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TITLE:

Application of Voltage in Dynamic Light Scattering Particle Size Analysis

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KEYWORDS:

dynamic light scattering (DLS), Lower critical solution temperature (LCST), applied voltage, analytical chemistry, poly(*N*-isopropylacrylamide), electrochemically-active block co-polymers

SUMMARY

Here, a protocol to apply voltage to solution during dynamic light scattering particle size measurements with the intent to explore the effect of voltage and temperature changes on polymer aggregation is presented.

ABSTRACT:

Dynamic light scattering (DLS) is a common method for characterizing the size distribution of polymers, proteins, and other nano- and microparticles. Modern instrumentation permits measurement of particle size as a function of time and/or temperature, but currently there is no simple method for performing DLS particle size distribution measurements in the presence of applied voltage. The ability to perform such measurements would be useful in the development of electroactive, stimuli-responsive polymers for applications such as sensing, soft robotics, and energy storage. Here, a technique using applied voltage coupled with DLS and a temperature ramp to observe changes in aggregation and particle size in thermoresponsive polymers with and without electroactive monomers is presented. The changes in aggregation behavior observed in these experiments were only possible through the combined application of voltage and temperature control. To obtain these results, a potentiostat was connected to a modified cuvette in order to apply voltage to a solution. Changes in polymer particle size were monitored using DLS in the presence of constant voltage. Simultaneously, current data were produced, which could be compared with particle size data, to understand the relationship between current and particle behavior. The polymer poly(*N*-isopropylacrylamide) (pNIPAM) served as a test polymer for this technique, as pNIPAM's response to temperature is well-studied. Changes in the lower-critical solution temperature (LCST) aggregation behavior of pNIPAM and poly(*N*-isopropylacrylamide)-*block*-poly(ferrocenylmethyl methacrylate), an electrochemically active block-copolymer, in the presence of applied voltage are observed. Understanding the

mechanisms behind such changes will be important when trying to achieve reversible polymer structures in the presence of applied voltage.

INTRODUCTION:

Dynamic light scattering (DLS) is a technique to determine particle size through the use of random changes in intensity of light scattered through solution[1]. DLS is capable of measuring aggregation of polymers by determining particle size. For this experiment, DLS was coupled with controlled temperature changes to observe when a polymer aggregates which is indicative of exceeding the lower critical solution temperature (LCST)[2,3]. Below the LCST, there exists one homogeneous liquid phase; above the LCST, the polymer becomes less soluble, aggregates, and condenses out of solution. An applied voltage (i.e., applied potential or electric field) was introduced across the scattering field to observe the effects of the electric field on aggregation behavior and LCST. The application of voltage in particle sizing measurements allows for new insights into particle behavior and subsequent applications in the fields of sensors, energy storage, drug delivery systems, soft robotics, and others.

In this protocol, two example polymers were used. Poly(*N*-isopropylacrylamide), or pNIPAM, is a thermal sensitive polymer, which contains both a hydrophilic amide group and a hydrophobic isopropyl group on the macromolecular chain[4,5]. Thermal-responsive polymer materials like pNIPAM have been widely used in controlled drug release, biochemical separation, and chemical sensors in recent years[3,4]. The LCST literature value of pNIPAM is around 30-35 °C[4]. pNIPAM is typically not electrochemically active. Therefore, as a second sample polymer an electrochemically-active block to the polymer, specifically ferrocenylmethyl methacrylate, is used to create a poly(*N*-isopropylacrylamide)-*block*-poly(ferrocenylmethyl methacrylate) block-copolymer, or p(NIPAM-*b*-FMMA)[6,7]. Both example polymers were synthesized by reversible addition fragmentation chain-transfer polymerization with controlled chain length[8–10]. The non-electrochemically active polymer, pNIPAM, was synthesized as 100 mer pure pNIPAM. The electrochemically active polymer, p(NIPAM-*b*-FMMA), was also 100 mer chain length, which contains 4% ferrocenylmethyl methacrylate (FMMA) and 96% NIPAM.

In this article, a protocol and methodology to study the effect of applied voltage on polymer aggregation is demonstrated. This method could also be extended to other applications of DLS, such as the analysis of protein folding/unfolding, protein-protein interactions, and agglomeration of electrostatically charged particles to name a few. The sample was heated from 20 °C to 40 °C to identify the LCST in the absence and presence of a 1 V applied field. Then, the sample was cooled from 40 °C to 20 °C without disrupting the applied field to study any hysteric or equilibrium effects.

PROTOCOL

1. Example polymer preparations

1.1. pNIPAM polymer synthesis

NOTE: This preparation produces 10 mL of 1 g/L solution, which is enough for 3-4 experiments.

1.1.1. Prepare the Schlenk line apparatus. Ensure the cold trap Dewar flask is filled with a slurry of dry ice and acetone, or if a mechanical refrigeration trap is used, ensure that the trap has reached an appropriate temperature.

1.1.2. In a 50 mL round-bottom flask, add 0.566 g of *N*-isopropylacrylamide (NIPAM) monomer, 0.016 g of reversible addition fragmentation chain transfer polymerization (RAFT) agent (phthalimidomethyl butyl trithiocarbonate), 0.0008 g of 2,2-azobis(2-methylpropionitrile) (AIBN) and 10 mL of 1,4-dioxane. Put a stir bar into the flask. Seal the flask with a rubber septum, wrap with vinyl tape, and dissolve the monomers in 1,4-dioxane.

1.1.3. Perform freeze-pump-thaw degassing as follows: Freeze the solution by immersing the round-bottom flask in a Dewar flask containing a slurry of dry ice and methanol. Once all material is frozen, use the vacuum manifold of the Schlenk line to evacuate the flask to an internal pressure under 100 kPa. Isolate the flask, and thaw under static vacuum, using warm water. Return the flask to atmospheric pressure using the nitrogen manifold of the Schlenk line.

1.1.4. Repeat step 1.1.3 three times to minimize the internal oxygen concentration.

1.1.5. Sparge the solution with nitrogen to balance the pressure to atmosphere. Heat the mixture to 85 °C using an oil bath and stir at 200 rpm for 36 h.

1.1.6. To a 50 mL beaker, add 40 mL of hexane. Then add the polymer mixture to the hexane dropwise. The pNIPAM should precipitate out as white floccule.

NOTE: NIPAM monomer is soluble in hexane, but pNIPAM has a poor solubility in hexane.

1.1.7. Pour the cloudy mixture into a Büchner funnel to collect the white pNIPAM powder. Transfer the powder to a 20 mL vial and put it in a vacuum oven overnight to remove leftover solvent. Store in a sealed container at room temperature until needed.

1.2. pNIPAM-*block*-poly(ferrocenylmethyl methacrylate) block-copolymer (p(NIPAM-*b*-FMMA)) synthesis

NOTE: This preparation produces 10 mL of 1 g/L solution, which is enough for 3-4 experiments.

1.2.1. Prepare the Schlenk line apparatus. Ensure the cold trap Dewar flask is filled with a slurry of dry ice and acetone, or if a mechanical refrigeration trap is used, ensure that the trap has reached an appropriate temperature.

1.2.2. In a 50 mL round-bottom flask, add 0.057g of ferrocenylmethyl methacrylate (FMMA) monomer, 0.016 g of RAFT agent, 0.0008 g of AIBN and 10 mL of 1,4-dioxane. Put a stir bar into the flask. Seal the flask with a rubber septum, wrap with vinyl tape, and dissolve the monomers in 1,4-dioxane.

1.2.3. Perform freeze-pump-thaw degassing as follows: Freeze the solution by immersing the round-bottom flask in a Dewar flask containing a slurry of dry ice and methanol. Once all material is frozen, use the vacuum manifold of the Schlenk line to evacuate the flask to an internal pressure under 100 kPa. Isolate the flask, and thaw under static vacuum, using warm water. Return the flask to atmospheric pressure using the nitrogen manifold of the Schlenk line.

1.2.4. Repeat step 1.2.3 three times to minimize the internal oxygen concentration.

1.2.5. Sparge the solution with nitrogen to balance the pressure to atmosphere. Heat the mixture to 85 °C using an oil bath and stir it for 10 h.

1.2.6. Dissolve 0.543 g of NIPAM and 0.0002 g of AIBN into 3 mL of 1,4-dioxane. Add the solution into the flask under nitrogen and sparge for 30 min. Heat the mixture to 85 °C using an oil bath and stir it at 200 rpm for another 36 h.

1.2.7. Add 40 mL of hexane to a 50 mL beaker. Then add the polymer mixture to the hexane dropwise. The p(NIPAM-b-FMMA) should precipitate out as brown powder because the FMMA monomer has a dark yellow color.

NOTE: NIPAM and FMMA monomers are soluble in hexane, but p(NIPAM-*b*-FMMA) has poor solubility in hexane.

1.2.8. Pour the yellow cloudy mixture into a Büchner funnel to collect the brown p(NIPAM-*b*-FMMA) powder. Transfer the powder to a 20 mL vial and put it in vacuum oven overnight to remove leftover solvent. Store in a sealed container at room temperature until needed.

2. DLS sample and cuvette preparation

NOTE: This section prepares the cuvette for applied voltage and the sample for DLS measurements.

2.1. Measure 10 mg of polymer powder and dissolve in 10 mL of filtered deionized (DI) water. Put the mixture in a refrigerator overnight. When ready to start the experiment, keep the sample on ice.

NOTE: The polymer concentration used in these experiments was 1 g/L, but the optimal range of concentrations for each sample will be unique. Also, best practice is to keep the polymer below the LCST until it is ready for testing.

2.2. Cut two pieces of 6.3 mm x 7 cm single-sided copper tape **(Figure 1)**. Use tweezers to stick each piece to opposite sides of the inside of the DLS sample cuvette, perpendicular to the light path. The bottom of the tape should reach near the bottom of the cuvette. Fold the edges of the copper tape over the top of the cuvette. Make sure the copper tape is near/wrapped on the top of the sample cuvette to ensure good electrical contact. Also make sure the copper tape does not connect with the metal contacts associated with the DLS equipment used for Zeta potential measurements.

