the illumination port using the blueprint design (**Supplemental Figure S1,** parts C, D, and E) and connect to the top left NPT screw.

2.3. Connect the pressure transducer to the top port NPT using the branch tee fitting and port connector fitting.

2.4. Connect the inlet needle valve in the left side NPT screw using a port connector fitting.

2.5. Install a pressure seal connector into the right-side port of the pressure cell. Insert three K-type thermocouple wires through the pressure seal connector with 3" of slack inside the cell and 3' slack outside the cell.

2.6. Polish the stage surface with sandpaper (**Supplemental Figure S1**, Part F).

2.7. Insert the thermocouples into the respective holes in the stage so that the tips are flush with the top of the stage. Use a small drop of glue in each hole to fix the thermocouples in place and allow them to dry.

2.8. Fit the acrylic disc on the back wall of the pressure cell to enhance light reflection. Fit the stage in the pressure cell.

2.9. Install the sapphire window.

2.9.1. Apply vacuum grease to two static sealing O-rings (one 1" and one 1-1/5"). Fit the O-rings into the grooves around the window hole on the pressure cell.

2.9.2. Insert the sapphire window. Cover the sapphire window with a 2-1/4" rubber washer and screw on the stainless-steel washer (**Supplemental Figure S1**, Part B) using eight M8 stainless steel screws (**Figure 2C)**.

3. Assemble the equipment in a large fume hood (Figure 2).

NOTE: As methane is a flammable gas under pressure, keep all methane-related tubing and vessels away from heat, sparks, open flame, and hot surfaces. Set all equipment up inside a well-ventilated area (e.g., a fume hood). Don safety glasses and lab coat before working with methane gas.

3.1. Carefully lift the pressure pump into a fume hood large enough for all the equipment to fit **(Figure 2A)**. Place the pump controller on top of the pump base. Connect the pump controller to the pump and plug it into a power strip.

3.2. Run a high-pressure-rated 1/4" copper pipe from the regulator on the methane gas cylinder to the fume hood next to the inlet of the pressure pump **(Figure 2A,B)**.

3.3. Place the data logger next to the pressure pump and set the laptop on the data logger **(Figure 2A)**. Plug both into a power strip. Connect the data logger to the laptop via the data logger USB.

3.4. On the laptop, install the proper software to control the data logger, camera, and pressure transducer on the pressure cell.

3.5. Set the aquarium beside the data logger and place non-leaching padding in the bottom of the aquarium to limit vibrations to the pressure cell **(Figure 2C)**.

3.6. Using a new 1/4" copper pipe, coil the copper pipe twice into an oval to fit in the aquarium, leaving room for the pressure cell to sit inside (**Figure 2D**). Ensure that the coil does

not block the sapphire window in the pressure cell. Elevate the pressure cell in the aquarium to view the sapphire window.

3.7. Place the circulating chiller on the floor near the fume hood **(Figure 2A)**. Fill the chiller with 50/50 v/v ethylene glycol/water.

NOTE: As ethylene glycol is hazardous, use appropriate safety attire, including gloves, lab coat, and goggles when pouring.

3.8. Cut two lengths of a 3/8" (inner diameter) plastic tubing to connect the chiller inlet and outlet to the copper pipe ends in the aquarium. Ensure there will be enough slack for the foam pipe insulation to fit before cutting.

3.9. Slide the plastic tubing through the foam pipe insulation.

3.10. Connect the insulated plastic tubing from the inlet and outlet on the circulating chiller to the ends of the copper coil inside the aquarium. Secure the seals by wrapping plumber's tape around the metal parts and tightening the connections with worm drive hose clamps. Turn the chiller on and set it to circulate at high speed. Ensure there are no leaks.

3.11. Apply underwater sealant around the copper coil/plastic tubing connections inside the aquarium. Allow the sealant to cure. Wrap the sealant with duct tape.

3.12. Install pressure pump tubing (**Figure 2E**).

NOTE: Always hand-tighten connections before using tools and never detach the NPT connections with plumber's tape because they will not re-seal well.

3.12.1. Install a 1/8" stainless steel pipe on either side of the pressure pump with the company fittings that came with the pump using plumber's tape **(Figure 2F)**.

3.12.2. With a tube bender, bend the 1/8" pipe forward at a 90° angle, approximately 2" away from the pump, to avoid bending at the connection.

3.12.3. With a tube bender, bend the 1/8" pipe downward at a 90° angle, approximately 2" away from the first bend.

3.12.4. Attach 1/8" to 1/4" adapter fitting to the 1/8" pipe on both sides **(Figure 2G)**.

3.12.5. Attach 1/4" pipe to adapter fitting on both sides.

NOTE: To affix the valve to the side of the pump, trim the 1/4" tubing so that the attached valve will sit next to the two screw holes.

3.12.6. Install the 1/4" needle valves (**Figure 2H**). If affixing valves to the pressure pump, machine a steel or plastic plate with two 1/16" holes for screws and one 1/2" hole to secure between needle valve connections. Insert the plate between the valve connections and screw the plate to the side of the pump.

NOTE: Ensure that arrows on the needle valves point from high pressure (inside the pressure pump) to low pressure (outside the pressure pump).

3.12.7. Connect one end of the 1/4" braided stainless steel flexible pressure-rated hose to the outlet valve on the pressure pump and the other end to the side valve of the pressure cell.

3.12.8. Connect thermocouples from the pressure cell to data logger channels using the data logger multichannel. Connect an additional thermocouple wire to measure the temperature of the tank solution and put the other end in the tank.

3.12.9. Connect the pressure transducer on the pressure cell to the laptop.

3.12.10. Set the pressure cell inside the aquarium, close to the front, for clearer imaging.

3.13. To insulate the aquarium, wrap the outside of the aquarium with foil-lined fiberglass, with a hole/slit for the camera to view the sapphire window of the pressure cell. Cover the top of the aquarium with insulating material to prevent evaporation during experiments.

NOTE: Avoid tightly sealing the aquarium top to avoid the buildup of heat from the light source.

3.14. To prevent the condensation of moist air on the front of the aquarium, run plastic tubing from the closest air valve to the front of the aquarium where the camera will be pointing so that the tubing will not be visible in the photographs.

3.15. Set the light source unit beside the aquarium and plug it into the power strip.

3.16. Set the camera in front of the aquarium, with the lens pointing towards the sapphire window. Plug the camera into the laptop and power strip.

3.17. Elevate all electronics from the hood surface to prevent potential leak damage. Double-check that power is distributed for the power capacity of the outlets.

4. Leak-test the pressure cell with water.

NOTE: To ensure all connections were sealed properly, leak-test the pressure cell with water any time the cell has been reassembled, especially after disconnecting the NPT screws. This is not necessary after removing the sapphire window or top valve. Water is safer under pressure than gas.

4.1. Open the pressure transducer software on the laptop and start collecting data at a scanning interval of 1 s.

4.2. Turn on the pressure pump and controller. Press **Pump A** on the pressure pump controller to monitor the pressure.

4.3. If there is pressure in the pump, decrease the pressure by pressing **Refill** on the pressure pump controller while both the pump inlet and outlet valves are still closed.

4.4. With both pressure cell valves open, open the pump outlet valve slightly by ~1/16" to slowly release the remaining pressure.

4.5. If connected, disconnect the 1/4" copper pipe from the inlet valve on the pressure pump.

4.6. Attach 1/4" flexible tubing to the pump inlet valve using a nut and ferrule set. Place the end of the tubing in a gallon of water.

4.7. Close the pump's outlet valve and open the pump's inlet valve.

4.8. Press **Refill** on the pressure pump controller to fill the pump piston with water.

4.9. Set the pressure cell in a shallow empty container outside of the aquarium.

4.10. Purge the air out of the pressure cell until water comes out of the top port and fills the pressure cell completely.

4.10.1. Close the pump's inlet valve and open the pump's outlet valve.

4.10.2. Ensure the valves on the pressure cell are still open.

4.10.3. Set the **maximum (max) flow** to **100 mL/min**: on the pressure pump controller, press **Limits**; press **3** for max flow; press **1** to set max flow; punch in **100**; press **Enter**.

