Developing a PT Standard using Polymer hydrogels

Zachary Vaz
Mentors: Dr. Susana Teixeira & Juscelino Leão
8/21/2024

Motivation for the Project

As a user facility, NCNR is always looking for ways to make SANS (pressure in particular) more accessible and user friendly.

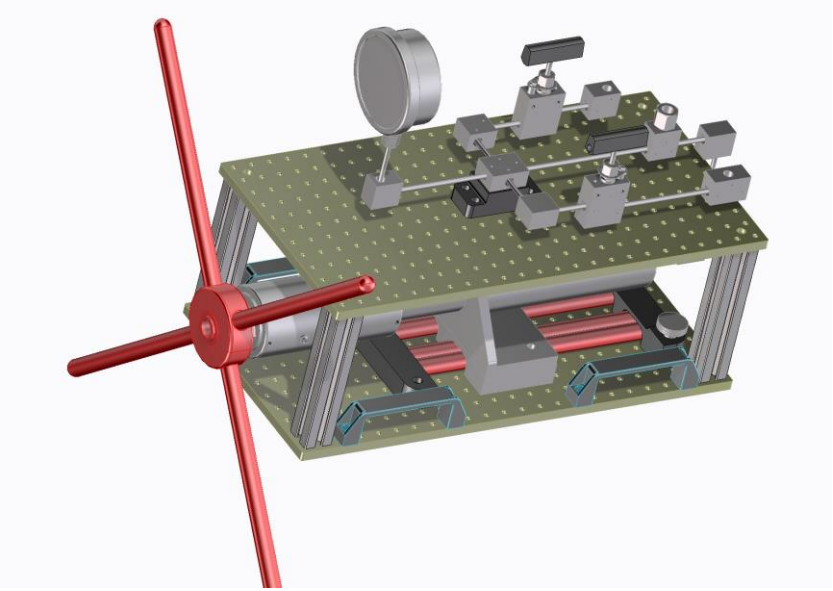
Looking for ways to optimize beamtime for HP SANS.

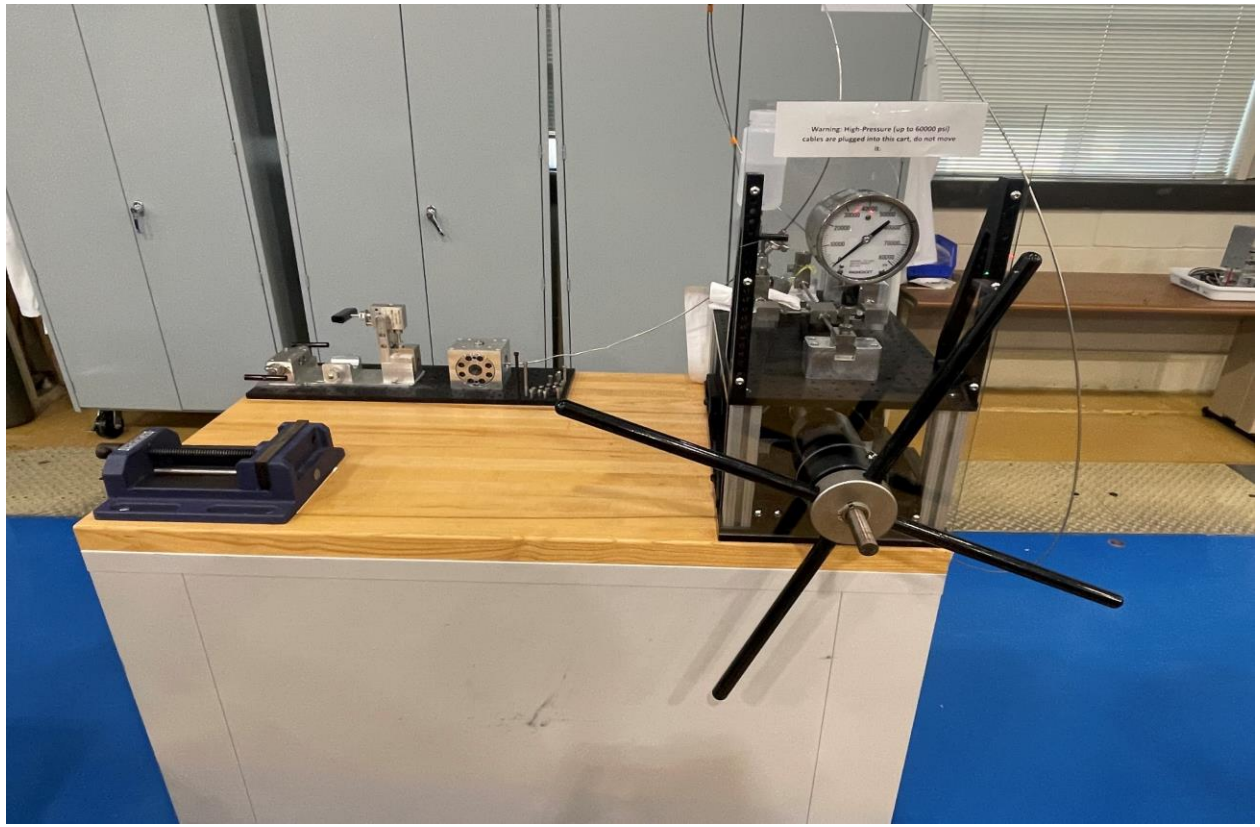
- Reduce *in-situ* measurements to only essential runs by making *ex-situ* and/or benchtop measurements more accessible.

Pre-incubation to probe irreversible changes before scheduling a neutron experiment.

High Pressure at the NCNR

Portable manual system capable of pressurizing samples up to ~60,000 psi (4.1 kbar).





Standards – Identifying a Candidate Material

There are currently no available pressure standards for Bio SANS instrument calibration or development.

We're at NIST. Ask yourself: What makes an ideal reference / standard material?

- Responsive to pressure, temperature – both *in-* and ***ex-situ*** *in a controllable manner.*
- Stable on the order of ~weeks
- Readily available (affordable)
- Reliability + Robust
- Easy / Time Efficient to verify

Poly(N-isopropylacrylamide) Hydrogels

Candidate for standardization

pNIPAM has both hydrophilic (-CONH) and hydrophobic (-$CH(CH_3)_2$) groups in a single monomer unit.

There is a coil-to-globule conformation change with an accompanying volume phase transition (VPT) just below human body temp (~32°C).

Termed the “lower critical solution temperature” (LCST).

Below LCST, the N-isopropyl groups are hydrated. Above, the structure collapses and NIPAM chains aggregate via hydrophobic interactions.