2.3. Wash the cuvette with DI water three times, then dab the excess water off with a Kimwipe.

3. DLS instrument controls and set up

NOTE: Three controls are recommended to complete before running each DLS experiment: (1) blank water solution; (2) a size standard; (3) measurement of the polymer before the start of a temperature ramp or applied voltage. Please consult the instrument manual before operation for guidance on preparing a sample, choosing settings, and assessing sample and data quality.

3.1. Transfer 1.5 mL of filtered solvent to the cuvette. Use DI water.

3.2. Insert the cuvette to the cuvette holder, ensuring the small arrow on the top of the cuvette is aligned with the cuvette holder. Close the lid.

3.3. Within the Zetasizer software, select **Measure** on the toolbar. Manual measurements were set up for the controls. Set the **Temperature** to the experimental starting point. Select 20 °C for this experiment.

3.4. Once the text at the bottom of the window says, **Insert cell and press start when ready**, hit the green triangle start button at the top of the screen. This starts the experiment, and the cuvette holder should not be opened after this.

3.5. Click on the tab **Multi-view** to observe real-time results. Continuously monitor the sample and data quality by observing count rate and the correlation function. Because this sample is just solvent, no clear signal corresponding to the presence of particles should be observed.

3.6. Add two drops of a standard solution to the cuvette or just use the water control, and repeat steps 3.2-3.6. Use a 20 nm NIST-traceable polystyrene size standard for this experiment.

NOTE: If the water or standard solution control runs show data that are inconsistent with the expected results, troubleshoot the error and repeat until the controls read as expected.

3.7. Rinse the cuvette and add the filtered polymer/test solution. Repeat steps 3.2-3.5. A clear measurement of the initial test solution should be observed. It is recommended to do this before any temperature ramp or applied voltage for a baseline measurement.

4. DLS SOP set up

NOTE: This section refers specifically to the temperature ramping operation of a Malvern Zetasizer NanoZS DLS instrument. Before starting experiments, it is strongly recommended to consult the instrument manual extensively for guidance on selecting a cell, preparing a sample, choosing measurement settings, and assessing sample and data quality.

4.1. Within the Zetasizer software (version 7.11), choose **File**, then click **New** to set up a new SOP **(Figure 2)**.

4.2. Click **Measurement type** to select **Trend > Temperature > Size**.

4.3. In **Material**, choose the appropriate material and refractive index. Choose **Protein** and the refractive index (RI) of 1.450 for this experiment. If exact values for refractive index are desired for more accurate calculation of volume distribution, the experimenter should determine the refractive index of their sample experimentally.

4.4. In **Dispersant**, choose the appropriate solvent. Choose **Water** as a solvent in this experiment.

4.5. In **Cell**, choose the cuvette being used. Use Disposable cuvettes (DTS0012) for this experiment.

4.6. In **Sequence**, set **Start temperature** and **End temperature**. For heating experiments, set the **Start temperature** at 20 °C and set the **End temperature** as 40 °C. For cooling experiments, choose the opposite. Uncheck the **Return to starting temperature** box.

4.7. Select an interval for each temperature step change. For these experiments, select 1.5 °C.

4.8. In **Size** measurement, set **Equilibrium time**. For these experiments, set the duration to 120 s. Choose number of measurements. Choose 3 measurements and **Automatic** for measurement duration.

4.9. Save SOP, then close the file.

4.10. If applied voltage is to be used, set up the potentiostat (**Section 5**) before continuing.

4.11. Once the potentiostat is set up, or if applied voltage is not used, return to the Zetasizer software and click **Measure** on the toolbar, then click **Start SOP**.

4.12. Once the text at the bottom of the SOP window says, **Insert cell and press start when ready**, hit the green triangle start button at the top of the screen. This starts the experiment, and the cuvette holder should not be opened after this.

4.13. Click on the tab **Multi-view** to observe real-time results. Continuously monitor the sample and data quality by observing count rate and the correlation function. See **Figures 3-5** for representative experimental results.

5. Potentiostat Setup

NOTE: It is recommended to use the same computer for particle size and applied voltage operations to time-sync the data, and therefore, easier to evaluate later. Please consult the applied voltage instrument manuals for guidance on wiring set up, software consultation, and choosing appropriate parameters. A Gamry potentiostat was used in these experiments.

5.1. Prepare two wires that are thin enough to fit through the small crevice on the upper right edge of the DLS cuvette holder area **(Figure 6)**. On one end of the prepared wire, strip off the insulation to allow for a connection to the potentiostat. On the opposite end, solder a short alligator clamp to the wire and connect to the cuvette. Make sure the DLS sample lid is closed.

5.2. Clamp the white reference potentiostat lead and the red counter potentiostat lead together to one of the prepared wires. Clamp the green working potentiostat lead and the blue working sense potentiostat lead to the other prepared wire. For this experiment, do not the orange counter sense and black ground potentiostat leads and leave them floating. To ensure the circuit does short, these wires should not touch any other lead or conductive surface.

NOTE: It does not matter which side each lead is connected to.

5.3. Within the software toolbar, click **Experiment**, then click option **E Physical Electrochemistry**, and select **Chronoamperometry**. For the purposes of this protocol, use a simple applied voltage by applying a single voltage with current response measured over time (i.e., chronoamperometry). Regardless of the specific electrochemical methodology, it is recommended to monitor the system response over time.

5.3.1. Set **Pre-step**, **Step 1**, and **Step 2 Voltage vs Reference**. This will be the applied voltage across the entire field/cuvette. Set **Voltage** to 1 V vs Reference for all three steps.

5.3.2. Set **Pre-step Delay Time**. For these experiments, set to 0.5 s to make sure a stable system at the desired voltage before recording a signal.

5.3.3. Set the time for both **Step 1 Time** and **Step 2 Time**. This controls how long the voltage will be applied. Set both to 14,400 s to make sure the applied voltage will continue throughout the DLS experiment.

5.3.4. Set **Sample Period**. This is how frequently the graph will read and record current and voltage values. Use 10.0 s in this experiment.

NTE: The other settings are not significant for the data presented here. The default values in the system were used.

5.4. Click **Collect**. Top toolbar will display an **Active** sign, indicating voltage is being applied. The current should give a moderate response (µA), and not overload the potentiostat. If no signal or excessive signal is observed, the system might be connected incorrectly, and therefore, troubleshoot the error and repeat until the expected current is observed.

5.5. Return to step 4.10 to start the DLS SOP.

6. Data analysis

NOTE: This section details preliminary analysis to understand the data obtained.

6.1. Import data into preferred data analysis and graphing software. Use **Excel** to analyze volume average sizes, derived from the main volume distribution peak, as a function of temperature.

6.2. For each run within a set of measurements at a given temperature, determine the particle volume size of the peak with the largest volume percent.

6.3. Calculate the average and standard deviation of the volume size over the three recorded measurements at a given temperature.

6.4. For each experiment, plot average size ± standard deviation on the y-axis (log scale) versus temperature on the x-axis (linear scale).

6.5. Import Gamry current data for analysis. Plot current data with time on the x-axis and current (in microamps) on the y-axis.

6.6. In order to relate current data to particle size data, compare the timestamp of the Zetasizer data to the Gamry current timestamp. This is possible if the two types of data are collected from the same computer. Otherwise, match recorded times as best as possible.

REPRESENTATIVE RESULTS:

The real-time file output of each run in the temperature ramp is presented as a table, as seen in **Figure 3**. Each record can be chosen independently to see the volume size (**Figure 4**) and correlation coefficient (**Figure 5**). Volume particle size distribution (PSD) is the most accurate data to interpret the overall distribution and LCST, but the quality of data should be assessed via correlation graph (**Figure 5**) to determine if any points should be excluded from analysis. Correlation graphs (**Figure 5**) that have a generally smooth curve are considered good quality, where non-smooth graphs or low-quality data should be considered for exclusion in the analysis. The curves at 24.5 °C have some bumps and minor peaks in the curves, but this can be attributed to the rapid change in polymer aggregation, and therefore these data were included. This confirms that DLS data collected in our modified system in the presence of voltage are of equivalent quality to normal DLS data.

As seen in **Figure 7** (red lines), pNIPAM exhibited an LCST at 30 °C, a temperature close to the literature-described values[4]. Without voltage, pNIPAM was able to aggregate and disaggregate within the tested temperature range, returning to its original size and indicating expected reversibility. With voltage (**Figure 7**, black lines) pNIPAM went from being soluble to aggregating to a size of 2000 nm, then being reduced to a size of around 1000 nm during cooling, never returning to the original soluble state. **Figure 8** shows the current data from pNIPAM with applied voltage and heating and cooling experiments corresponding to **Figure 7** (black lines). The first vertical red line, at 26 °C, is a key transition point of pNIPAM where a phase change is observed with DLS. The second vertical line, at 40 °C, shows the maximum temperature in our measurement before the cooling cycle.

As seen in **Figure 9** (red lines), the p(NIPAM-*b*-FMMA) polymer containing an electroactive FMMA block exhibited an LCST at 33 °C. Without voltage, p(NIPAM-*b*-FMMA) was able to aggregate and disaggregate, returning to its original size. With voltage (**Figure 9**, black lines), the LCST of p(NIPAM-*b*-FMMA) shifted to 28 °C. Again, with applied voltage, the p(NIPAM-*b*-FMMA) was not able to disaggregate and return to its original size during the cooling cycle. **Figure 10** shows the current data from p(NIPAM-*b*-FMMA) with applied voltage and heating and cooling experiments corresponding to **Figure 9** (black lines). The first vertical red line, at 28 °C, is just above the phase change observed with DLS. The second vertical line, at 40 °C, is at the maximum temperature in our measurement before cooling.

Evaluation of the current response data from the applied voltage is crucial to understanding the size response. If current is not carefully monitored, data will be misconstrued and potentially misunderstood. In one trial displayed in **Figure 11**, the voltage cut in and out as a result of accidental short circuiting. As a result of a short circuit, the voltage was only applied randomly and sporadically, and this resulted in a trend more similar to the no-voltage condition.