4.10.4. Press **D** to reach the previous page.

4.10.5. Set the constant flow rate to 100 mL/min: on the pressure pump controller, press **Const Flow**; press **A** for flowrate; punch in **100**; press **Enter**. Press **Run**.

4.10.6. If water does not come out or if the volume in the piston is insufficient, refill the piston again by closing the pump outlet valve, opening the pump inlet valve with tubing in water, and press **Refill**. Then, purge the air out by closing the pump inlet valve, opening the pump outlet valve, setting the flow rate to **100**, and pressing **Run**.

4.10.7. Once water comes out of the top port, check for leaks and tighten any leaking connections. Press **Stop**. Close the pressure cell outlet (top) valve.

4.11. Pressurize the pressure cell.

NOTE: Don safety glasses before pressurizing the pressure cell.

4.11.1. Set the max flow limit to 10 mL/min to prevent fast pressurization of the cell: on the pressure pump controller, press **Limits**; press **3** for max flow; press **1** to set max flow; punch in **10**; press **Enter**.

4.11.2. Pressurize the cell to 100 kPa: on the pressure pump controller, press **Const Press**; press **A**; punch in **100**; press **Enter**. Press **Run**.

4.11.3. Check for leaks. If there is a leak, press **Stop** on the pump controller, tighten the leaking components, press **Run**, and repeat until there are no leaks at 100 kPa. Ensure there are no leaks by closing the pump outlet valve and monitoring the pressure cell's pressure in the pressure transducer software.

NOTE: If the pressure decreases consistently and is not normal fluctuation due to room temperature variation, there is a leak.

4.11.4. Increase the pressure in increments of 50 kPa from 100 kPa to 500 kPa, and then in increments of ~1,000 kPa from 500 kPa to ~10,000 kPa. Do this by changing the **Const Press** setting as before. Between pressure settings, close the pump outlet valve and monitor the cell's pressure like before to ensure that the pressure is constant. If the pressure drops, carefully tighten the leaking components.

4.12. Upon reaching 10,000 kPa, close the pump outlet valve and observe how well the pressure cell holds pressure according to the pressure transducer. As a consistent drop in pressure indicates a leak, tighten connections at a lower pressure, ~1,000 kPa.

4.13. To depressurize, open the pump outlet valve and set the pressure to 100 kPa. Once the pressure plateaus, slightly open the pressure cell outlet valve.

4.14. To remove water from the pressure pump, close the pump inlet valve, change the max flow and **Const Flow** settings to **100 mL/min**, and press **Run** until the pump is empty.

4.15. Disconnect the 1/4" flexible tubing from the pump inlet. Disconnect the braided stainless-steel hosing from the pressure cell. Open both valves and drain the water. Remove the sapphire window to allow the cell to completely dry.

5. Form a methane hydrate shell on the droplet surface.

5.1. Prepare the equipment.

5.1.1. Connect the methane cylinder regulator to the pump with the 1/4" copper pipe using a new nut and ferrule set. Ensure that the gas cylinder is closed.

5.1.2. Practice droplet insertion technique.

5.1.2.1 Glue a flexible tip, such as IV tubing, cut at an angle to the end of the cannula to help direct the droplet toward the sapphire window. Attach a 1 mL syringe to the cannula and pull in the desired volume of deionized water (~50–300 µL). Without the needle valve or sapphire window attached, insert the end of the cannula into the top port and practice expelling the droplet onto the center stage.

NOTE: In this protocol, 250 µL of deionized water was taken into the syringe.

5.1.3. Reattach the sapphire window and washers with M8 screws. Connect the braided stainless-steel hose from the pressure pump to the pressure cell, and double-check that all connections from the gas cylinder to the pressure cell are tight. Open the pressure cell inlet valve (side valve), and set the pressure cell in the aquarium. Insert a fiber optic light source cable into the pressure cell illumination port.

5.1.4. Add 50/50 ethanol/water (v/v) to the aquarium until it is level with the top of the pressure cell, just below the light source connection. Ensure that the hood flow is turned on. When the solution level falls before future trials in the following weeks, add more ethanol. Replace the solution monthly.

5.1.5. Set the chiller to the temperature that will achieve ~0 °C to -3 °C inside the cell (~-4 °C) and start circulating through coils. Turn on the airflow to the front of the aquarium to prevent condensation on the aquarium surface.

5.1.6. Start a temperature log in the data logger software. Set the scanning interval to 30 s. Wait until the temperature inside the pressure cell is stable at 2 °C (~6–24 h).

5.2. Add a water droplet into the pressure cell using the camera view on the laptop.

5.2.1. Turn on the light source to ~80%. Open the camera software. In live view, focus the camera lens at the cell's inner chamber. Adjust the light source for best imaging.

5.2.2. Start a new temperature log with a 1 s scanning interval.

5.2.3. If attached, detach the outlet needle valve in the top port of the pressure cell. Attach a 1 mL syringe to the cannula and pull in the desired volume of deionized water (~50–300 µL).

NOTE: In this protocol, 250 µL of deionized water was pulled into the syringe.

5.2.4. Insert the cannula through the top port until the tip is visible in the camera software in live view mode. Expel the fluid droplet from the syringe over the central thermocouple. Reattach the needle valve.

5.3. Focus the camera on the droplet in the pressure cell. Begin time-lapse imaging every ~60 s.

5.4. Open the pressure transducer software on the laptop and start collecting data on the chart and the data log at a scanning interval of 1 s (same as the temperature scanning interval). Wait until the droplet temperature is stable between 0–3 °C.

5.5. Pressurize the pressure cell to the desired pressure.

NOTE: Don safety glasses before pressurizing the cell.

5.5.1. Turn on the pump and the controller. Close the pressure pump's inlet valve.

5.5.2. Open the pump's outlet valve and the pressure cell's valves.

NOTE: The pressure cell inlet valve should always be open.

5.5.3. Tare the pump pressure by pressing **Zero** on the pressure pump controller. Select **Pump A** on the pressure pump controller to monitor the pressure.

5.5.4. Ensure that the pressure pump is empty if a different fluid other than methane gas was present in the pump. Do this by setting the max flow and **Const Flow** to **100 mL/min** and pressing **Run**. Leave it running until the pump is empty. Close the pump outlet valve and open the pump inlet valve.

5.5.5. Open the gas cylinder and set the gas cylinder regulator to 1,000 kPa.

5.5.6. Press **Refill** on the pressure pump controller. When the pump is full and near 1,000 kPa, close the pump inlet valve and the gas cylinder.

5.5.7. Slightly open (~1/16" turn) the pump outlet valve to the cell. Monitor the pressure cell pressure in the pressure transducer software as the pressure may decrease due to the relatively lower temperature in the pressure cell.

5.5.8. Set the max flow to **10 mL/min**: on the pressure pump controller, press **Limits**; press **3** for max flow; press **1** to set max flow; punch in **10**; press **Enter**.

5.5.9. Set the max pressure to **5,000 kPa**: on the pressure pump controller, press **Limits**; press **1**; punch in **5000**; press **Enter**.

5.5.10. Set the constant pressure to **1,000 kPa**: on the pressure pump controller, press **Const Press**; press **A**; punch in **1000**; press **Enter**. Press **Run**.

5.5.11. When 1,000 kPa is reached, press **Stop** on the pump controller and close the pump's outlet valve. Monitor the pressure in the pressure cell to ensure there are no leaks. If the pressure drops, use the liquid leak detector to find the leak at the connections and carefully tighten the leaking components.

5.5.12. If the cell is stable, open the pump outlet and set the **Const Press** to **2,000 kPa**. Press **Stop** and monitor. If stable at 2,000 kPa, set **Const Press** to **3,000 kPa**. Press **Stop** and monitor. If stable at 3,000 kPa, set **Const Press** to **4,000 kPa**.

5.5.13. Press **Stop** and monitor. If stable at 4,000 kPa, set **Const Press** to **5,000 kPa**. Press **Stop** and monitor. If the pressure is stable, close the pump outlet.

NOTE: If the pump volume runs out, close the pump outlet and slightly open the pump inlet. Slowly open the gas cylinder and set the gas regulator to 1,000 kPa. Press **Refill** on the pump controller. When the pump is refilled, close the gas cylinder and the pump inlet. Pressurize the pump to match the pressure cell pressure.