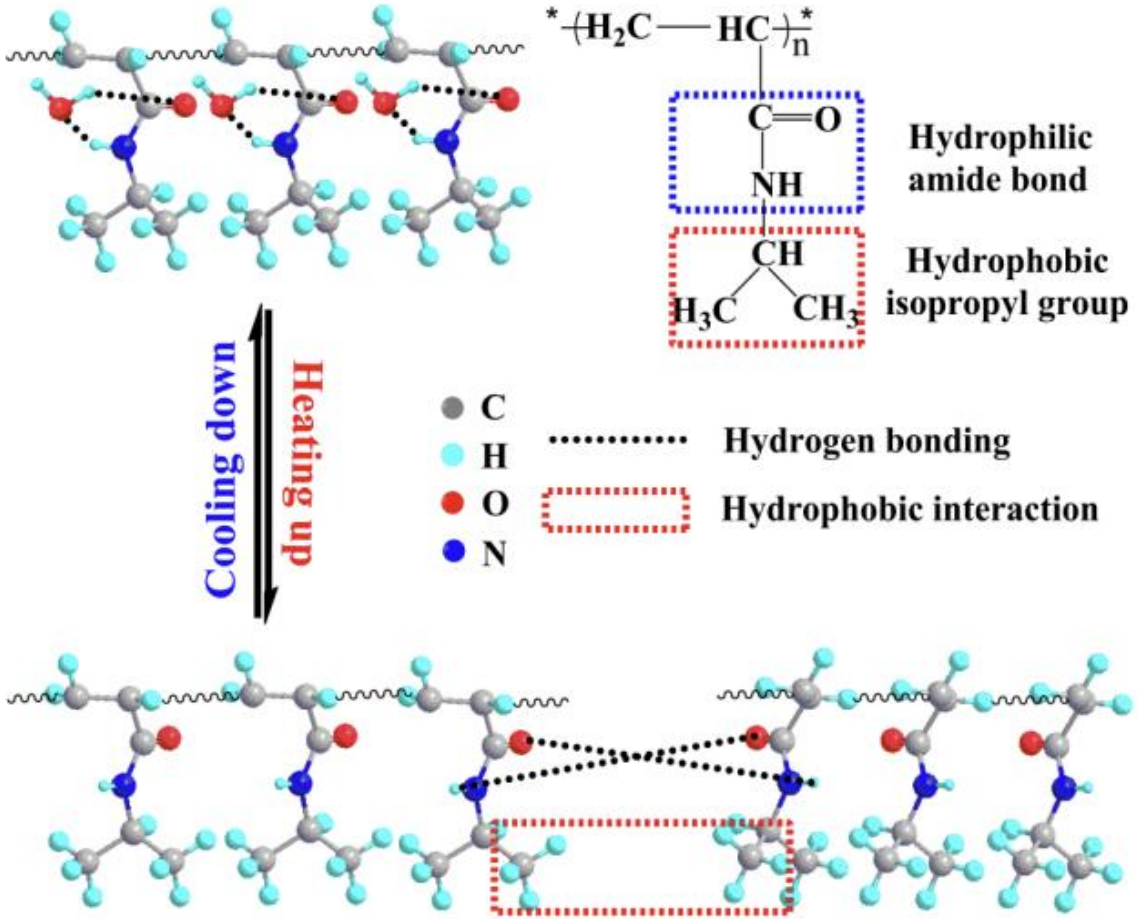


Figure 1. Molecular structure and thermoresponsive mechanism of PNIPAAm.

Image from:
Yang et al. (2020). ***Polymers*** 12, 389; doi:10.3390/polym12020389

Poly(N-isopropylacrylamide) Hydrogels

Applications

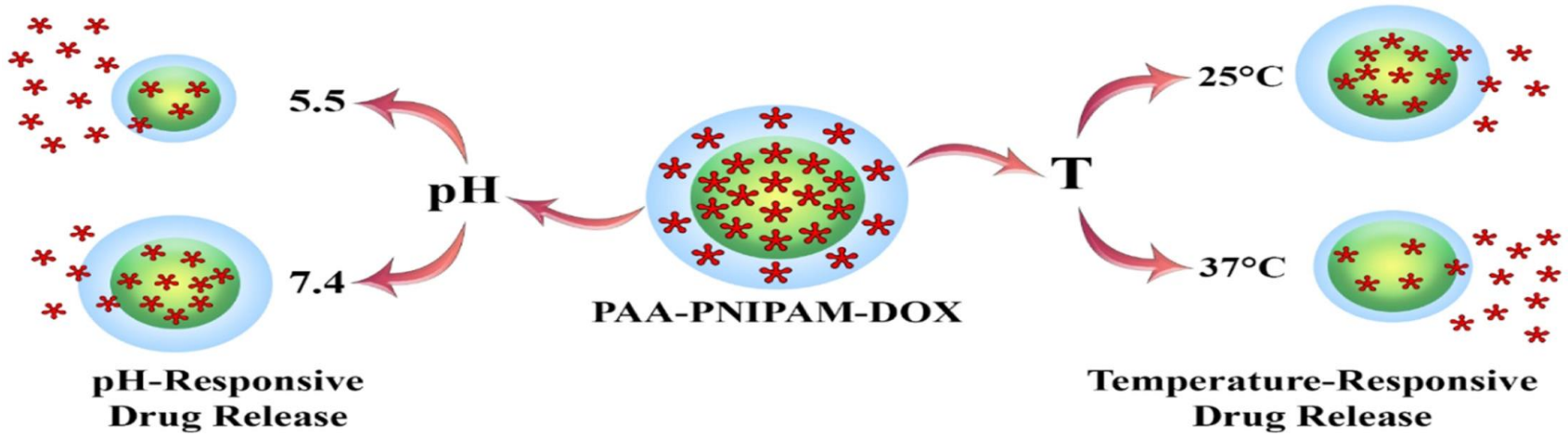
LCTS just below human body temp exploited in applications such as:

-Drug Delivery

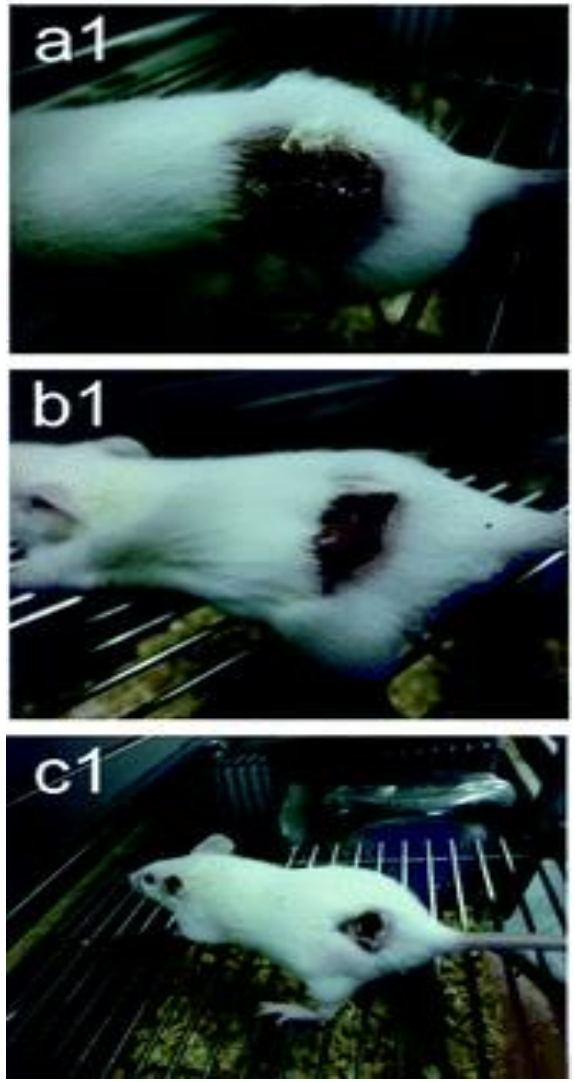
-Tissue Engineering

-Wound Dressing (“artificial skin”)

PNIPAM is a well studied and characterized compound, but much work remains to be done.