FIGURE LEGENDS:

Figure 1. Disposable DLS cuvettes modified by adding copper tape to the sides to allow for applied voltage. The copper tape extends to the bottom and is wrapped around the top to ensure a good connection.

Figure 2. **A screenshot of DLS SOP setup, including sequence set up, size measurement specifications, and trend set up procedures**. The screen depicted here acts as a main page where all other, more specific aspects and subpages of the data, can be observed.

Figure 3. Example of record view of data collection in Zetasizer software. These records contain in-depth measurements of factors like intensity, volume size, correlation data, and quality of data.

Figure 4. Volume particle size distribution (PSD) for a single measurement at 31 °C of pNIPAM

with 1 V of applied voltage. This screen can be accessed by selecting the desired data point found in the records view tab (**Figure 4**) and provides detailed sizing information.

Figure 5. Correlation data of an experiment with acceptable, reproducible correlation functions for each temperature in the presence of applied voltage. The correlation functions of three repeated runs at three different temperatures are plotted.

Figure 6. DLS experiment setup using Gamry Potentiostat to apply constant voltage to the solution. This image depicts the wiring setup and general assembly of the circuitry required for this system.

Figure 7. Plot of pNIPAM particle size versus temperature. Black lines = applied voltage, red lines = no voltage, square data points = heating trend, triangle data points = cooling trend. Without applied voltage, the LCST was 30 °C during heating and 24 °C during cooling. With applied voltage, the LCST was 26 °C during heating and no disaggregation was observed during cooling.

Figure 8. Current data from pNIPAM with heating and cooling. The first vertical red line is the LCST of pNIPAM where the phase change is observed in the DLS data (**Figure 7**). The second vertical line shows the time at which heating was completed and cooling was initiated. The x-axis indicates time since start of experiment, as well as temperature at various time points.

Figure 9. Plot of p(NIPAM-*b*-FMMA) particle size versus temperature. Black lines = applied voltage, red lines = no voltage, square data points = heating trend, triangle data points = cooling trend. Without applied voltage, the LCST was 33 °C during heating and 28 °C during cooling. With applied voltage, the LCST was 28 °C during heating and no disaggregation was observed during cooling.

Figure 10. Current data from the p(NIPAM-*b*-FMMA) with heating and cooling. The first vertical red line is just above the LCST of p(NIPAM-*b*-FMMA) where the phase change is observed in the DLS data (**Figure 9**). The x-axis indicates time since start of experiment, as well as temperature at various time points.

Figure 11. Poorly connected circuit leading to error in data in a pNIPAM trial. The DLS data, pictured left, is similar to data from trials without voltage, which is explained by a disconnected and incomplete circuit. This theory of a poorly connected circuit is supported by the scattered current data, pictured right.

DISCUSSION:

Applying voltage to either pNIPAM or p(NIPAM-*b*-FMMA) solutions changed the polymer aggregation behavior in response to temperature. With both materials, when an applied voltage was present, the polymers’ volume size remained high even when the solutions were cooled below their LCST. This was an unexpected result, as the trials with no voltage showed the polymers returning to their original sizes. These experiments allow us to conclude that for our temperature range, and with an applied voltage, polymer aggregation is not fully reversible, regardless of electroactive monomers added to the pNIPAM.

Another interesting result can be seen upon further inspection of **Figure 9** and p(NIPAM-*b*-FMMA) LCST changes. Without voltage, the maximum volume size is around 1000 nm, and aggregation is reversible. However, with applied voltage, the stable agglomeration is approximately 100 nm and non-reversible. This would indicate a new stable agglomeration state formed with applied voltage compared to the absence of voltage.

The current response from the constant applied voltage may also provide insights into aggregation response. Because the files are timestamped, the current with relative changes in temperature can be matched, although there is not equal spacing between the temperature and time due to the automatic optimization at each step based on the scattering intensity and attenuation settings in the DLS. Our data indicate the current increases with temperature, then starts decreasing immediately after the LCST is passed. Clear trends are associated with the approximate time of aggregation, showing a low resistance in solution, resulting in less current. During cooling, current increases, but does not increase as rapidly as it did during heating. The current data adds information and possible insight into the polymer behavior.

The methodology of applying a voltage to dynamic light scattering particle size distribution measurements was successful. Differences in polymer aggregation behavior associated with particle size distribution measurements were observed when an applied voltage was present during a temperature ramp trend compared to the no-voltage case. The observed behavior was only present when both an applied voltage and temperature ramp were used.

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DISCLOSURES:

The authors declare no conflicts of interest.

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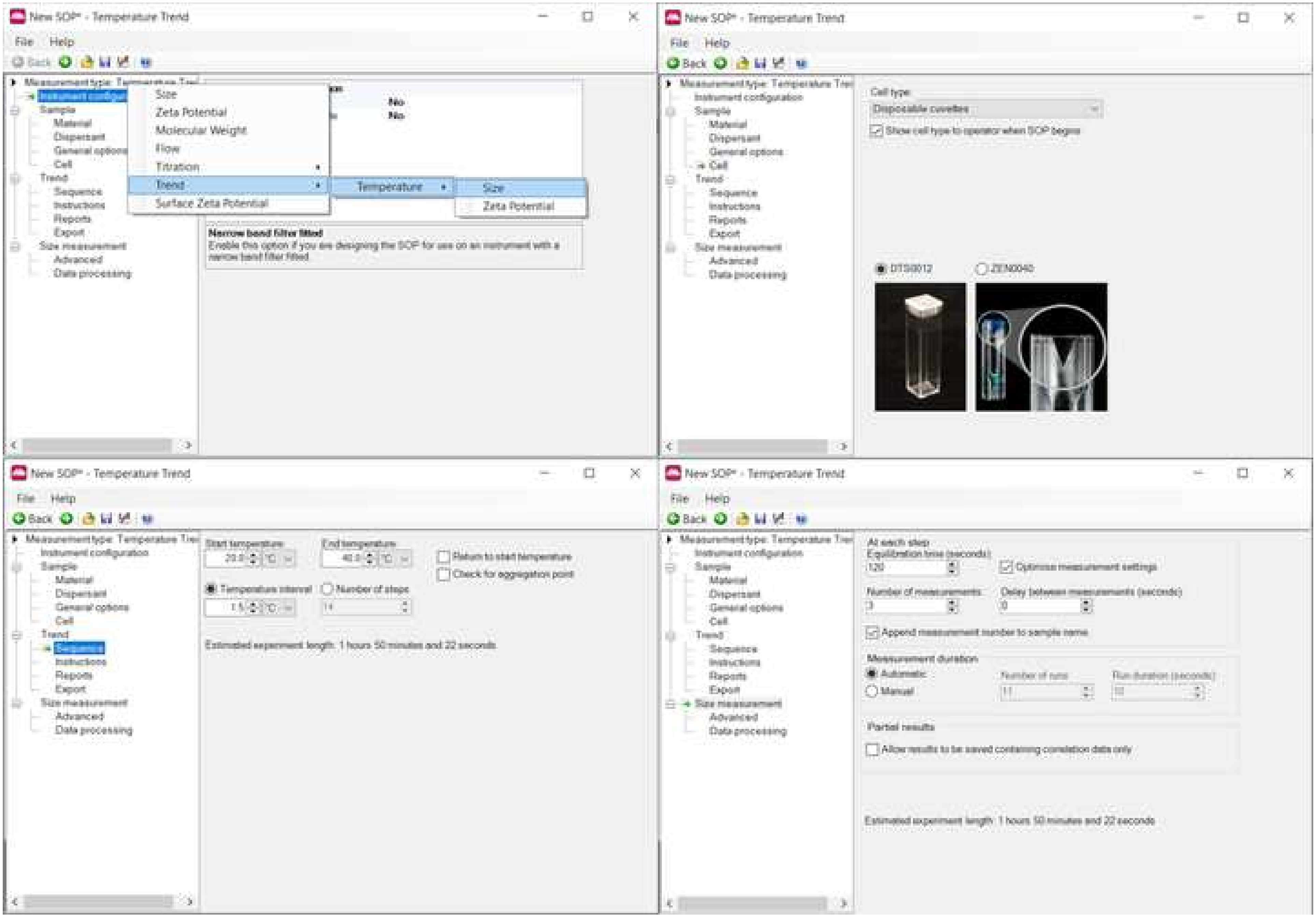
Figure 2



No
No

Narrow band filter fitted
Enable this option if you are designing the SOP for use on an instrument with a narrow band filter fitted

Cell type
Disposable cuvettes
☑ Show cell type to operator when SOP begins
● DTS0012 ○ ZEN0040

Start temperature 20.0 °C
End temperature 40.0 °C
☐ Return to start temperature
☐ Check for aggregation point
● Temperature interval 1.5 °C
○ Number of steps 14