5.5.14. Wait for ~12–24 h for the gas to permeate the droplet.

5.6. Nucleate the hydrate shell using dry ice.

5.6.1. Switch the time-lapse to take images every 2–5 s. Add dry ice to the top of the cell until the hydrate shell is seen in time-lapse. If the dry ice slides, affix tape around the top of the cell.

5.7. Observe the progress of the methane hydrate formation through time-lapse photos for ~2–6 h.

5.8. Depressurize the cell to 2,000 kPa by opening the pump outlet and setting the **Const Press** to 2,000 kPa. Note when melting occurs.

NOTE: Bubbling in the sessile droplet may occur due to the escape of the dissolved gas.

5.9. After ~30 min, repressurize the pressure cell to 5,000 kPa to observe the memory effect. Note when a hydrate shell begins to reform. Allow the shell to form for ~30 min to 2 h.

5.10. Depressurize the cell by opening the pump outlet and setting the **Const Press** to 0 kPa. If there is residual pressure in the pressure cell, slightly open the pressure cell top valve by ~1/16".

5.11. Save the pressure and temperature data as .csv files.

5.12. Remove the droplet by removing the top pressure cell valve as before and extracting the droplet with the syringe/cannula/IV tube. If there is a concern for contamination between trials, remove the sapphire window and sanitize the stage and replace the vacuum grease. Use a suction cup to remove the sapphire window once the pressure cell has warmed to room temperature.

6. Analyze the data

6.1. Open the temperature and pressure .csv files.

6.2. Make a new spreadsheet. Copy the time and pressure columns from the pressure .csv and the time and temperature from the temperature .csv file into the new spreadsheet.

6.3. Make a scatter plot with time on the x-axis and two y-axes with temperature and pressure (**Supplemental Figure S2**).

6.4. Make two more columns for the hydrate stability curve. In the first column, input the temperatures from 273.15 K to ~279.15 K at 0.1 K intervals. In the second column, calculate the pressure by using formula (1) from Sloan & Koh[13].

P [kPa] = exp(a+b/T [K]) where a = 38.98 and b = -8533.80 (**1**)

6.5. Make a scatter plot of the hydrate stability boundary, with temperature (K) on the x-axis and pressure (kPa) on the y-axis. Add a second series on the scatter plot with experimental temperature and pressure on the x and y axes, respectively (**Figure 4**).

6.6. Note on the graphs where a hydrate shell became visible, according to the time-lapse imaging.

REPRESENTATIVE RESULTS:

With this method, a gas hydrate shell on a droplet can be monitored visually through a sapphire window of the pressure cell and via temperature and pressure transducers. To nucleate the hydrate shell after pressurizing to 5 MPa, dry ice can be added to the top of the pressure cell to induce a thermal shock to trigger rapid hydrate crystallization. There is a clear morphologic difference upon dry ice-forced hydrate shell formation. The water droplet transitioned from a smooth, reflective surface (**Figure 3A**) to an opaque hydrate shell with a slightly dendritic surface (**Figure 3B**). The addition of 100 µg mL^{-1} Type I AFP altered the hydrate morphology by inducing ridged edges along the droplet and protrusions from the top of the droplet (**Figure 3C,D**).

After the hydrate shell developed for ~1 h, the cell was depressurized to 2 MPa (**Supplemental Video S3**). During depressurization, there was a 0.2 °C to 0.5 °C drop in temperature near the P/T stability curve[13] (**Figure 4**) due to exothermic hydrate dissociation. Hydrate dissociation was confirmed by visual melting through time-lapse imaging at the beginning of the decrease in temperature, noted by stars in **Figure 4**. After complete hydrate dissociation, we repressurized the cell to observe the morphology and melting temperature with the "memory effect"[14], the

phenomenon in which hydrate forms faster after hydrate has already formed in the system (**Supplemental Video S4**). Upon re-pressurization, a hydrate shell reformed within a couple of minutes after reaching 5 MPa, and we observed the same temperature decrease at the stability curve during dissociation.

Negative controls with no droplet and with a droplet that did not form a hydrate shell (**Figure 4**, Trials 4 and 5) showed no decrease in temperature during depressurization. Upon depressurization below 2 MPa, we observed gas bubbling within the droplet from rapid degassing. Because the apex of each temperature decrease was above the previously established P/T stability curve[13] (hydrate stability curve #1 in **Figure 4)**, a regression curve was calculated based on the apex P/T of these trials (P [kPa] = EXP(38.98+-8533.8/T [K]), hydrate stability curve #2 in **Figure 4)**.

FIGURE AND TABLE LEGENDS:

Figure 1: Pressure cell. The stage on which the droplet sits and the embedded thermocouples are revealed by removing the sapphire window and overlying rubber and steel washers. All parts and connections are labeled. **Top left inset**: stage shown from above with central and side stage embedded thermocouples.

Figure 2: Methane hydrate experimental setup. (**A**) The fume hood in which the experimental setup is located. (**B**) The gas cylinder is connected via a copper coil to the pressure pump. Highlighted from panel (**A**) are (**C**) the assembled pressure cell, (**D**) the 10-gallon (37.85 L) tank without the insulation or solution, (**E**) the pressure pump, and (**F, G, H**) zoomed-in images of pressure pump connections.

Figure 3: Methane hydrate shells. Representative images of the droplet before (**A**) and after (**B**) a methane hydrate shell formed on a deionized water droplet and before (**C**) and after (**D**) a hydrate shell formed on a droplet containing 100 µg mL^{-1} Type I antifreeze protein. Scale bars = 5 mm.

Figure 4: Pressure-temperature stability diagram. Pressure and temperature data during depressurization are shown with P/T stability curves of methane hydrate (#1 from Sloan and Koh 2007[13] and #2 calculated from taking a regression curve from hydrate melting peaks from this study). Trials with successfully formed hydrate shells on DI water droplets are Trials 1, 2, and 3. Trial 4 was a negative control with no droplet on the stage. The droplet in trial 5 was another negative control in which no hydrate shell was formed. Stars indicate when visual hydrate melting began during depressurization. Trial 1 has a resolution of 30 s (a data point every 30 s); other trials have a resolution of 1 s. Abbreviations: T = trial; M.E. = memory effect; P/T = pressure-temperature; DI = deionized; res = resolution.

Supplemental Figure S1: CAD images for machining the pressure cell. Parts A–F of the pressure cell are labeled with their part letter and dimensions. Abbreviation: CAD = computer-aided design.

Supplemental Figure S2: Pressure and temperature data over time for Trials 2–4. Trials 2 and 3 were regular deionized water droplets that formed hydrate shells. Trial 4 was a negative control in which no droplet was present. The trials are lined up at the first depressurization, which occurs at time zero. A small drop in temperature occurs at the beginning of depressurization due to the gas mixing with the pressure pump. A larger temperature drop occurs due to the hydrate melting after the initial pressure drop, as shown in trials 2 and 3. The temperature fluctuation at the end of trial 4 is due to the opening of the valve leading to complete depressurization, which also occurs at the end of trials 2 and 3.

Supplemental Table S1: Allowable stress (MPa) of the machined pressure cell. Abbreviation: FS = factor of safety.

Supplemental Table S2: Factor of safety for the machined pressure cell. Abbreviation: FS = factor of safety.

Supplemental Video S1: Strain. Video of the strain simulation on machined pressure cell.

Supplemental Video S2: Stress. Video of the stress simulation on machined pressure cell.

Supplemental Video S3: Trial 3 of hydrate shell dissociation. Time-lapse video of hydrate shell dissociation at 25x speed.

Supplemental Video S3: Trial 3 of memory effect nucleation. Time-lapse video of hydrate shell formation by memory effect after repressurizing from 2 MPa to 5 MPa at 10x speed.