Journal of Pharmaceutical Sciences. 2023; 112(7): 1957–1966. Temperature and pH-responsive PNIPAM@PAA Nanospheres with a Core-Shell Structure for Controlled Release of Doxorubicin in Breast Cancer Treatment.



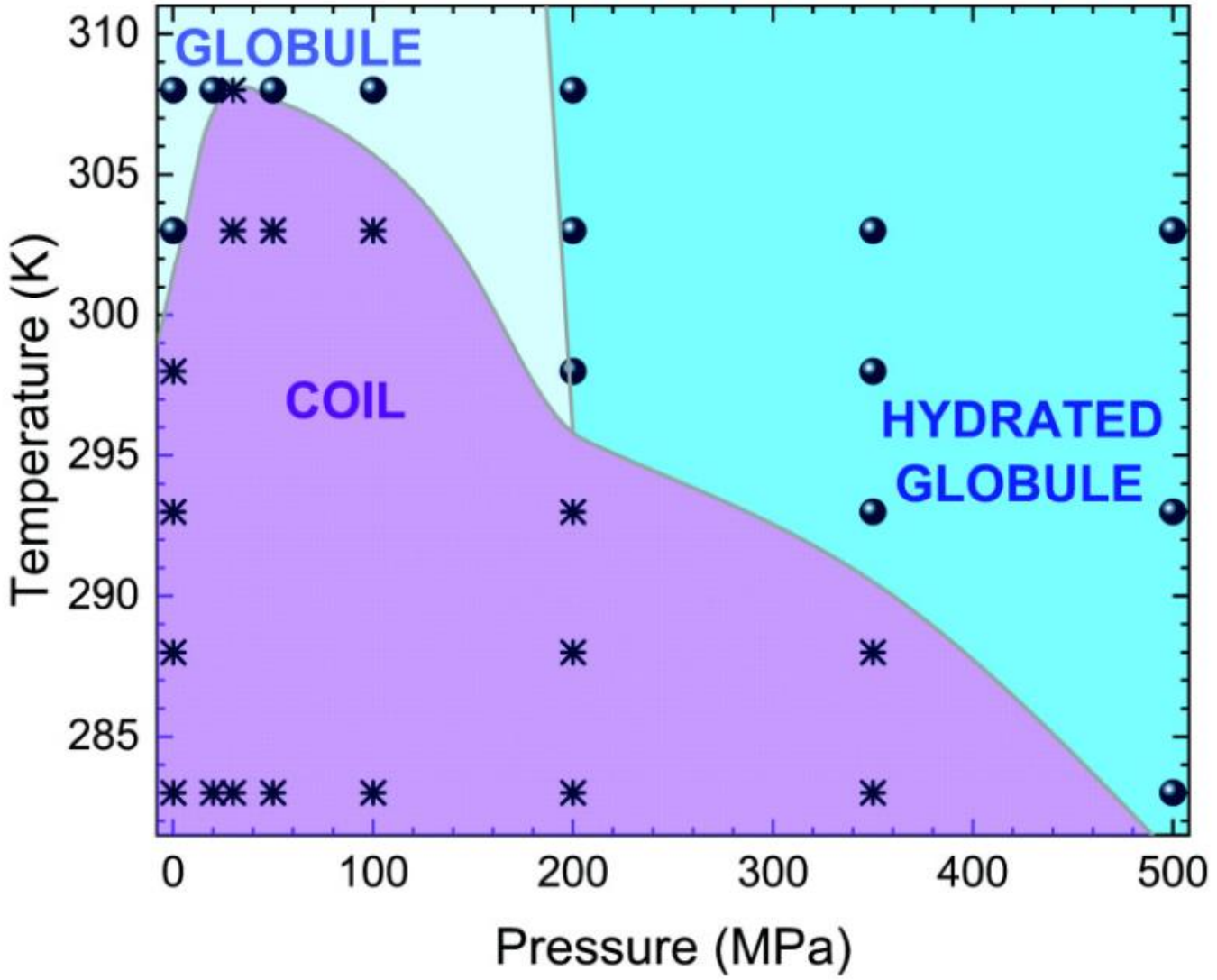
Royal Soc of Chem. 2015; 5: 25870–25876. Novel conjugated Ag@PNIPAM nanocomposites for an effective antibacterial wound dressing.

Poly(N-isopropylacrylamide) Hydrogels

Lack of experimental, but…

Phase diagram resulting from simulation of 30 repeating units at infinite dilution.

Assumptions of the model may not neatly apply to our sample, but this simulation gave us a good starting point for choosing P-T conditions to explore.



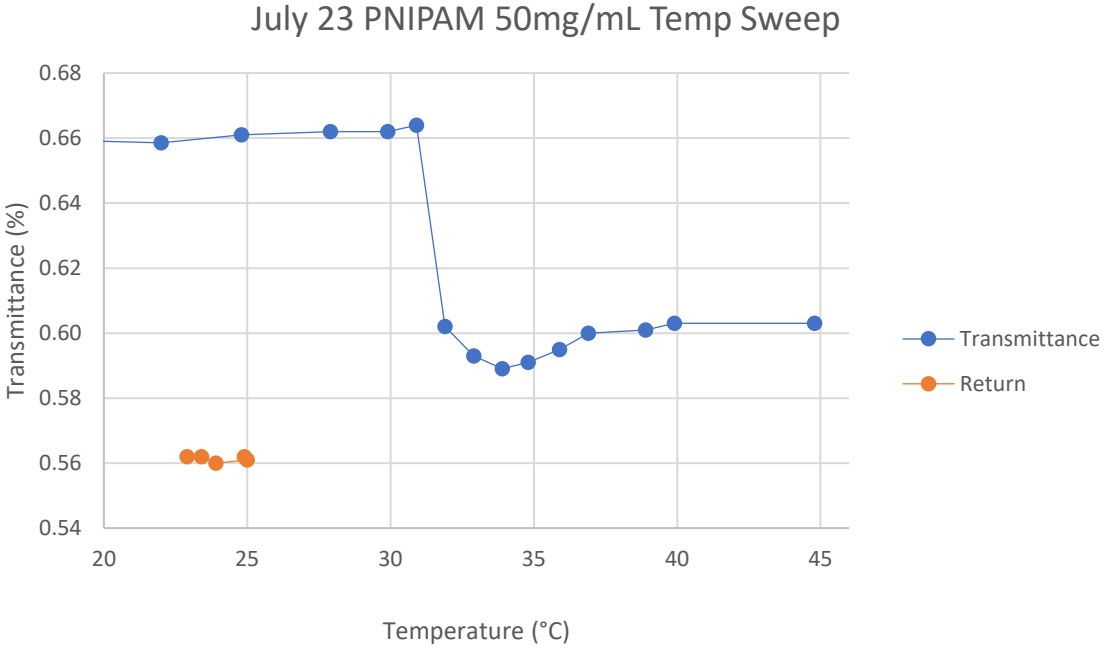
Phys Chem Phys. 2021 Mar 18; 23(10): 5984–5991. Molecular insights on poly(N-isopropylacrylamide) coil-to-globule transition induced by pressure