Estimated experiment length: 1 hours 50 minutes and 22 seconds

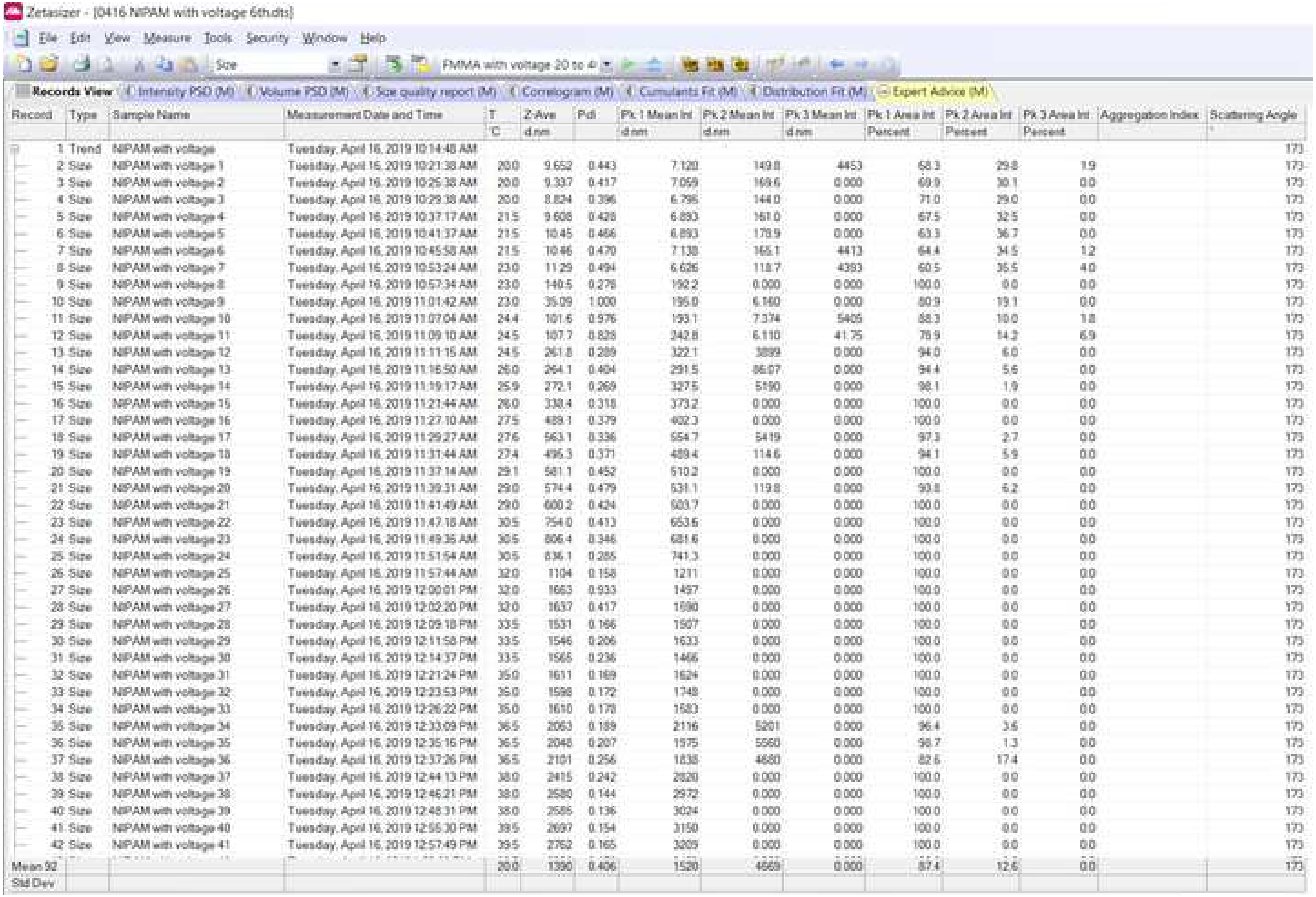
At each step
Equilibration time (seconds) 120
☑ Optimise measurement settings
Number of measurements 3
Delay between measurements (seconds) 0
☑ Append measurement number to sample name

Measurement duration
● Automatic
○ Manual
Number of runs 11
Run duration (seconds) 10

Partial results
☐ Allow results to be saved containing correlation data only

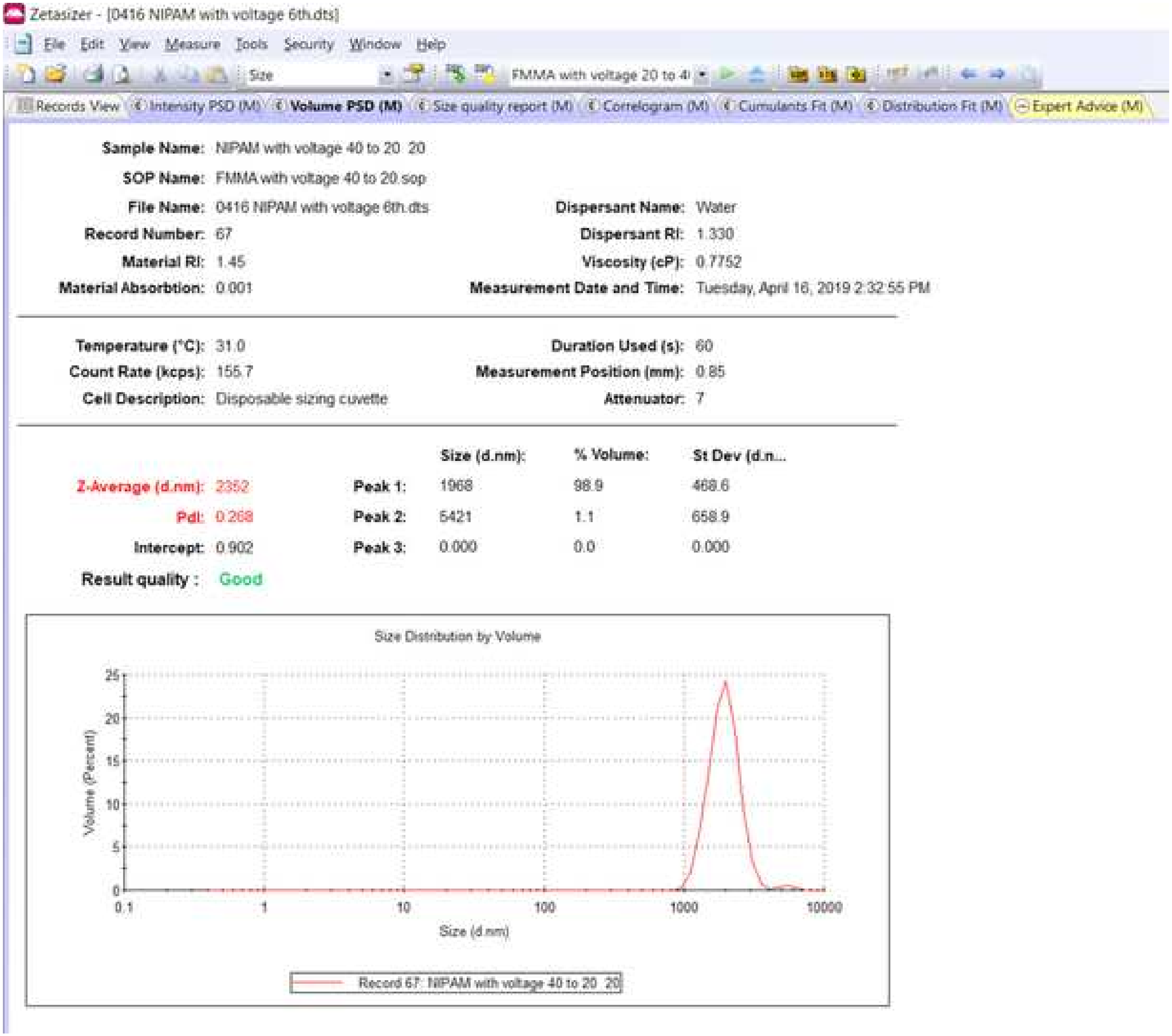
Estimated experiment length: 1 hours 50 minutes and 22 seconds