DISCUSSION:

We have developed a method to form methane hydrate shells on sessile water droplets safely and share this method to machine and assemble a pressure cell rated to 10 MPa working pressure, as well as the pressurizing and cooling systems. The pressure cell is outfitted with a stage for the droplet containing embedded thermocouples, a sapphire window for visualizing the droplet, and a pressure transducer fixed to the top of the cell. The cooling system includes chilled ethylene glycol circulating through copper coils in a tank with 50% ethanol solution, in which the pressure cell is placed. A pressure pump pressurizes the gas from the cylinder to the pressure cell. The hydrate shell forms upon rapid temperature decrease with the addition of dry ice to the top of the pressure cell. We allow the shell to form for 2 h, during which we believe the gas permeates through stochastic cracking of the hydrate shell, and Ostwald ripening over a longer period. Indeed, this device could be used to study these phenomena.

The critical steps for this protocol include: 1) leak-test the pressure cell with water before pressurizing it with gas, 2) practice adding the water droplet onto the stage before inserting the sapphire window, 3) cool the droplet to be stable at ~2 °C before pressurizing, 4) pressurize with a max flow rate of 10 mL min^{-1} to 5 MPa in 1 MPa increments, 5) close the outlet valve on the pressure pump (to the cell) to limit gas exchange with the pressure pump, 6) set the temperature,

pressure, and time-lapse software to log every 1 s, 1 s, and 5 s (or less), respectively, before adding dry ice, 7) apply dry ice to the top of the cell continuously until a hydrate shell is observed in the time-lapse, 8) allow the hydrate shell to form for at least 1 h, 9) depressurize at the same speed as pressurizing.

During method development, we optimized variables and techniques, including the timing of cooling, pressurizing, depressurizing, droplet size, and the droplet insertion technique. There are a few limitations in using this method. One limitation is the resolution of droplet imaging due to the camera resolution and materials between the camera and droplet (tank, ethanol solution, thick sapphire window). Additionally, while other studies observe the surface droplet on a microscale[7,9,10], this method only allows for macro-scale observations. A microscope lens attachment could be installed if there was interest in micro observations.

Another limitation to this method is not being able to measure the hydrate shell thickness precisely. However, the hydrate thickness can be estimated by subtracting the cross-sectional area before and after hydrate formation and calculating the gas consumption using the change in temperature during depressurization to determine the volume of hydrate formed. Another limitation is that this droplet cannot be viewed in 3D because there is only one side of the pressure cell containing a sapphire window. In contrast, other studies have machined sapphire cells completely to observe the droplet[7]. We also did not install a temperature-controlling stage[10] or spectroscopic techniques; however, these could certainly be installed using this setup.

With this method, the morphology, dissociation pressure and temperature, and the change in temperature during hydrate dissociation can be observed with droplets containing additives or alternative stage substrates. This method is relatively cheap, and there are few thorough protocols for forming gas hydrate shells. Because high-pressure systems can be dangerous, we include safety tips for pressurizing and leak testing. Additionally, many setups do not allow the visualization of gas hydrate formation, or do so on a much smaller or much larger scale. Laboratory experiments are a major contributor to the understanding of naturally occurring gas hydrates and natural gas hydrates that can cause lethal gas pipeline explosions. This method can be used to quickly assess the effects of additives on the dissociation temperature and morphology and the ability of additives to eliminate the memory effect. Effective additives could be used as inhibitors in natural gas pipelines or to study the biological activity of deep-sea bacterial proteins[6,15].

ACKNOWLEDGMENTS:

NASA Exobiology grant 80NSSC19K0477 funded this research. We thank William Waite and Nicolas Espinoza for valuable discussions.

DISCLOSURES:

There are no competing financial interests.

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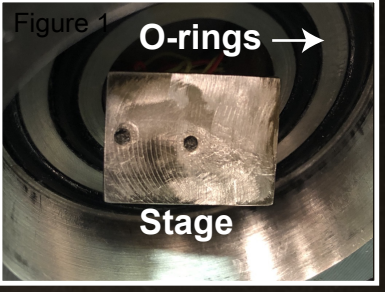
Figure 1



Click here to access/download;Figure;Figure1_pressure_cell.pdf

Outlet Valve

Illumination Port

Pressure Transducer

O-rings

NPT Screws

Inlet Valve

Pressure Seal Connector

Stage

NPT Screw

Thermocouples

Thermocouples

Steel Washer

Rubber Washer

Sapphire Window

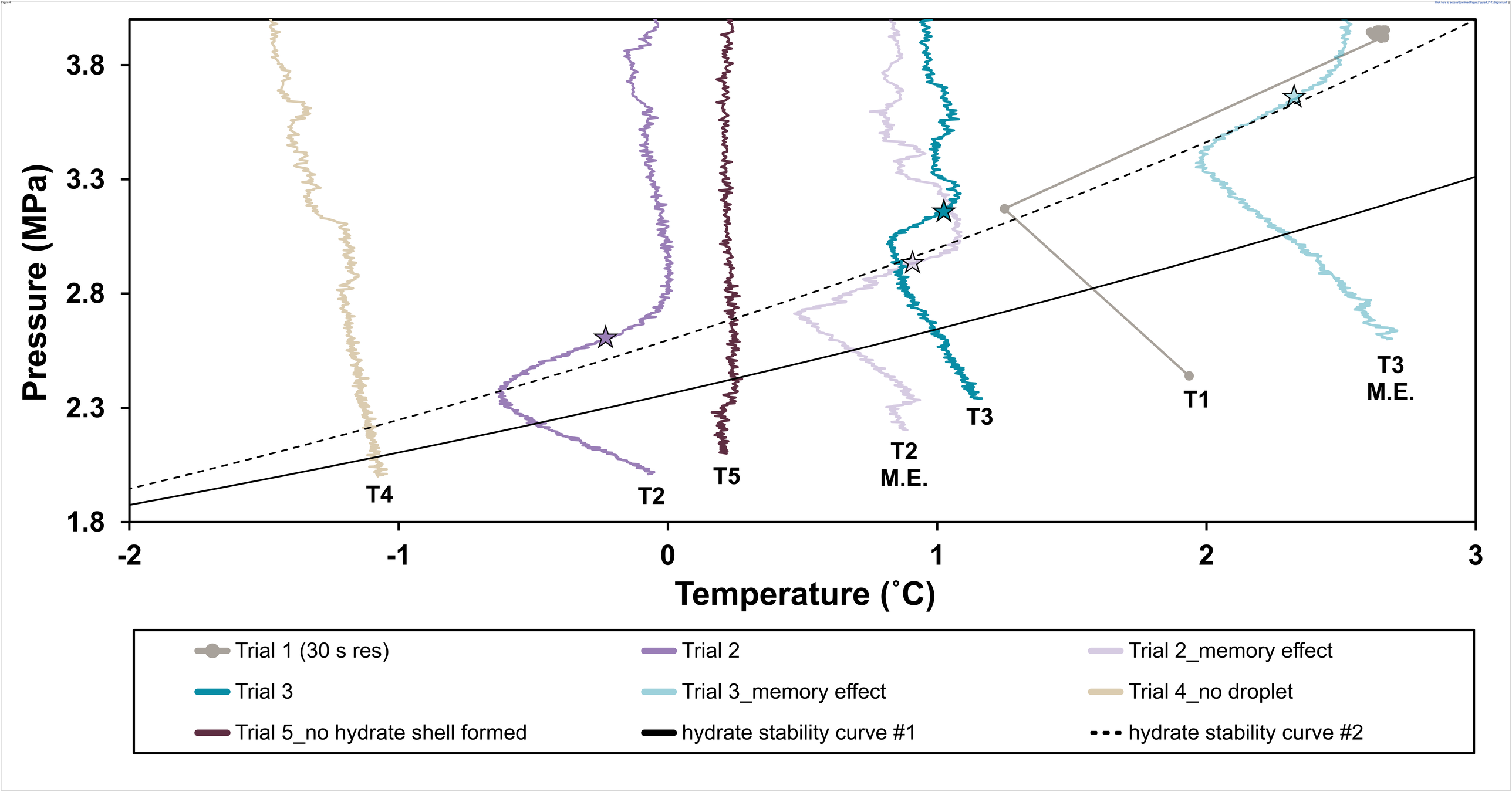

Pressure (MPa)
3.8
3.3
2.8
2.3
1.8
-2
-1
0
1
2
3
Temperature (˚C)
T4
T2
T5
T2
M.E.
T3
T1
T3
M.E.
Trial 1 (30 s res)
Trial 2
Trial 2_memory effect
Trial 3
Trial 3_memory effect
Trial 4_no droplet
Trial 5_no hydrate shell formed
hydrate stability curve #1
hydrate stability curve #2