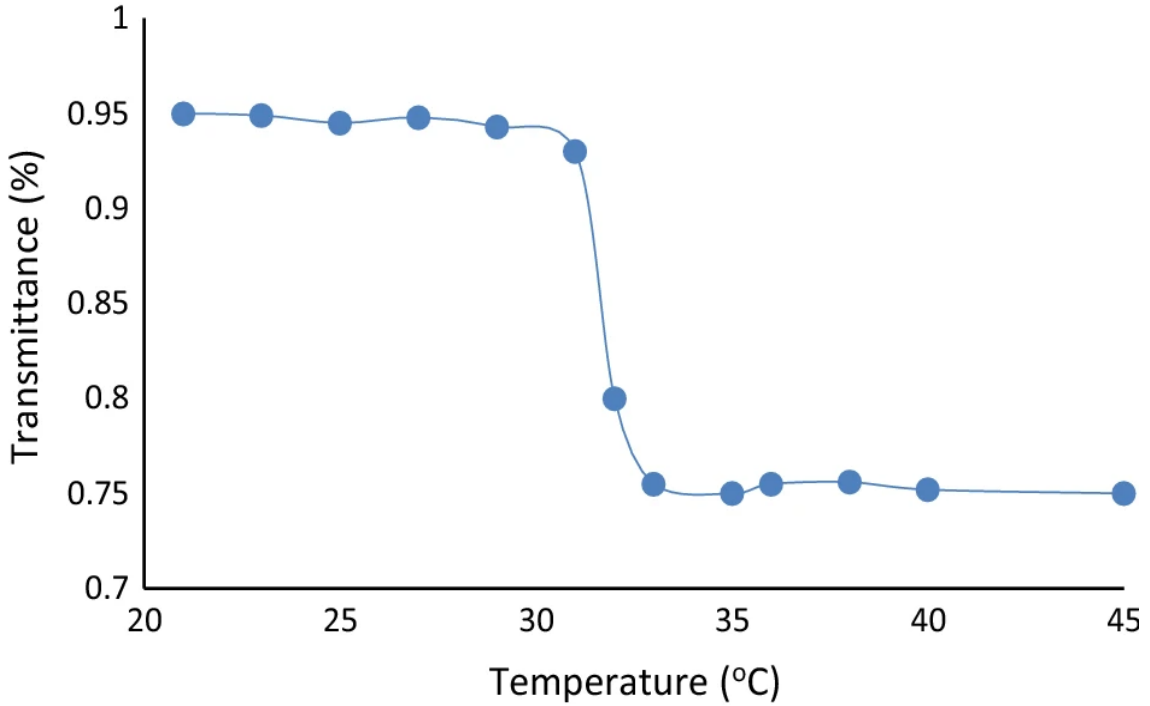
Getting to know Our Sample

UV Vis Experiments

Wanted to confirm what was known using our specific samples and define boundaries we could work within. Literature agrees that the LCST for PNIPAM is reversible.

Wanted to confirm this using UV-vis as done in the plot from publication referenced to the right.





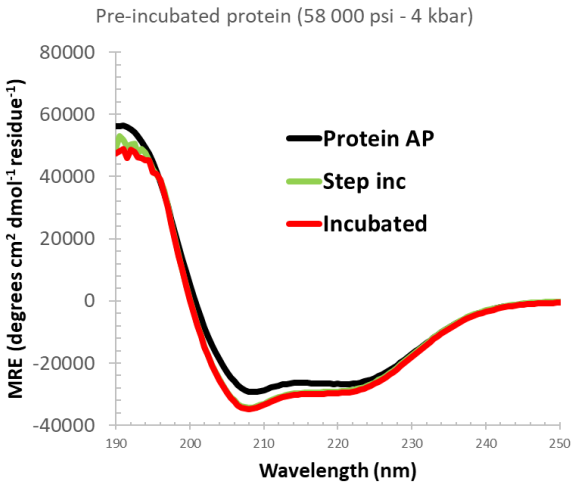
Polym. Bull. 2018 Sept 18; 76: 2819–2834. PdNPs@thermo-responsive block copolymers composed of PNIPAM and poly(ionic liquid) via RAFT polymerization.

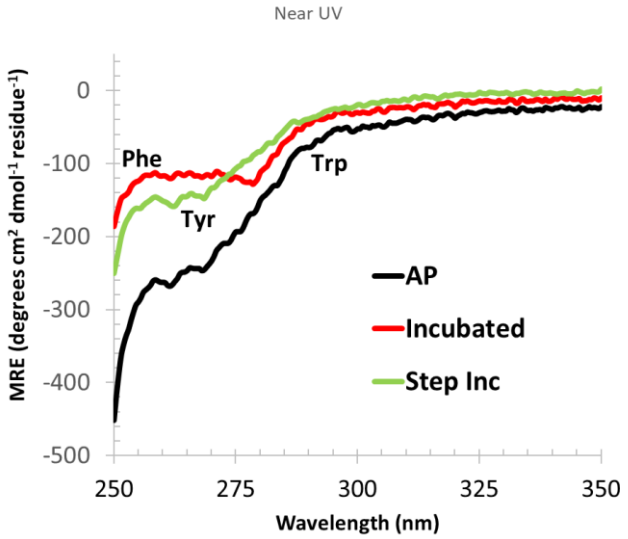
I saw a *non-reversible* change on time scale ~10min. Suspicious. Wanted to retest at different concentration. UV-vis stopped producing reliable data. Trouble shot with Donna for a few days but had to step away for more pressing work.