Figure 3



Record	Type	Sample Name	Measurement Date and Time	T	Z-Ave	PdI	Pk 1 Mean Int	Pk 2 Mean Int	Pk 3 Mean Int	Pk 1 Area Int	Pk 2 Area Int	Pk 3 Area Int	Aggregation Index	Scattering Angle
				°C	d.nm		d.nm	d.nm	d.nm	Percent	Percent	Percent		°
1	Trend	NIPAM with voltage	Tuesday, April 16, 2019 10:14:48 AM											173
2	Size	NIPAM with voltage 1	Tuesday, April 16, 2019 10:21:38 AM	20.0	9.652	0.443	7.120	149.0	4453	68.3	29.8	1.9		173
3	Size	NIPAM with voltage 2	Tuesday, April 16, 2019 10:25:38 AM	20.0	9.337	0.417	7.059	169.6	0.000	69.9	30.1	0.0		173
4	Size	NIPAM with voltage 3	Tuesday, April 16, 2019 10:29:38 AM	20.0	8.824	0.396	6.796	144.0	0.000	71.0	29.0	0.0		173
5	Size	NIPAM with voltage 4	Tuesday, April 16, 2019 10:37:17 AM	21.5	9.608	0.428	6.893	161.0	0.000	67.5	32.5	0.0		173
6	Size	NIPAM with voltage 5	Tuesday, April 16, 2019 10:41:37 AM	21.5	10.45	0.466	6.893	178.9	0.000	63.3	36.7	0.0		173
7	Size	NIPAM with voltage 6	Tuesday, April 16, 2019 10:45:58 AM	21.5	10.46	0.470	7.138	165.1	4413	64.4	34.5	1.2		173
8	Size	NIPAM with voltage 7	Tuesday, April 16, 2019 10:53:24 AM	23.0	11.29	0.494	6.626	118.7	4393	60.5	35.5	4.0		173
9	Size	NIPAM with voltage 8	Tuesday, April 16, 2019 10:57:34 AM	23.0	140.5	0.278	192.2	0.000	0.000	100.0	0.0	0.0		173
10	Size	NIPAM with voltage 9	Tuesday, April 16, 2019 11:01:42 AM	23.0	35.09	1.000	195.0	6.160	0.000	80.9	19.1	0.0		173
11	Size	NIPAM with voltage 10	Tuesday, April 16, 2019 11:07:04 AM	24.4	101.6	0.976	193.1	7.374	5405	88.3	10.0	1.8		173
12	Size	NIPAM with voltage 11	Tuesday, April 16, 2019 11:09:10 AM	24.5	107.7	0.828	242.8	6.110	41.75	78.9	14.2	6.9		173
13	Size	NIPAM with voltage 12	Tuesday, April 16, 2019 11:11:15 AM	24.5	261.8	0.289	322.1	3899	0.000	94.0	6.0	0.0		173
14	Size	NIPAM with voltage 13	Tuesday, April 16, 2019 11:16:50 AM	26.0	264.1	0.404	291.5	86.07	0.000	94.4	5.6	0.0		173
15	Size	NIPAM with voltage 14	Tuesday, April 16, 2019 11:19:17 AM	25.9	272.1	0.269	327.5	5190	0.000	98.1	1.9	0.0		173
16	Size	NIPAM with voltage 15	Tuesday, April 16, 2019 11:21:44 AM	26.0	338.4	0.318	373.2	0.000	0.000	100.0	0.0	0.0		173
17	Size	NIPAM with voltage 16	Tuesday, April 16, 2019 11:27:10 AM	27.5	489.1	0.379	402.3	0.000	0.000	100.0	0.0	0.0		173
18	Size	NIPAM with voltage 17	Tuesday, April 16, 2019 11:29:27 AM	27.6	563.1	0.336	554.7	5419	0.000	97.3	2.7	0.0		173
19	Size	NIPAM with voltage 18	Tuesday, April 16, 2019 11:31:44 AM	27.4	496.3	0.371	489.4	114.6	0.000	94.1	5.9	0.0		173
20	Size	NIPAM with voltage 19	Tuesday, April 16, 2019 11:37:14 AM	29.1	581.1	0.452	510.2	0.000	0.000	100.0	0.0	0.0		173
21	Size	NIPAM with voltage 20	Tuesday, April 16, 2019 11:39:31 AM	29.0	574.4	0.479	531.1	119.8	0.000	93.8	6.2	0.0		173
22	Size	NIPAM with voltage 21	Tuesday, April 16, 2019 11:41:49 AM	29.0	600.2	0.424	503.7	0.000	0.000	100.0	0.0	0.0		173
23	Size	NIPAM with voltage 22	Tuesday, April 16, 2019 11:47:18 AM	30.5	754.0	0.413	653.6	0.000	0.000	100.0	0.0	0.0		173
24	Size	NIPAM with voltage 23	Tuesday, April 16, 2019 11:49:36 AM	30.5	806.4	0.346	681.6	0.000	0.000	100.0	0.0	0.0		173
25	Size	NIPAM with voltage 24	Tuesday, April 16, 2019 11:51:54 AM	30.5	836.1	0.285	741.3	0.000	0.000	100.0	0.0	0.0		173
26	Size	NIPAM with voltage 25	Tuesday, April 16, 2019 11:57:44 AM	32.0	1104	0.158	1211	0.000	0.000	100.0	0.0	0.0		173
27	Size	NIPAM with voltage 26	Tuesday, April 16, 2019 12:00:01 PM	32.0	1663	0.933	1497	0.000	0.000	100.0	0.0	0.0		173
28	Size	NIPAM with voltage 27	Tuesday, April 16, 2019 12:02:20 PM	32.0	1637	0.417	1590	0.000	0.000	100.0	0.0	0.0		173
29	Size	NIPAM with voltage 28	Tuesday, April 16, 2019 12:09:18 PM	33.5	1531	0.166	1507	0.000	0.000	100.0	0.0	0.0		173
30	Size	NIPAM with voltage 29	Tuesday, April 16, 2019 12:11:58 PM	33.5	1546	0.206	1633	0.000	0.000	100.0	0.0	0.0		173
31	Size	NIPAM with voltage 30	Tuesday, April 16, 2019 12:14:37 PM	33.5	1565	0.236	1466	0.000	0.000	100.0	0.0	0.0		173
32	Size	NIPAM with voltage 31	Tuesday, April 16, 2019 12:21:24 PM	35.0	1611	0.169	1624	0.000	0.000	100.0	0.0	0.0		173
33	Size	NIPAM with voltage 32	Tuesday, April 16, 2019 12:23:53 PM	35.0	1598	0.172	1748	0.000	0.000	100.0	0.0	0.0		173
34	Size	NIPAM with voltage 33	Tuesday, April 16, 2019 12:26:22 PM	35.0	1610	0.178	1583	0.000	0.000	100.0	0.0	0.0		173
35	Size	NIPAM with voltage 34	Tuesday, April 16, 2019 12:33:09 PM	36.5	2063	0.189	2116	5201	0.000	96.4	3.6	0.0		173
36	Size	NIPAM with voltage 35	Tuesday, April 16, 2019 12:35:16 PM	36.5	2048	0.207	1975	5560	0.000	98.7	1.3	0.0		173
37	Size	NIPAM with voltage 36	Tuesday, April 16, 2019 12:37:26 PM	36.5	2101	0.256	1838	4680	0.000	82.6	17.4	0.0		173
38	Size	NIPAM with voltage 37	Tuesday, April 16, 2019 12:44:13 PM	38.0	2415	0.242	2820	0.000	0.000	100.0	0.0	0.0		173
39	Size	NIPAM with voltage 38	Tuesday, April 16, 2019 12:46:21 PM	38.0	2580	0.144	2972	0.000	0.000	100.0	0.0	0.0		173
40	Size	NIPAM with voltage 39	Tuesday, April 16, 2019 12:48:31 PM	38.0	2585	0.136	3024	0.000	0.000	100.0	0.0	0.0		173
41	Size	NIPAM with voltage 40	Tuesday, April 16, 2019 12:55:30 PM	39.5	2697	0.154	3150	0.000	0.000	100.0	0.0	0.0		173
42	Size	NIPAM with voltage 41	Tuesday, April 16, 2019 12:57:49 PM	39.5	2762	0.165	3209	0.000	0.000	100.0	0.0	0.0		173
Mean 92				20.0	1390	0.406	1520	4669	0.000	87.4	12.6	0.0		173
Std Dev														

Figure 4



Sample Name: NIPAM with voltage 40 to 20 20
SOP Name: FMMA with voltage 40 to 20.sop
File Name: 0416 NIPAM with voltage 6th.dts
Record Number: 67
Material RI: 1.45
Material Absorbtion: 0.001
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.7752
Measurement Date and Time: Tuesday, April 16, 2019 2:32:55 PM

Temperature (°C): 31.0
Count Rate (kcps): 155.7
Cell Description: Disposable sizing cuvette
Duration Used (s): 60
Measurement Position (mm): 0.85
Attenuator: 7

Z-Average (d.nm): 2352
PdI: 0.268
Intercept: 0.902
Result quality : Good

	Size (d.nm):	% Volume:	St Dev (d.n...
Peak 1:	1968	98.9	468.6
Peak 2:	5421	1.1	658.9
Peak 3:	0.000	0.0	0.000