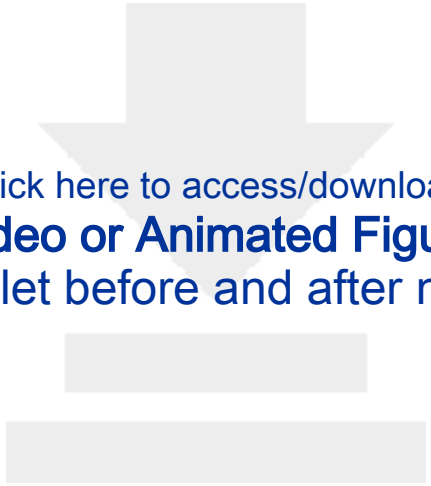
Figure 2

Click here to access/download

Video or Animated Figure

Figure2_Basic Setup.svg

Figure 3



Click here to access/download

Video or Animated Figure

Figure3_Droplet before and after nucleation.svg

Table of Materials

Click here to access/download;Table of Materials;JoVE_Materials (2).xlsx

Name of Material/ Equipment	Name in Protocol	Company	Catalog Number
CAMERA AND LAPTOP			
Camera Body	camera	Nikon	D7200
Camera Control Pro 2 Software	camera software	Nikon	
Laptop	laptop	HP Pavilion	hp-pavilion-laptop-14-ce0068st
Macrophotography Lens	lens	Nikon	AF-S MICRO 105mm f/2.8G IF-ED Lens
CONSUMABLES			
Deionized water	DI water		
Dry Ice	dry ice	VWR or grocery store	
Ethanol	ethanol		
Ethylene Glycol	ethylene glycol		
COOLING SYSTEM			
1/2 in. O.D. x 3/8 in. I.D. x 25 ft. Polyethylene Tubing	3/8” (inner diameter) plastic tubir	Everbilt	Model # 301844
Circulating chiller	chiller	Polyscience	
Economical Flexible Polyethylene Foam Pipe Insulation	foam pipe insulation	McMaster-Carr	4530K162
Plastic tubing			
DATALOGGER			
34970A/ 34972A, 20-Channel	datalogger multichannel	Keysight Technologies	34901A
Benchvue or Benchlink software	temperature transducer software	Benchlink	
RS232	datalogger	Keysight Technologies	34970A
USB/GPIB interface	datalogger USB	Keysight Technologies	82357B
datalogger multichannel Schott Fostec -Llc 20510 Ace Fiber Optic Light Source	light source unit	Schott Fostec	A20500

Schott Fostec light source guide - single bundle	fiber optic light source cable	Schott Fostec	A08031.40
METHANE GAS AND REGULATOR			
1/4 OD in. x 20 ft. Copper Soft Refrigeration Coil	high pressure-rated 1/4” copper	Everbilt	Model # D 04020PS
Methane cylinder regulator	methane cylinder regulator	Airgas	Y11N114G350-AG
Methane gas cylinder	methane gas cylinder	Airgas	ME UHP300
PRESSURE PUMP			
1/4 in. flexible tubing, ~ 3 ft.	1/4" flexible tubing		
260D Syringe Pump W/Controller	pressure pump	Teledyne Instruments Inc.	67-1240-520
Controller − Ethernet/USB		Teledyne Instruments Inc.	62-1240-114
Smooth-Bore Seamless 316 Stainless Steel Tubing, 1/4" OD, 0.035" Wall Thickness, 1 Foot Long (x5)	1/4" pipe	McMaster-Carr	89785K824
Smooth-Bore Seamless 316 Stainless Steel Tubing, 1/8" OD, 0.02" Wall Thickness, 1 Foot Long (x4)	1/8" pipe	McMaster-Carr	89785K811
Stainless Steel Swagelok Tube Fitting, Reducing Union, 1/4 in. x 1/8 in. Tube OD (x4)	1/8” to 1/4” adapter	Swagelok	SS-400-6-2
PRESSURE CELL			
316 Stainless Steel Nut and Ferrule Set (1 Nut/1 Front Ferrule/1 Back Ferrule) for 1/4 in. Tube Fitting (20)	ferrule set	Swagelok	SS-400-NFSET

316L Stainless Steel Convoluted (FM) Hose, 1/4 in., 316L Stainless Steel Braid, 1/4 in. Tube Adapters, 60 in. (1.5 m) Length	1/4" braided stainless steel flexible pressure-rated hose	Swagelok	SS-FM4TA4TA4-60
ABAQUS	simulation software	ABAQUS FEA	
Abrasion-Resistant Cushioning Washer for 7/8" Screw Size, 0.875" ID, 2.25" OD, packs of 10 (x1)	2.25" rubber washer	McMaster-Carr	90131A107
Abrasion-Resistant Sealing Washer, Aramid Fabric/Buna-N Rubber, 3/8" Screw Size, 0.625" OD, packs of 10 (x1)		McMaster-Carr	93303A105
Acrylic Sheet | White 2447 / WRT31 Extruded Paper-Masked (Translucent 55% (0.118 x 12 x 12)	acrylic disc	Interstate Plastics	ACRW7EPSH
AutoCAD	engineering design software	AutoCAD	
Conax fitting	pressure seal connector	Conax Technologies	311401-011
High Accuracy Oil Filled Pressure Transducers/Transmitters for General industrial applications (x2)	pressure transducer	Omega Engineering, Inc.	PX409-3.5KGUSBH
HIGH PRESSURE CHAMBER PARTS	Part B = stainless steel washer	Wither Tool, Die and Manufacturing Company	
High-Strength 316 Stainless Steel Socket Head Screw, M5 x 0.80 mm Thread, 14 mm Long (x20)		McMaster-Carr	90037A119

High-Strength 316 Stainless Steel Socket Head Screw, M8 x 1.25 mm Thread, 25 mm Long (x20)	M8 stainless steel screws	McMaster-Carr	90037A133
Oil-Resistant Hard Buna-N O-Ring, 3/32 Fractional Width, Dash Number 120, packs of 50 (x1)	1" o-ring	McMaster-Carr	5308T178
Oil-Resistant Hard Buna-N O-Ring, 3/32 Fractional Width, Dash Number 128, packs of 50 (x1)	1.5" o-ring	McMaster-Carr	5308T186
software	pressure transducer software	Inc.	
Polycarbonate Disc		McMaster-Carr	8571K31
Sapphire windows (x3)	sapphire window	Guild Optical Associates, Inc.	Optical Grade Sapphire Window, C-Plane Diameter: 1.811” ±.005” Thickness: .590” ±.005” Surface Quality: 60/40 Edges ground and safety chamfered
Solid Thermocouple Wire FEP Insulation and Jacket, Type K, 24 Gauge, 50 ft. Length (x1)	thermocouples	McMaster-Carr	3870K32
Stainless Steel Integral Bonnet Needle Valve, 0.37 Cv, 1/4 in. Swagelok Tube Fitting, Regulating Stem (x4)	1/4" needle valves	Swagelok	SS-1RS4
Stainless Steel Pipe Fitting, Hex Nipple, 1/4 in. Male NPT (x2)		Swagelok	SS-4-HN
Stainless Steel Swagelok Tube Fitting, Female Branch Tee, 1/4 in. Tube OD x 1/4 in. Tube OD x 1/4 in. Female NPT (x2)	branch tee fitting	Swagelok	SS-400-3-4TTF