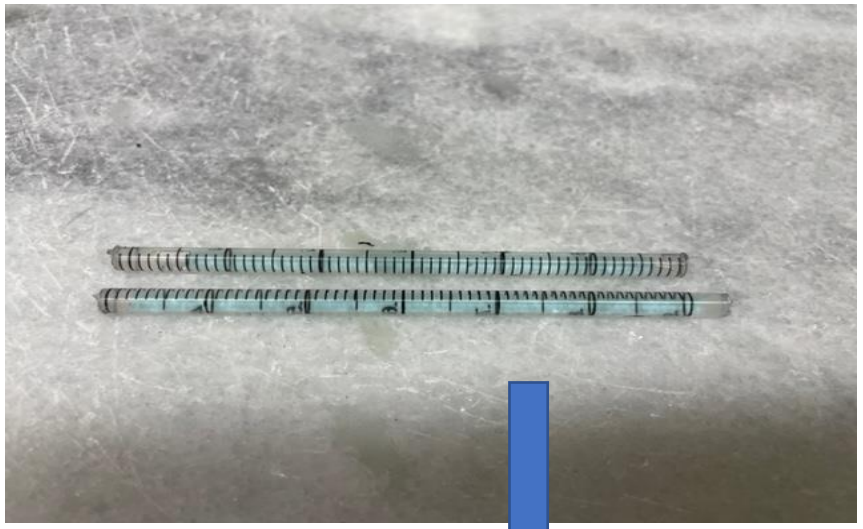
Last Summer (2023): Pre-Incubation Vessel

Previously, developed a rudimentary pre-incubation vessel from pipettes and vacuum grease in the LIPSS separator.

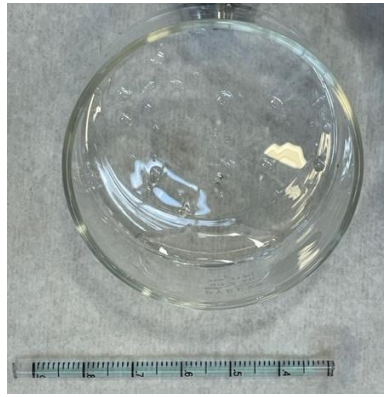
Preliminary CD measurements on BSA protein showed proof of concept for pressure incubation







Vessel does not leak confirmed with UV-Vis



This Summer (2024): <Name of Pressure Vessel>

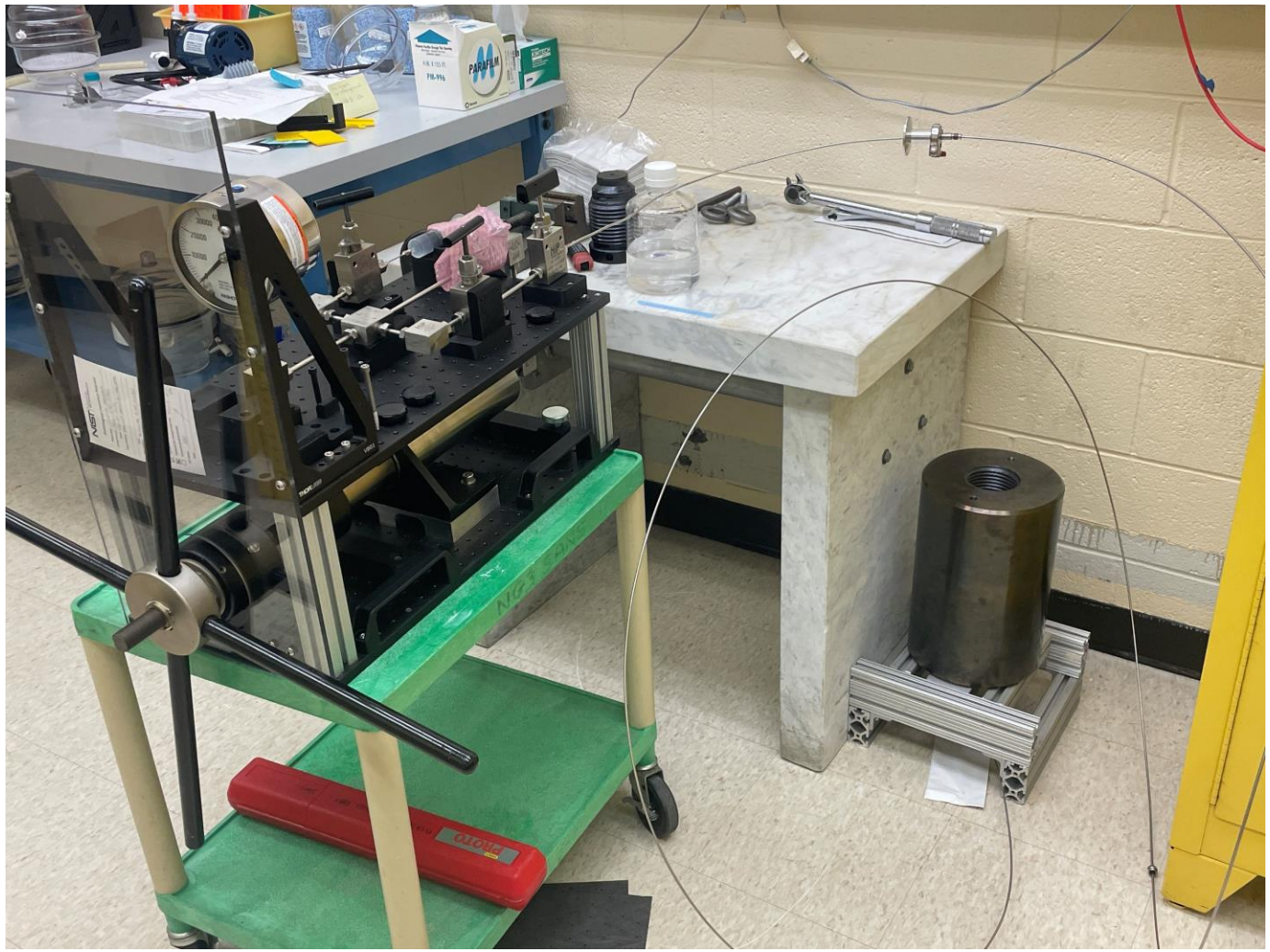
Now have access to commercial pressure vessel. Installed and commissioned with Susana.

The “Incubus”

Capable of pressurizing up to 4 kbar (as before), but much more convenient with larger incubation volume.

<u>Ahoy, captain!</u>

Pressurized using manual system. Relative difficulty in pressurizing compared to McHugh cell due to larger volume.