Figure 5

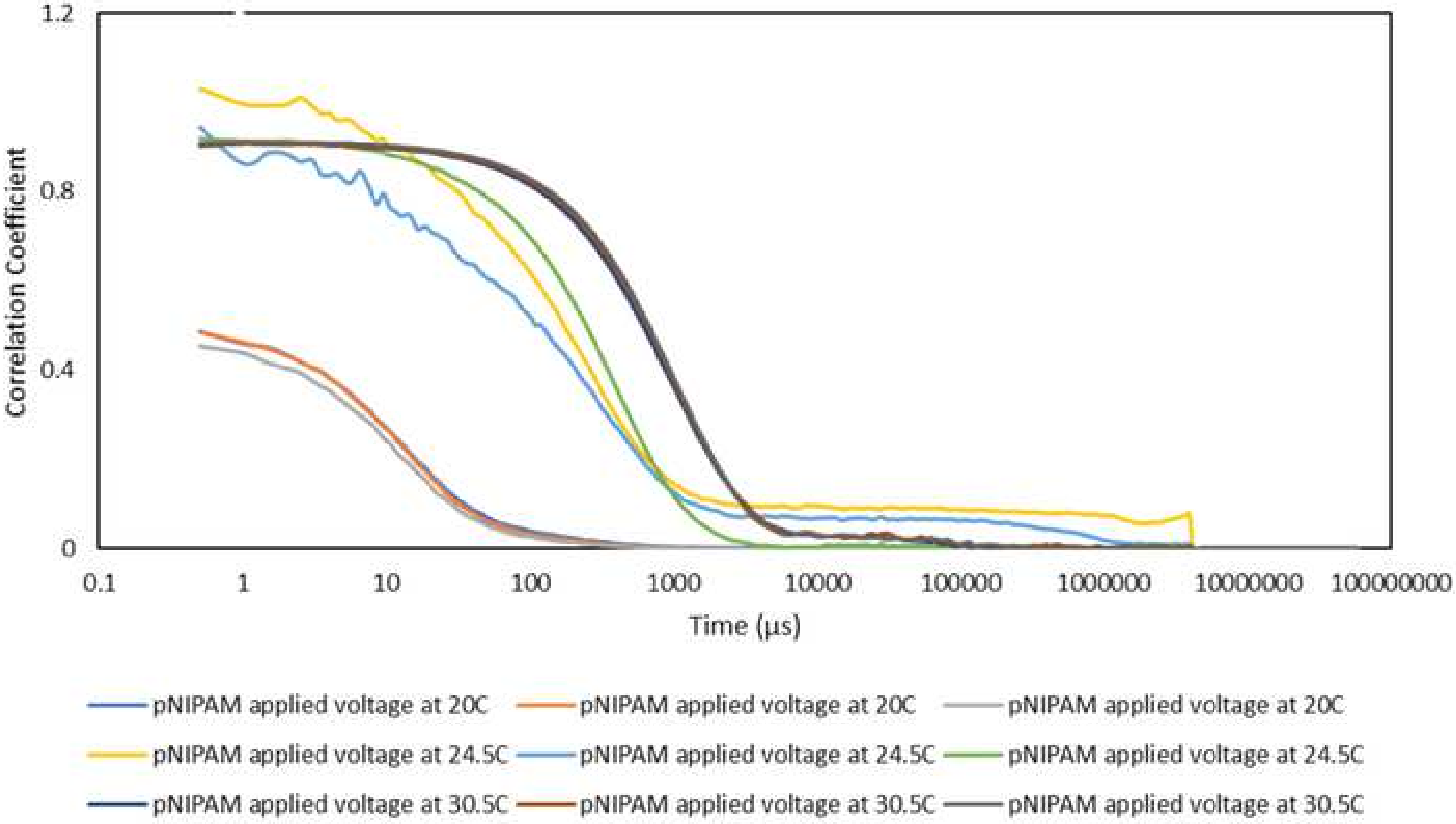
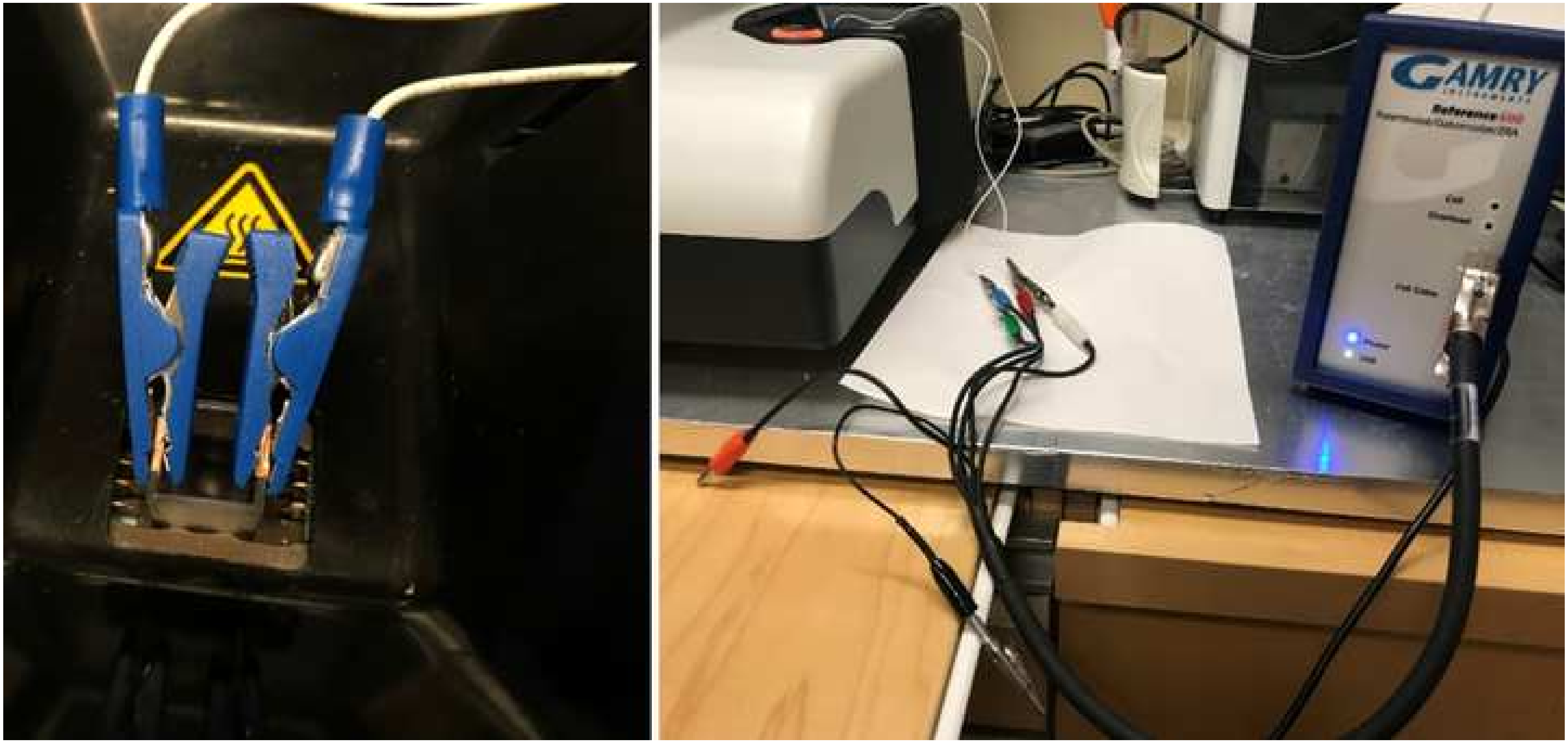
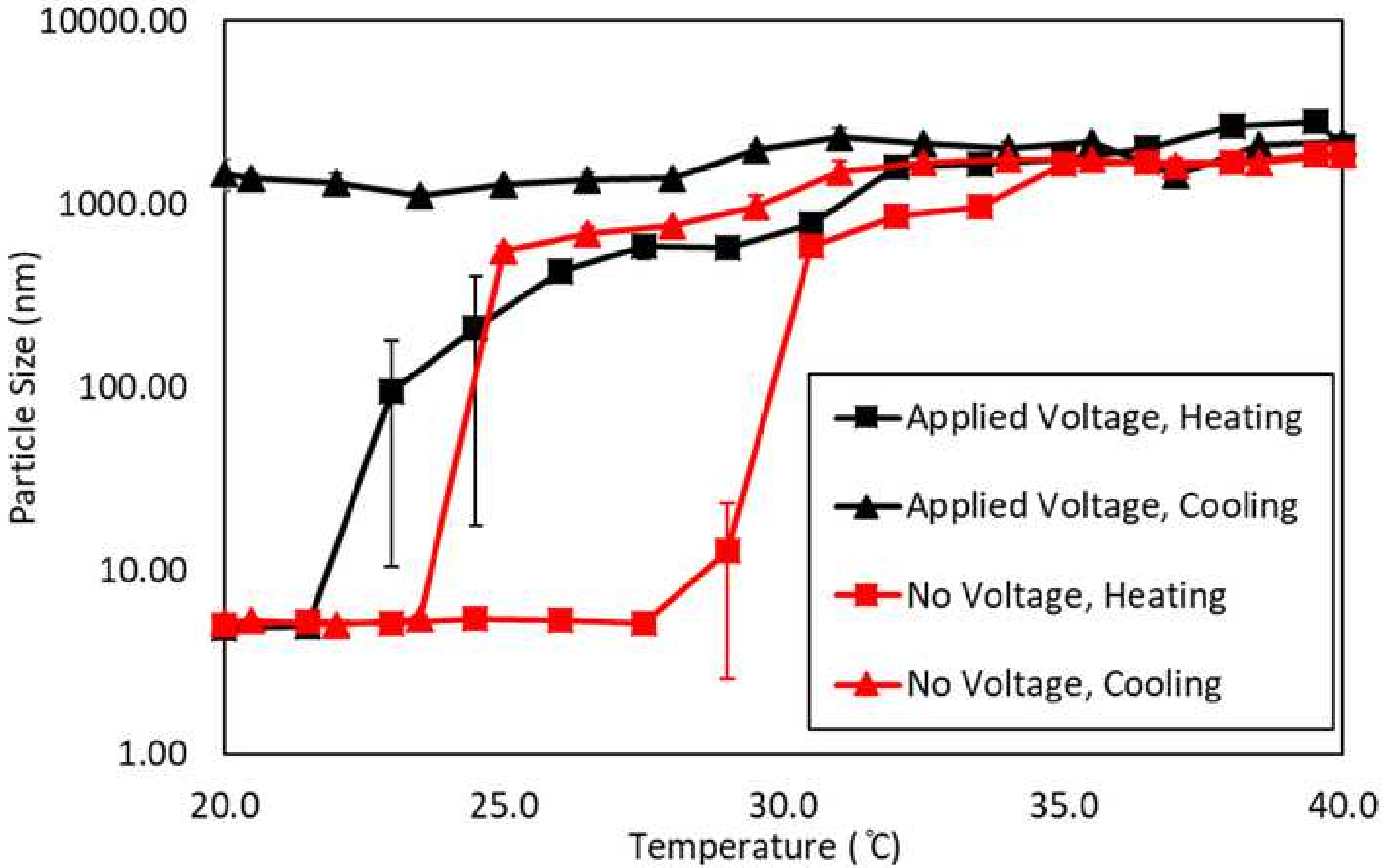
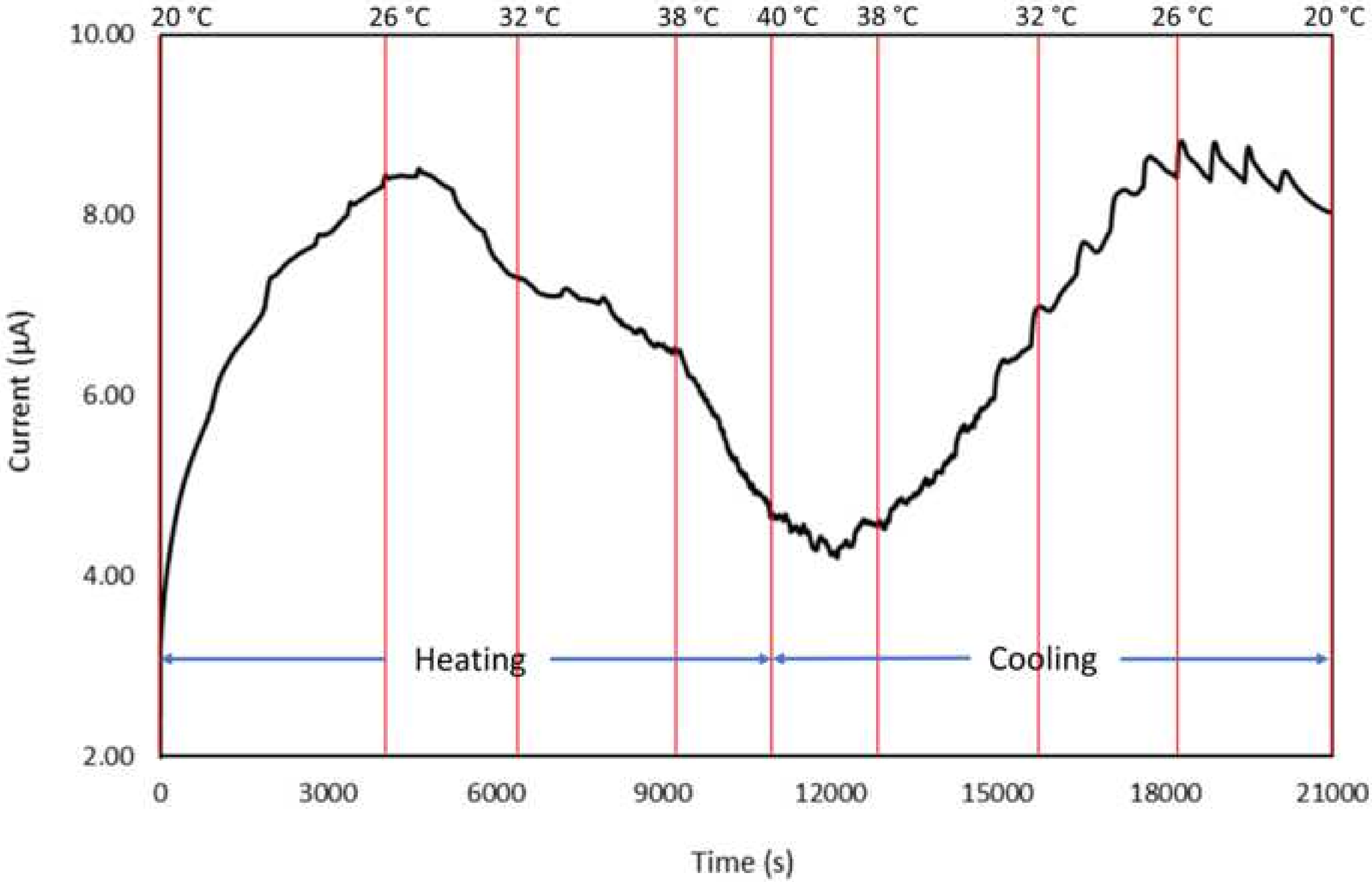
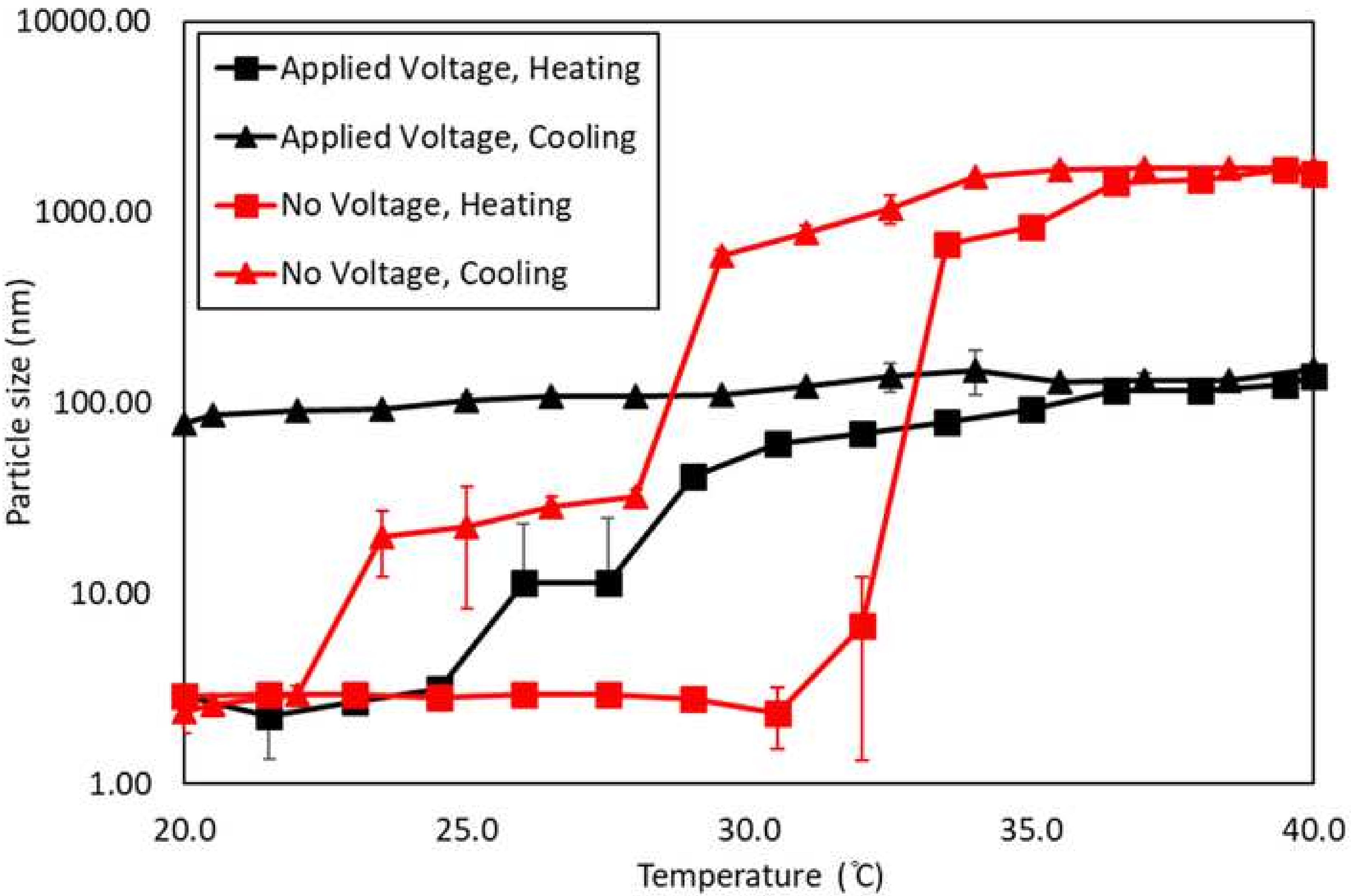