Stainless Steel Swagelok Tube Fitting, Male Connector, 1/4 in. Tube OD x 1/4 in. Male NPT (x4)	NPT screws	Swagelok	SS-400-1-4
Stainless Steel Swagelok Tube Fitting, Port Connector, 1/4 in. Tube OD (x8)	port connector fitting	Swagelok	SS-401-PC
TANK			
1/4 OD in. x 20 ft. Copper Soft Refrigeration Coil	1/4" copper pipe	Everbilt	Model # D 04020PS
10 gallon aquarium	10 gallon tank	Tetra	
2 oz. Waterweld	underwater sealant	J-B Weld	Model # 8277
Pipe Wrap Insulation	foil-lined fiberglass	Frost King	Model # SP42X/16
3/8 7/8 in. Stainless Steel Hose Clamp (10 pack)	worm drive hose clamps	Everbilt	Model # 670655E
Styrofoam	insulating material		
TOOLS			
1-1/8 in. Ratcheting Tube Cutter		Husky	Model # 86-036-0111
1/2 in. to 1 in. Pipe Cutter		Apollo	Model # 69PTKC001
Adjustable wrench (x2)		Steel Core	Model # 31899
Allen wrench set		Home Depot	
Duct tape	duct tape		
Flexible tubing, like an IV line, to fit on the end of grainger probe (canula)	IV tube		
Grainger 18 gauge probe	cannula	Grainger	
High Vacuum Grease	vacuum grease	Dow corning	
Klein Tools Professional 90 Degree 4-in-1 Tube Bender	tube bender	Klein Tools	Model # 89030
Snoop liquid leak detector	liquid leak detector	Swagelok	MS-SNOOP-8OZ
Suction cup	suction cup	Home Depot	
Teflon Tape	plumber's tape		
Temflex 3/4 in. x 60 ft. 1700 Electrical Tape Black	electrical tape	3M	Model # 1700-1PK-BB40

Comments/Description
Needs to be PC with plenty of storage (~ 1 Tb)
Buy just before nucleation
For circulating coolant from chiller to copper coils in aquarium
3/4" thick wall; 1/2" inner diameter; R Value 3; 6' long
use any tubing that fits the airline connection in the lab and long enough to travel from the airline connection to the front of the aquarium
3115PS-12W-B20 115 V ~AC 50/60Hz 5/4.5 W

For pressurizing ISCO pressure pump. An additional pack is needed for coolant circulation, as listed below.

Connect to pump inlet for leak test

Purchase if you would like to install Labview onto computer and control pressure pump remotely. We did not do this.

Used for fitting connections where necessary

Connects pressure pump to pressure cell

Used for illumination port

Machine a circle of acrylic to fit in the inner chamber of the pressure cell to serve as the background for imaging

TG(PTM2/)-24-A6-T, OPTIONAL 1/4" NPT

Buy two so there is a backup.

Machining for pressure cell parts as listed in CAD drawings (Figure S1)

Used for illumination port

Listed in CAD drawings for illumination port,
Fig. S1 Part E

Buy three so there are two backups.

Two will be used for the pressure pump as well.

Used for illumination port

Used with pressure transducer

Used on top port and side port leading to needle valves

Use as tube connections between NTP and
valve connections

For circulating coolant

For wrapping around aquarium

Need two wrenches with jaw at least 1"

For inserting droplet
Apply to o-rings before inserting sapphire window

To detect leaks when pressurized when methane
For removing tight fitting sapphire window

Response to Reviewers JOVE manuscript JoVE62686

All comments are listed below in black and our responses in red.

We thank the editor for their helpful suggestions, which resulted in a much-improved manuscript.

Editorial comments:
Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please define all abbreviations at first use.
We defined antifreeze proteins (AFPs) after first use (line 50). We also corrected few grammar issues.

2. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.
For example: AutoCAD, ABAQUS, Keysight datalogger; BenchVue or BenchLink, Camera Control Pro 2, TRH Central – Omega Engineering Inc.; Teflon; Styrofoam; Omega Inc., ISCO pump etc
We replaced all commercial language in the main text and in figures with generic terms.

3. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.
We adjusted the language in several steps. We reduced our number of "Notes" to seven. We included additional safety procedures.

Line 117 in clean version: STEP 3.1. "As methane is a flammable gas under pressure, keep all methane-related tubing and vessels away from heat, sparks, open flame, and hot surfaces. Set all equipment up inside a well-ventilated area (e.g. fume hood). Don safety glasses and lab coat before working with methane gas."

Line 137 in clean version: STEP 3.8 "As ethylene glycol is hazardous, use appropriate safety attire, including gloves, lab coat, and goggles when pouring."

Line 189 in clean version: STEP 3.18 "Elevate all electronics from the hood surface to prevent potential leak damage. Double check that power is distributed for power capacity of outlets."

Line 232 and 305 in clean version: STEP 4.11.1 and 5.6.1 "Don safety glasses before pressurizing the pressure cell."

4. After including a one line space between each protocol step, highlight up to 3 pages of protocol text for inclusion in the protocol section of the video. This will clarify what needs to be filmed.
Line spacing setting was switched to "don't add spacing between paragraphs" and then a single space was added between each step. Step 4 was highlighted to clarify what needs to be filmed.

5. Please include a scale bar for all images taken with a microscope to provide context to the magnification used. Define the scale in the appropriate Figure Legend (fig 3).
We added a description of the scale in the caption of Figure 3. Figure 3 has a scale bar of 5 mm.

6. Please rearrange the panels of Fig 2 so that it's easier to follow the sequence.
We rearranged the panels in Fig 2 and deleted superfluous panels so that the image is easier to follow. We reflected these changes in figure calls throughout the text.

7. Please add legends of all supplemental figures, videos (supplemental or otherwise), and tables (supplemental or otherwise) to the figure and table legends section.
Done!

8. As we are a methods journal, please add any limitations of the technique to the Discussion.
We added additional limitations to this method.

Line 484 in clean version: "There are a few limitations in using this method. One limitation is the resolution of droplet imaging due to the camera resolution and materials between the camera and droplet (tank, ethanol solution, thick sapphire window). Additionally, while other studies observe the surface droplet on a microscale7,9,10, this method only allows for macro-scale observations. A microscope lens attachment could be installed if there was interest in micro observations. Another limitation to this method is not being able to precisely measure the hydrate shell thickness. However, we can estimate hydrate thickness by subtracting the cross-sectional area before and after hydrate formation, as well as calculate the gas consumption using the change in temperature during depressurization to determine the volume of hydrate formed. Another limitation is that we are not able to view this droplet in 3D because there is only one side of the pressure cell containing a sapphire window, whereas other studies have machined completely sapphire cells to observe the droplet7. We also did not install a temperature-controlling stage10 or spectroscopic techniques, but these could certainly be installed using this setup."

9. Please include a Disclosures section, providing information regarding the authors' competing financial interests or other conflicts of interest. If authors have no competing financial interests, then a statement indicating no competing financial interests must be included.
Done!

10. Please sort the Materials Table alphabetically by the name of the material.
We alphabetized the name of materials by section and within sections.

11. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source (ital.). Volume (bold) (Issue), FirstPage–LastPage (YEAR).] For more than 6 authors, list only the first author then et al. Please include volume and issue numbers for all references, and do not abbreviate journal names.
We corrected the formatting of the Endnote references.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

The authors are describing a method to form methane hydrate shells on water droplets. They provide a

step by step description for building a pressurized cell in which hydrate. crystal are formed on the surface of a sessile drop.
We thank Reviewer 1 for their helpful suggestions, which resulted in a much-improved manuscript.

Major Concerns:
The authors claim that using this method the morphology of the hydrate can be detected. It is not clear how.
This is a great point - we included an additive (Type I AFP) to show the morphologic difference between control and additive in Fig. 3 and explained the effects in the results section.

Moreover, the viewing window is only showing a 2D image of the drop. How are they compensating for that in the analysis?
Another excellent observation – through time-lapse imaging, we can visualize the surface of the hydrate from viewing window. To show how morphology can be altered with additives using only a 2D approach, we included images of the Type I AFP treatment in Fig. 3. We also discussed measuring hydrate thickness using the cross-sectional area of the droplet, as well as measuring gas consumption to predict the volume of hydrate formed.

Line 489 in clean version: “Another limitation to this method is not being able to precisely measure the hydrate shell thickness. However, we can estimate hydrate thickness by subtracting the cross-sectional area before and after hydrate formation, as well as calculate the gas consumption to determine the volume of hydrate formed.”

Minor Concerns:
It will be beneficial to add a bit more analysis to the results. For example, what kind of information can we get from the pressure read. Also, since the authors mentioned the use of this system to determine effectivity of inhibitors they should comment on how the procedure should be conducted.
We agree! Per reviewer #2’s suggestion, we added instructions for measuring hydrate thickness by comparing the droplet cross-sectional area before and after hydrate formation.