PARAFILM

Hydrogel incubations

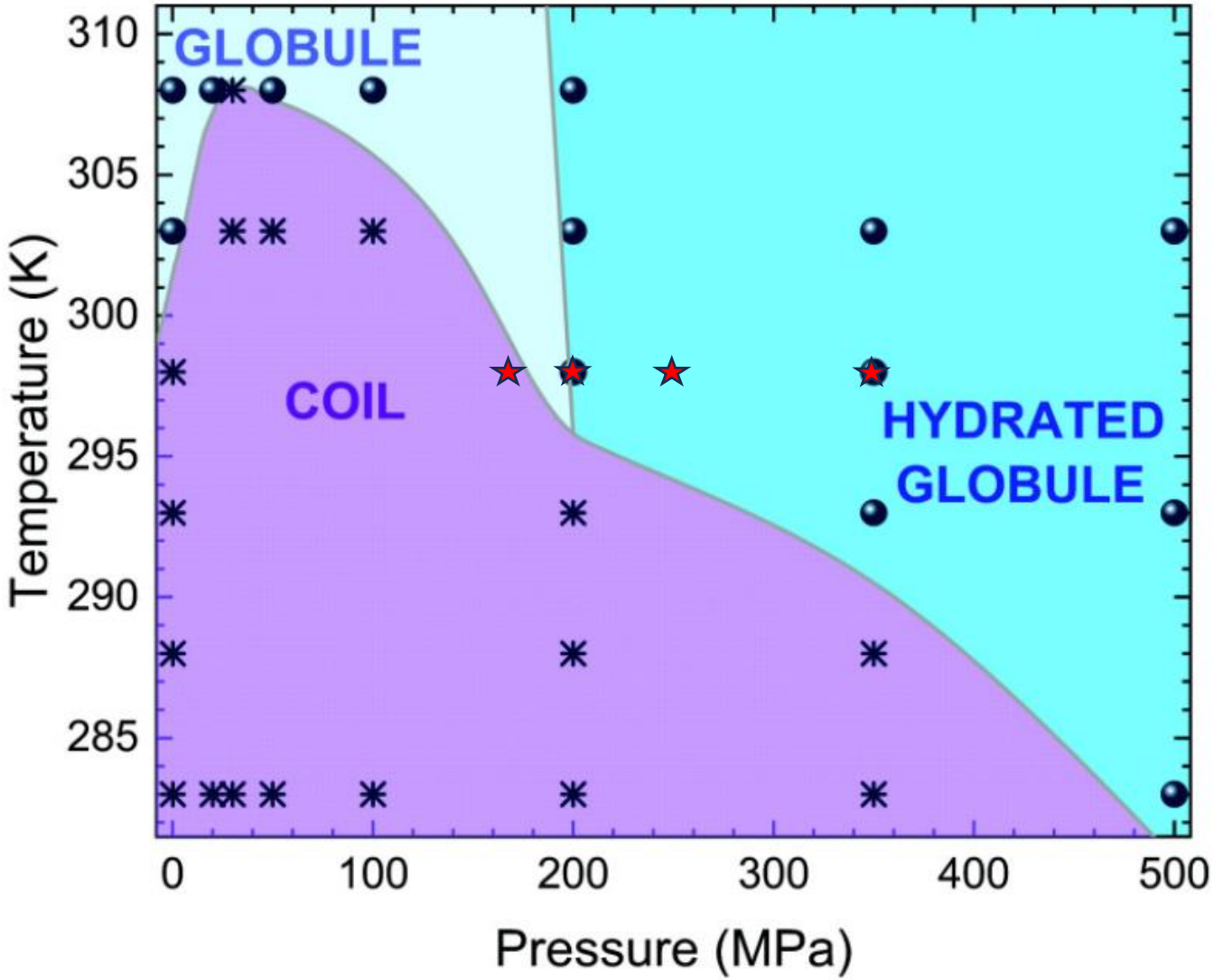
10mg/mL and 50 mg/mL PNIPAM solutions in H_20 and 150mM salt were pressurized in steps to 4 different final pressures: 180 MPa, 200 MPa, 250 MPa, and 350 MPa.

Goal was to explore phase plane and investigate reversibility of pressure induced phase changes.

Incubations were carried out at a nominal ambient temperature of ~25 °C.

Why only an isotherm?

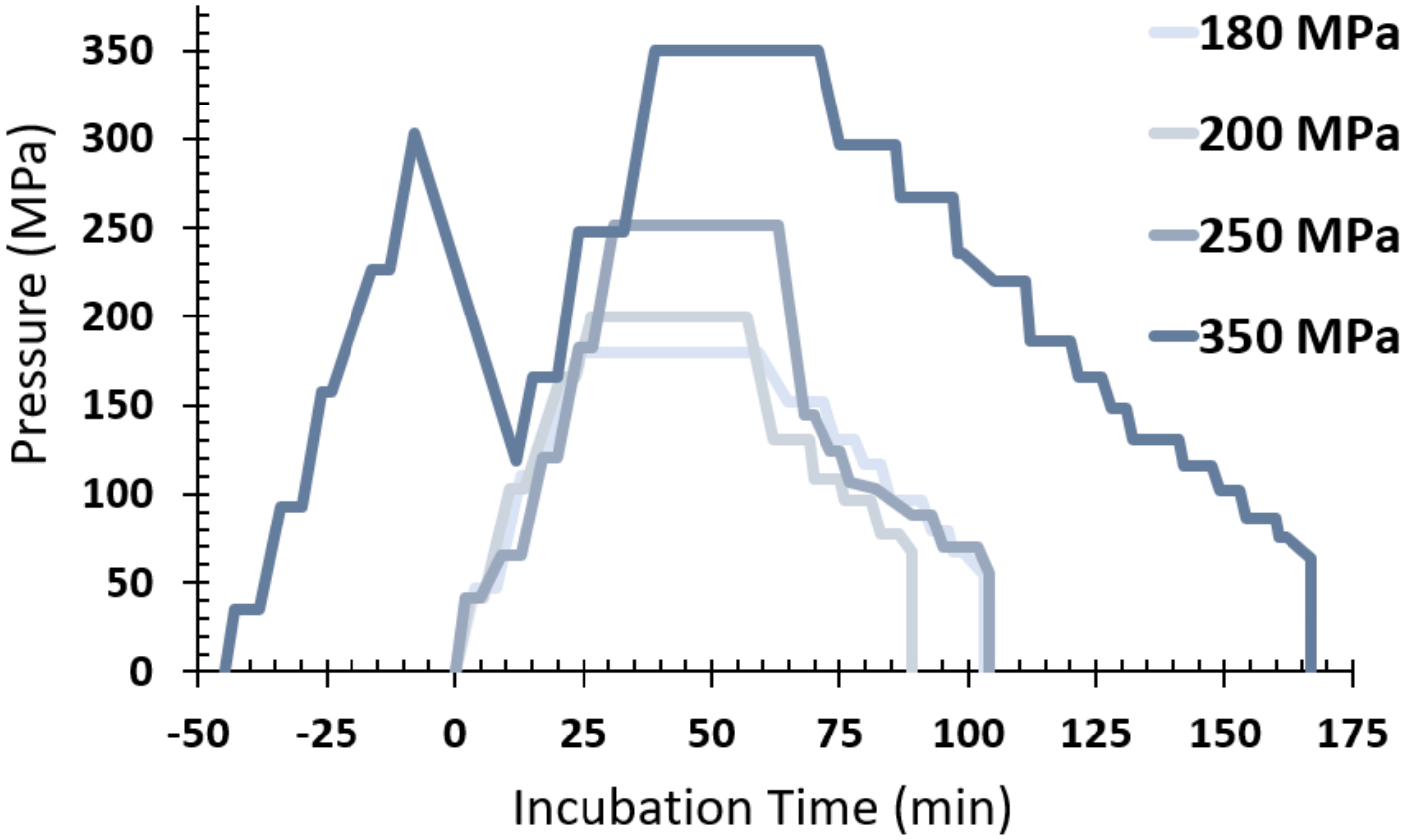
An attempt was made to heat samples during incubation (e.g. pre-heating reservoir water, mixing ethanol with water in reservoir), but samples thermalized relatively quickly and the incubation was evidently a bit of a thermal sink.