1.2
0.8
0.4
0
Correlation Coefficient
0.1
1
10
100
1000
10000
100000
1000000
10000000
100000000
Time (μs)
pNIPAM applied voltage at 20C
pNIPAM applied voltage at 20C
pNIPAM applied voltage at 20C
pNIPAM applied voltage at 24.5C
pNIPAM applied voltage at 24.5C
pNIPAM applied voltage at 24.5C
pNIPAM applied voltage at 30.5C
pNIPAM applied voltage at 30.5C
pNIPAM applied voltage at 30.5C

Figure 6




10000.00
1000.00
100.00
10.00
1.00
Particle Size (nm)
20.0
25.0
30.0
35.0
40.0
Temperature (℃)
Applied Voltage, Heating
Applied Voltage, Cooling
No Voltage, Heating
No Voltage, Cooling


20 °C
26 °C
32 °C
38 °C
40 °C
38 °C
32 °C
26 °C
20 °C
10.00
8.00
6.00
4.00
2.00
Current (µA)
Heating
Cooling
0
3000
6000
9000
12000
15000
18000
21000
Time (s)


10000.00
1000.00
100.00
10.00
1.00
Particle size (nm)
20.0
25.0
30.0
35.0
40.0
Temperature (℃)
Applied Voltage, Heating
Applied Voltage, Cooling
No Voltage, Heating
No Voltage, Cooling

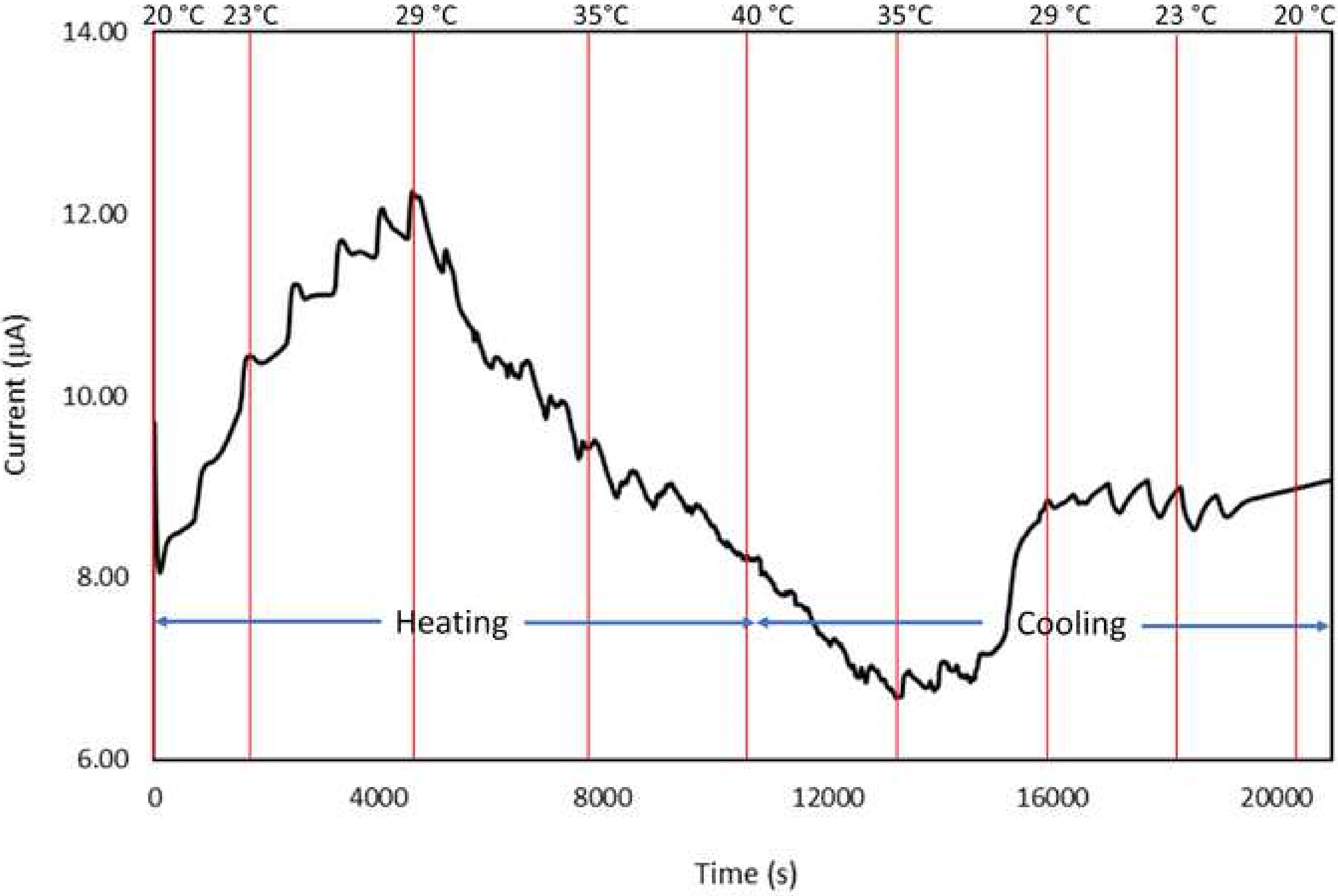
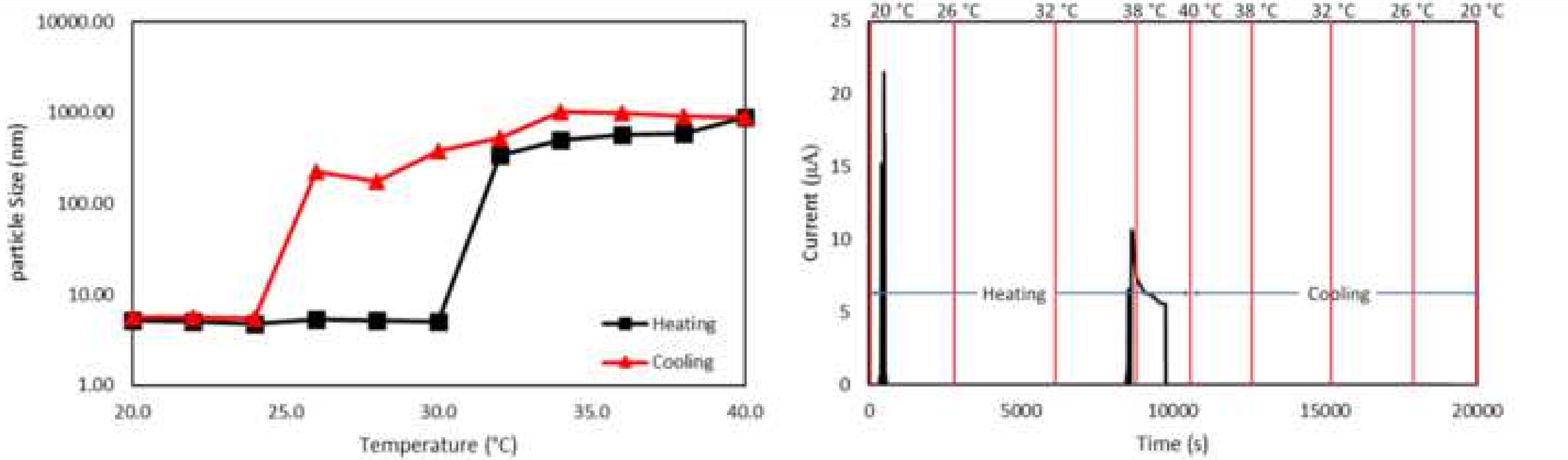