Reviewer #2:
Manuscript Summary:
The manuscript portion reads very well and presents sufficient background information that supports the ensuing discussion and description of the pressure vessel and methodology. In some respects the sapphire-plate optical cell described here is similar to some of the optical cells designed and made by Uri Makogon, but the authors do mention that many other pressure vessels are (and have been) used by others, and the placement of the window and specific design application of this cell make it notable. The detailed description of protocols, methodology, CAD designs, and equipment list is extremely thorough and will be useful for gas hydrate researchers who choose to build a similar cell. The occasional casual comment ("Buy three so there are two backups") offers practical information not ordinarily provided in reports. The overall pressure cell design and apparatus lay-out are very clearly shown here and the videos obviously speak to the success of the design and methodology. The figures (and tables) are well organized and clearly convey the necessary information.

Overall this is an excellent methods article. From the perspective of presenting a thorough description of methodology and instrument design, the current report reads fine as is. Some comments that the authors might want to address are listed below.
We thank Reviewer 2 for their helpful suggestions, which resulted in a much-improved manuscript.

Major Concerns:
None.

Minor Concerns:
* Line 38. Suggest starting the sentence with "Gas hydrates, also called clathrate hydrates, are…." Or similar. The terms "methane hydrate" and "methane clathrate" are used interchangeably throughout the text and figure captions, and I wouldn't presume that all of your audience will know that they're the same thing. Alternatively, the authors could leave the opening sentence as is and just use one term ("methane hydrate" or "methane clathrate") consistently throughout.
We left the first line as is and consistently used “hydrate” throughout. We changed the figure 4 legend to “hydrate stability curve”.

* Line 49. Add "(AFPs)" after "antifreeze proteins", as the acronym is used later in line 57.
Done!

* Line 58. Shouldn't "polyPVP" just say "PVP"? (Isn't "poly" redundant here?)
Yes, thank you. This has been changed.

* Line 428. Check wording ("…and memory effect elimination potential of additives…")
We adjusted this wording to flow better.

Line 504 in clean version: “This method can be used to quickly assess the effect of additives on the dissociation temperature and morphology, and the ability of additives to eliminate memory effect”

* In Figure 3a, perhaps add a couple of words (such as "edge of window" or "edge of acrylic disc", and "droplet" or similar) and arrows to make it immediately apparent which surface corresponds to the droplet. To a first-timer looking at these images, it's easy to mistake the window edge (or disc) for the surface of the drop, because the portion of the image between the window (or disc) and the droplet surface looks quite like a partial coating. It's difficult to judge depth in any of the photos or videos, and simply pointing out what's what in Fig 3a would help prevent any confusion.
Great idea! We added labels in Fig 3a to avoid first-timer confusion.

* Somewhere in the "Representative results" section, it should be noted what the vertically-curved light-colored streaks are, that are inside the drop at the end of the dissociation video (Vid 3), and at the start of the memory-effect video (Vid 4) when the droplet is pure liquid at the start. I viewed both videos several times and still don't have a clear idea of what the "streaks" are.
Great point! These “streaks” are gas bubbles, which form during depressurization. We added an explanation in the results.

Line 402 in clean version: “Upon depressurization below 2 MPa, we observed gas bubbling within the droplet from rapid degassing.”

Line 352 in clean version: STEP 5.9: “Bubbling in the sessile droplet may occur due to dissolved gas escaping.”

* Is the pressure vessel itself 316? I don't see it specified. I would assume 316 for optimal corrosion resistance, or perhaps 304 for easier machining. Section 1.1.2 ("Pressure cell design and validation")

assigns 301 for analysis (why 301?). Later in the Table of Materials, many of the tubing, tube fittings, nuts, screws, etc., are listed as 316 or 316L.
You're correct! It's actually stainless steel 316 rather than 301. We fixed this.

* The authors state that one limitation of the cell is the inability to measure the thickness of the hydrate film (Lines 416-417). This is indeed a limitation as even incipient development of a shell obscures any visual observation of what happens underneath. (In this regard, a high-pressure optical cell based around a Si capillary tube like those designed by I-M Chou et al., that allows for direct loading of fluids and that has almost no optical distortion is quite helpful as one can continually observe what happens within the liquid phase after the hydrate coating forms. But that is not the same as observation of hydrate films on a sessile water droplet.) I would think that if the cross sectional area of the droplet was measured precisely, and assuming the initial mass of H2O in the droplet was known, that a good approximation of the shell thickness could be easily calculated from the cross-sectional change from start to finish simply based on the known densities of liquid water (1.0 g/cm3) versus the density of an empty hydrate lattice (~0.79 g/cm3 at T near 0°C). That's just over 20% volumetric expansion that attends methane clathrate formation from liquid water, i.e. easily visible. The dendritic "whiskers" forming along the right-hand side of the droplet would slightly complicate the calculation, but those whiskers are extremely fine and I doubt would change the calculation significantly manner. An additional assumption would be that the diameter of the droplet is essentially uniform, which is not easily verified by imaging in one plane only as here, but again likely wouldn't change the calculation significantly. Determining extent of reaction (and by correlation shell thickness if hydrate formation only occurs on the surface of droplets) through visual assessment of volume change might be problematic for very thin shells, but could be quite useful for experiments in which reaction is planned to continue for longer periods of time, for instance in determining time to, say, 25% or 50% conversion of water to hydrate in the presence of any given (or no) additives.
This is a great idea! We added this method for estimating hydrate thickness to our discussion.

Line 489 in clean version: "Another limitation to this method is not being able to precisely measure the hydrate shell thickness. However, we can estimate hydrate thickness by subtracting the cross-sectional area before and after hydrate formation, as well as calculate the gas consumption to determine the volume of hydrate formed."

* Once the hydrate rind forms, how is the reaction expected to proceed from there? By gas diffusion (and diffusion limited) through the outer hydrate shell? By migration of either gas or liquid through microcracks that should presumably form as a result of the volume change that attends reaction? In the videos, it appears that the shell forms first (and develops a bit thicker) on the left-side of the droplet, which may explain why the dendritic whiskers of hydrate form more at the right side of the droplet, if water was expelled after partial development of the shell. With the exception of the brief mention of Ostwald ripening (observed by Chen et al), the subject of nucleation and subsequent growth is notably missing.
Given that this is a methods article I would not expect an in-depth discussion of the methane hydrate formation process on sessile water drops, but even one or two sentences that discuss the author's thoughts on the hydrate formation process could be helpful and would speak to additional uses of the apparatus.
Great point! We believe both processes could be happening here, which we summarized in the discussion.

Line 469 in clean version: “The hydrate shell forms upon rapid temperature decrease with the addition of dry ice to the top of the pressure cell. We allow the shell to form for 2 hours, during which we believe gas permeates through stochastic cracking of the hydrate shell, as well as Ostwald ripening over a longer time period. Indeed, this device could be used to study these phenomena.”

Reviewer #3:

Manuscript Summary:

A protocol is presented for the formation of methane-hydrate on sessile water drops, which can assist other scientists in performing this type of experiments.

We thank Reviewer 3 for their helpful suggestions, which resulted in a much-improved manuscript.