Phys Chem Phys. 2021 Mar 18; 23(10): 5984–5991. Molecular insights on poly(N-isopropylacrylamide) coil-to-globule transition induced by pressure

Hydrogel incubations

Samples were incubated in the pressure steps shown and frozen within ~30 min after unloading. Small sample volumes of ~1 mL were cooled in a -80 °C freezer.



Critical to collect data of this kind of potential standardization procedure. Our pressure steps were *not* performed in a manner consistent with a rigid procedure (notice interruption in the 350 MPa due to leak.

Alternative pressurizing system would be ideal for a process such as this.

Additionally, freezing could be done more promptly (e.g. immersing in liquid nitrogen. More on this later.

Asking ourselves:

What would be the best concentration and ionic strength for our PNIPAM standard?

SAXS Data

Collected small angle x-ray scattering data from IBBR.

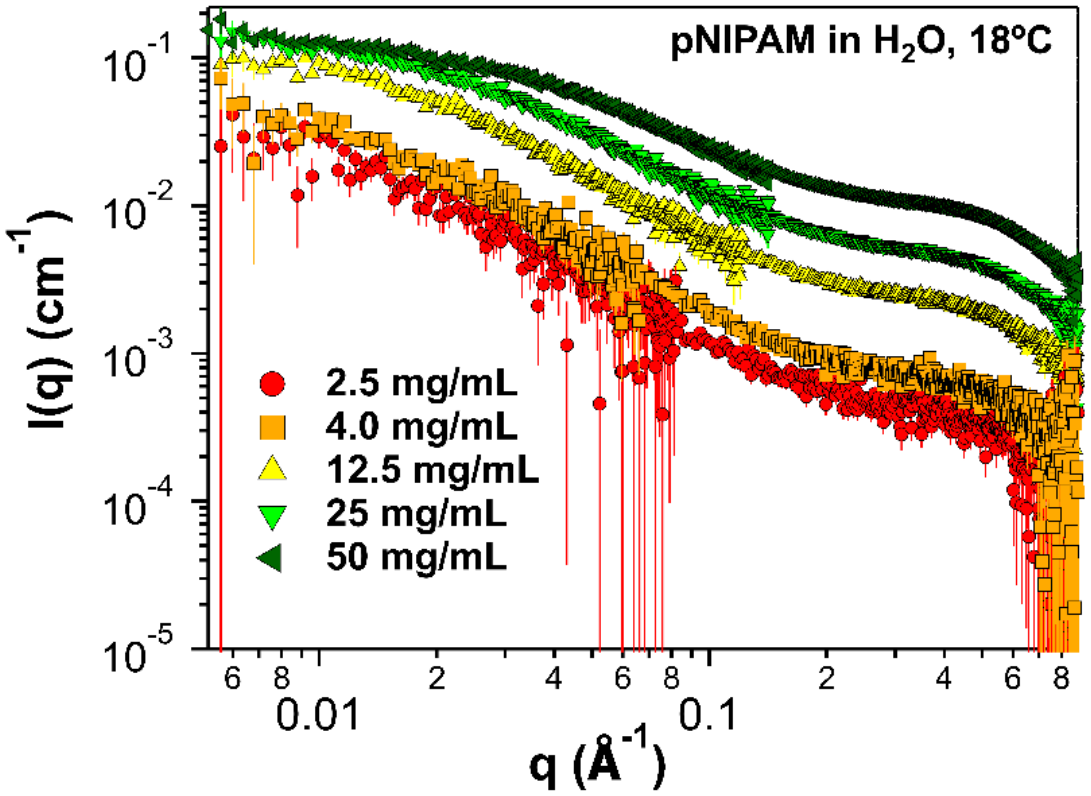
Before incubations, did a preliminary run in June to get a sense of what we wanted to look at for incubated samples in the future (concentrations, salt vs no salt, temperature, etc.)

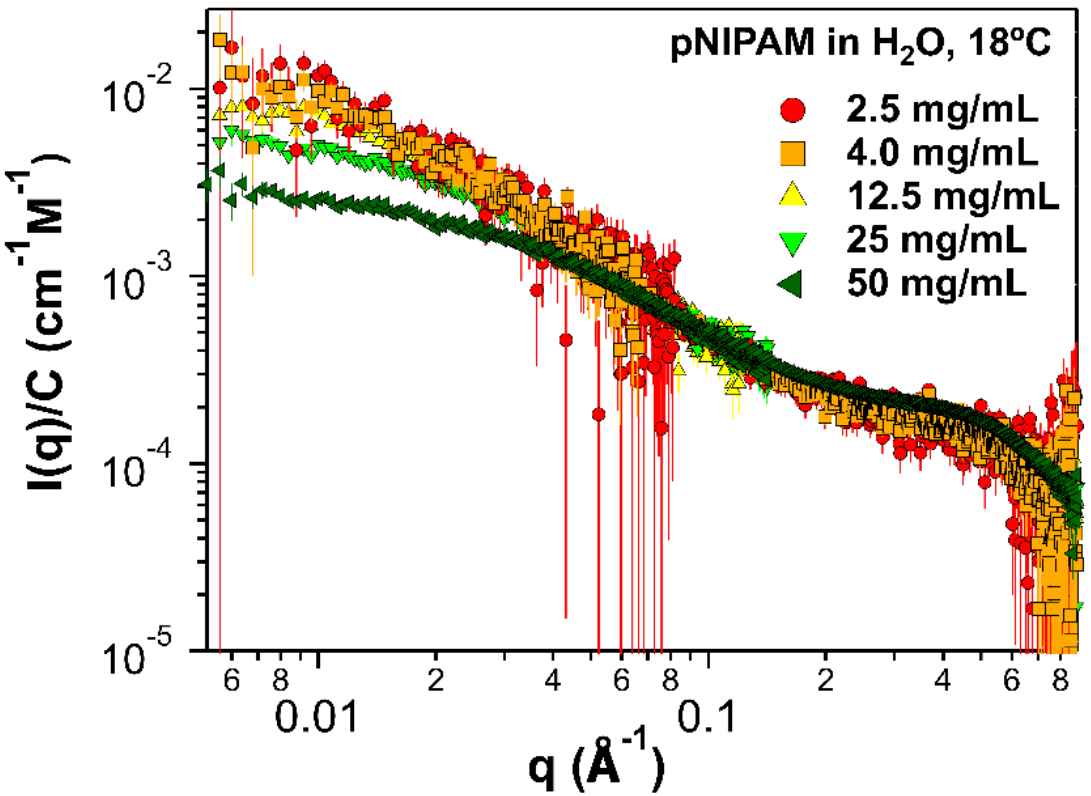
Prepared initial stock of 4 mg/mL and a 50 mg/mL which was diluted for the concentration series on the following slide.