20 °C
23°C
29 °C
35°C
40 °C
35°C
29 °C
23 °C
20 °C
14.00
12.00
10.00
8.00
6.00
Current (µA)
Heating
Cooling
0
4000
8000
12000
16000
20000
Time (s)

Figure 11


10000.00
1000.00
100.00
10.00
1.00
particle Size (nm)
20.0
25.0
30.0
35.0
40.0
Temperature (°C)
Heating
Cooling
20 °C
26 °C
32 °C
38 °C
40 °C
38 °C
32 °C
26 °C
20 °C
25
20
15
10
5
0
Current (µA)
Heating
Cooling
0
5000
10000
15000
20000
Time (s)

Name of Material/Equipment	Company	Catalog Number
N -Isopropylacrylamide	Tokyo Chemical Industry CO.,LTD	I0401-500G
1,4-Dioxane	Alfa Aesar	39118
2,2′-Azobis(2-methylpropionitrile)	SIGMA-ALDRICH	441090-100G
Cuvette	Malvern	DTS0012
Dynamic Light Scattering	Malvern	Zetasizer NanoZS
Ferrocenylmethyl methacrylate	ASTATECH	FD13136-1G
Phthalimidomethyl butyl trithiocarbonate	SIGMA-ALDRICH	777072-1G
Potentiostat	Gamry	Reference 600

Comments/Description

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Author(s):	Tianyu Ren, Emma J. Roberge, John R. Csoros, W. Rudolph Seitz, Eva Rose M. Balog, Jeffrey Mark Halpern

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Dear Editor,

We want to thank the reviewers for taking the time to provide an in-depth response to our manuscript. In this latest submission, we addressed all of the concerns. We provide additional information below in red. In addition, we also addressed all the editorial comments and edits requested. Please accept this draft for publication.

Reviewers' comments:
Reviewer #1:
Manuscript Summary:
This manuscript described an interesting experiment utilizing DLS to monitor aggregation process in presence of an electric field at various temperature.

Thank you for your summary and review.

Reviewer #2:
Manuscript Summary:
This manuscript describes an experimental procedure to carry out a DLS measurement under applied potential conditions in solution. The protocols are well written. The figures are clear. The instruments used in this work are commercially available. Because of these reasons, I think the readers will be able to carry out the measurements using the protocols provided.

Thank you for this summary and review.

Minor Concerns:
The experiment presented is based on a two-electrode configuration, would it be easier to control the applied potential using a three-electrode configuration?

A three-electrode configuration, often encouraged for traditional electrochemical cells, does allow for a better interpretation of the system. In this configuration, the potential isn't necessarily better controlled, but the current response would be easier to interpret. Yet, we predict no electrochemical measurements in the system. The same metal is chosen for the anode and cathode (copper) to try and create an even electric field distribution over the entire 1 cm cuvette distance. If three-electrode configuration, or even a proper reference electrode is use, the distribution would not be uniform, and favored towards one electrode. More information can be requested on this subject if needed.

Reviewer #3:
Manuscript Summary:
This work shows results from looking at the effects on the hydrodynamic size as a result of a constant applied electric field. The authors modified a commercial DLS system to allow the application of an electric field within the sample cuvette. The PNIPAM based sample system exhibits a temperature specific aggregation behavior which they show is altered in the presence of the applied field. The steps to repeat the experiments are listed in detail. In addition to cited interest, this work may have potential applications in the development of battery materials.

Thank you for this summary and review.

Major Concerns:
None

Minor Concerns:
Line 84: you use the abbreviation "RAFT" and have never before introduced it. Please state the full description reversible addition-fragmentation chain-transfer polymerization to introduce the term.
Line 195: might be beneficial to add the Zetasizer software version, e.g. v 7.12

Thank you for the above suggestions, we have added these valuable suggestions.

Line 199: the refractive index of PNIPAM may be different from protein, see for example https://refractiveindex.info/?shelf=organic&book=poly(N-isopropylacrylamide)&page=Brasse - this will not have a substantial affect on your conclusions, but may alter the size values

You are correct that the refractive index could alter the size values but should not substantially effect your conclusions. While the pNIPAM refractive index is known, as cited in the link above as 1.5031, the refractive answered can also change as a function of temperature. Because the refractive answered can be looked up or measured, and entered manually, we added the language "If exact values for refractive index are desired for more accurate calculation of volume distribution, the experimenter should determine the refractive index of their sample experimentally."

Line 271:you indicated using the "volume average size" . Please clarify - is this the z-average size (which is an intensity average) or is this the mean size of the main volume distribution peak?
Line 300: instead of "double-clicking" just "selecting"

Thank you for the above suggestions, we have added these valuable suggestions.

Line 303/304: possibly append "The Zetasizer software indicates good size result quality (as shown in Figure 5)."

For dissolved pNIPAM, the Zetasizer software does not always assign a "good size result quality" for low signal-to-noise measurements, even when correlation graphs are reproducible and interpretable. We do not recommend relying on this output for assessment of measurement quality. We prefer to recommend interpretation of the correlation graphs as a best practice.

Line 304: suggest to add: The correlation functions of three repeated runs at three different temperatures are plotted.

This has been added

Line 306/7: The colors red and black appear to be opposite in the figure caption of what is used in the graph legend. Please triple check this!

Thank you for discovering this. As you indicated, we originally had different colors. The latest draft has been corrected.

Line 313: suggested addition, after modification of Fig 8: x-axis indicates time since start of experiment, as well as temperature at various time points.

Great suggestion. We updated the figures and added the line to the caption

Line 315: figure caption says "black lines - no voltage", whereas graph legend shows black data applied voltage. Please triple check this!

Thank you for discovering this. The latest draft has been corrected.

Line 325: suggested addition, after modification of Fig 10: x-axis indicates time since start of experiment, as well as temperature at various time points.

Great suggestion. We updated the figures and added the line to the caption

Line 332: suggest to extend the sentence "...good quality, as also confirmed by the Zetasizer software result quality indicator."

For dissolved pNIPAM, the Zetasizer software does not always assign a “good size result quality” for low signal-to-noise measurements, even when correlation graphs are reproducible and interpretable. We do not recommend relying on this output for assessment of measurement quality. We prefer to recommend interpretation of the correlation graphs as a best practice.

Line 336: "black lines" should be "red lines". Please triple check this!
Line 339: "red lines" should be "black lines". Please triple check this!
Line 342: "red lines" should be "black lines". Please triple check this!
Line 347: "black lines" should be "red lines". Please triple check this!
Line 349: "red lines" should be "black lines". Please triple check this!
Line 353: "red lines" should be "black lines". Please triple check this!
Figure 7: You appear to have changed the color scheme at some time in your manuscript preparation. Please triple check that colors in text and in graph are consistent!

Thank you for discovering this. As you indicated, we originally had different colors. The latest draft has been corrected.

Figure 8: I think the readability of this figure could be improved significantly by adding some temperature points to the time scale, i.e. 20, 25, 30 35 40 35, 30, 25 20 maybe above the x-axis

Great suggestion. We updated the figures and added the line to the caption

Figure 9: You appear to have changed the color scheme at some time in your manuscript preparation. Please triple check that colors in text and in graph are consistent!

Thank you for discovering this. As you indicated, we originally had different colors. The latest draft has been corrected.

Figure 10: I think the readability of this figure could be improved significantly by adding some temperature points to the time scale, i.e. 20, 25, 30 35 40 35, 30, 25 20 maybe above the x-axis
Figure 11: Figure 8: I think the readability of this figure could be improved significantly by adding some temperature points to the time scale, i.e. 20, 25, 30 35 40 35, 30, 25 20 maybe above the x-axis of the graph on the right side.

Great suggestion. We updated the figures and added the line to the caption