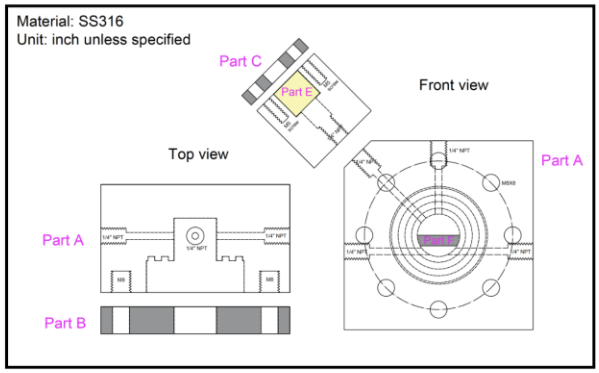
Minor Concerns:

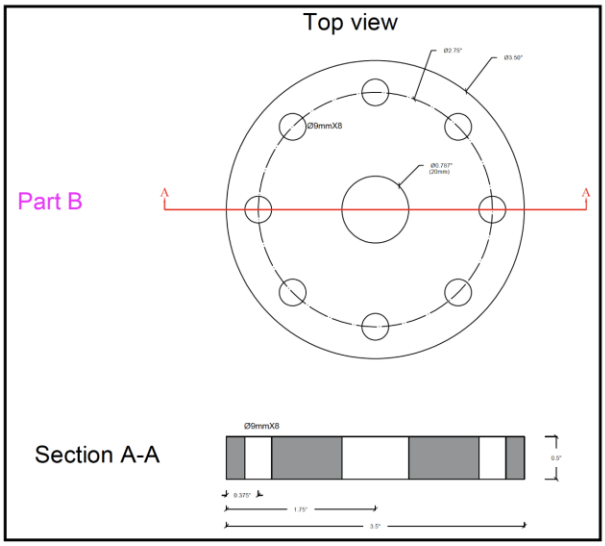
Subsection 1.12 - Isn't treating it as a static problem a rigorous approach?

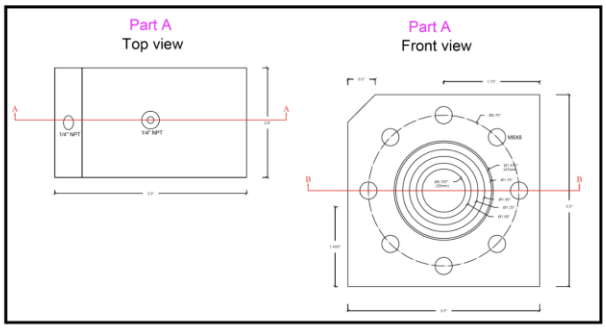
What is the timescale of "slow pressure changes"?

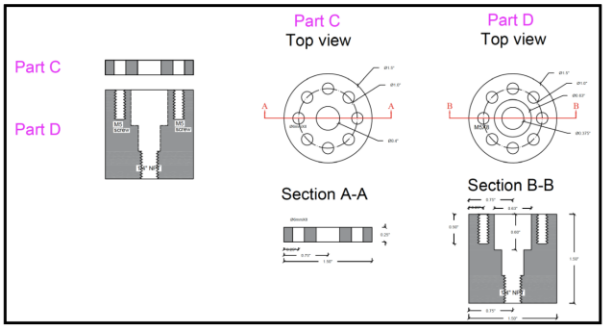
International codes for pressure vessels using austenitic stainless steel (300 series group) allow static analysis with recommended factors of safety (FS). For instance, ASME Section VIII Divisions 1 recommends FS=3.5 tensile strength and FS=1.12 yield strength for elastic analysis; ASME Section VIII Divisions 2 recommends FS=2.4 tensile strength for locally elastic-plastic analysis; EN 13445 recommends FS=3 tensile strength; Japanese codes on boilers and pressure vessels are developed largely based on ASME codes and with the same FS values. The pressure validation simulation complies with these codes.

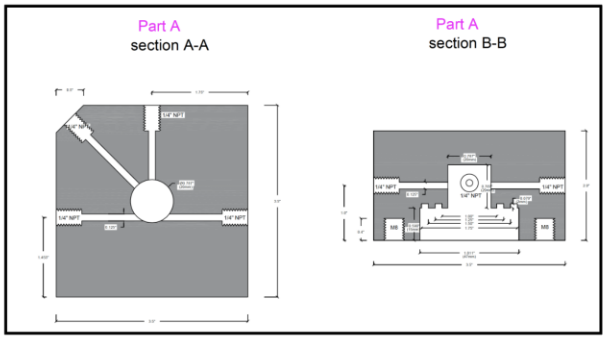
Supplemental Figure S1

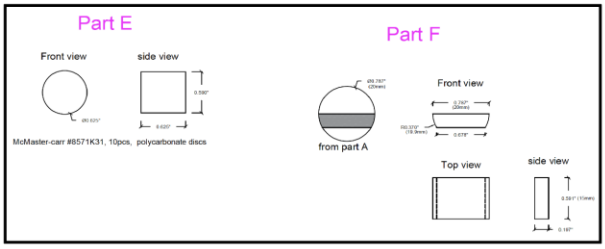












Supplemental Figure S2

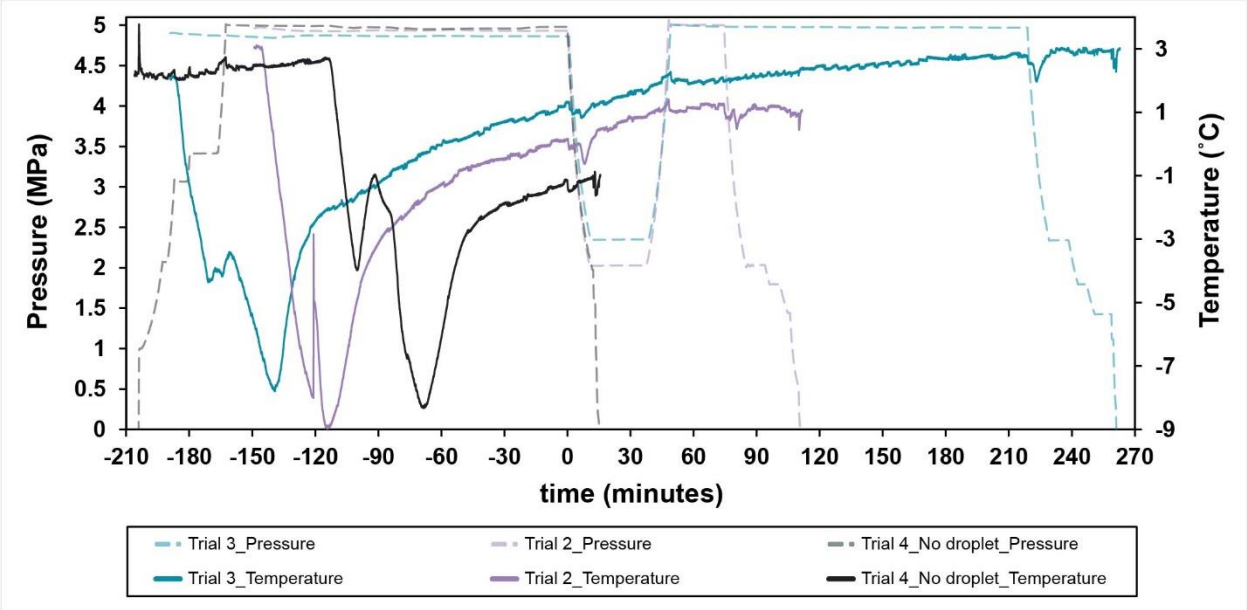


Table S1. Allowable stress (MPa) of the machined pressure cell.

Code	Allowable stress [Mpa]		Remarks
	Tensile strength σ_t	**Tensile strength σ_y**	
	σ_{allow} = tensile strength/FS	σ_{allow} = yield strength/FS	
ASME Section VIII Divisions 1	147.1	183.0	Elastic analysis
ASME Section VIII Divisions 2	214.6	183.0	Locally elastic-plastic analysis
ASME Section VIII Divisions 3	Design-by-analysis Code		Elastic-plastic analysis
EN 13445	214.6 (@ 20 °C)	136.7	

Table S2. Factor of safety for the machined pressure cell.

Code	Factor of Safety FS		Remarks
	Tensile strength σ_t	**Tensile strength σ_y**	
	σ_{allow} = tensile strength/FS	σ_{allow} = yield strength/FS	
ASME Section VIII Divisions 1	3.5	1.12	Elastic analysis
ASME Section VIII Divisions 2	2.4	1.12	Locally elastic-plastic analysis
ASME Section VIII Divisions 3	Design-by-analysis Code		Elastic-plastic analysis
EN 13445	3.0 (@ 20 °C)	1.5 (0.1% offset)	

Video S1

Video S2

Click here to access/download

Supplemental Coding Files

Video_S2_stress.mov

Video S3

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Supplemental Coding Files

Video_S3_Trial 3 dissociation_25x.mp4

Video S4

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Supplemental Coding Files

Video_S4_Trial 3 memory effect nucleation_10x.mp4