SAXS Data

Preliminary small angle x-ray data was collected at IBBR to get a sense of what we wanted to look at (concentrations, salt vs no salt, temperature, etc.)

Concentration Series





Diff. at low q → Potentially due to excluded volume effects or repulsive interactions

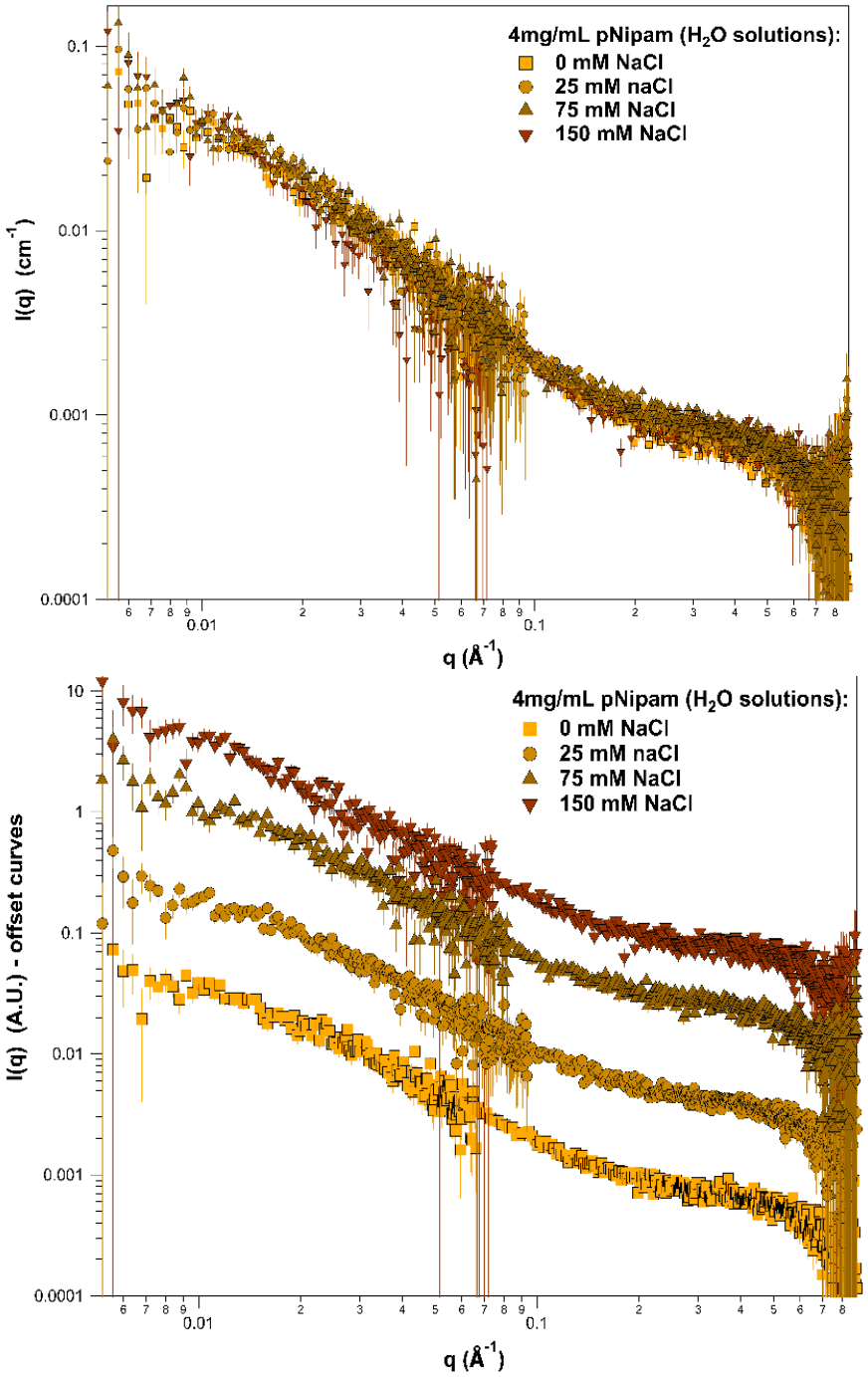
- Being able to use a higher concentration would improve time efficiency for scattering

SAXS Data

We also investigated the effects of salt.

Explored up to 150 mM NaCl to try and see any effects, but did not want to go too high such that SAXS contrast was reduce compromising signal to noise.

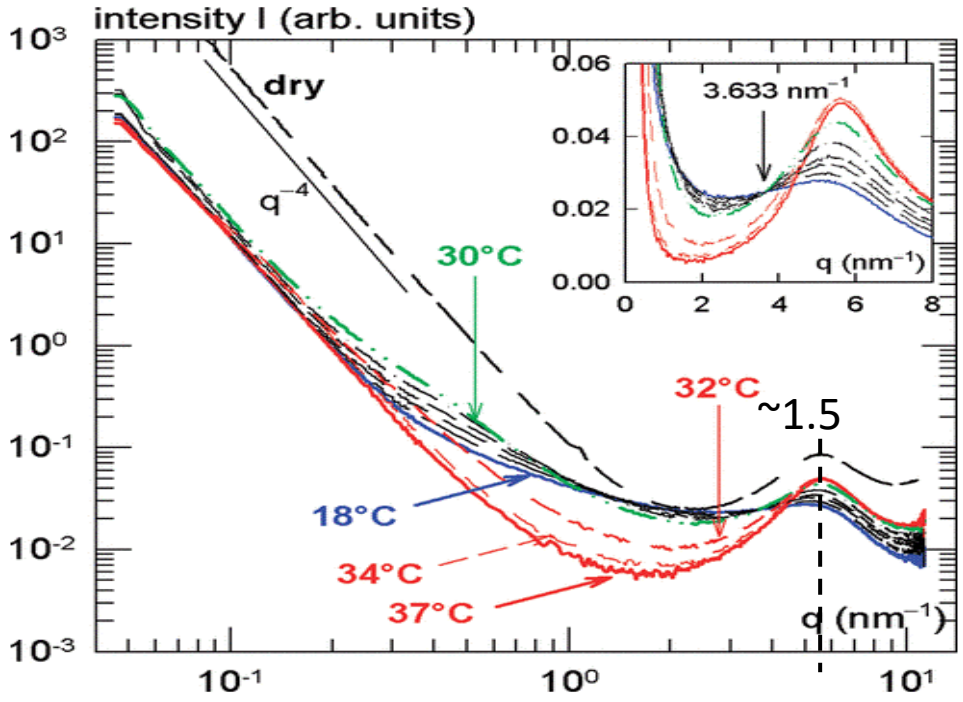
This drove our decision to choose a relatively high salt concentration, 150 mM, for our later measurements.

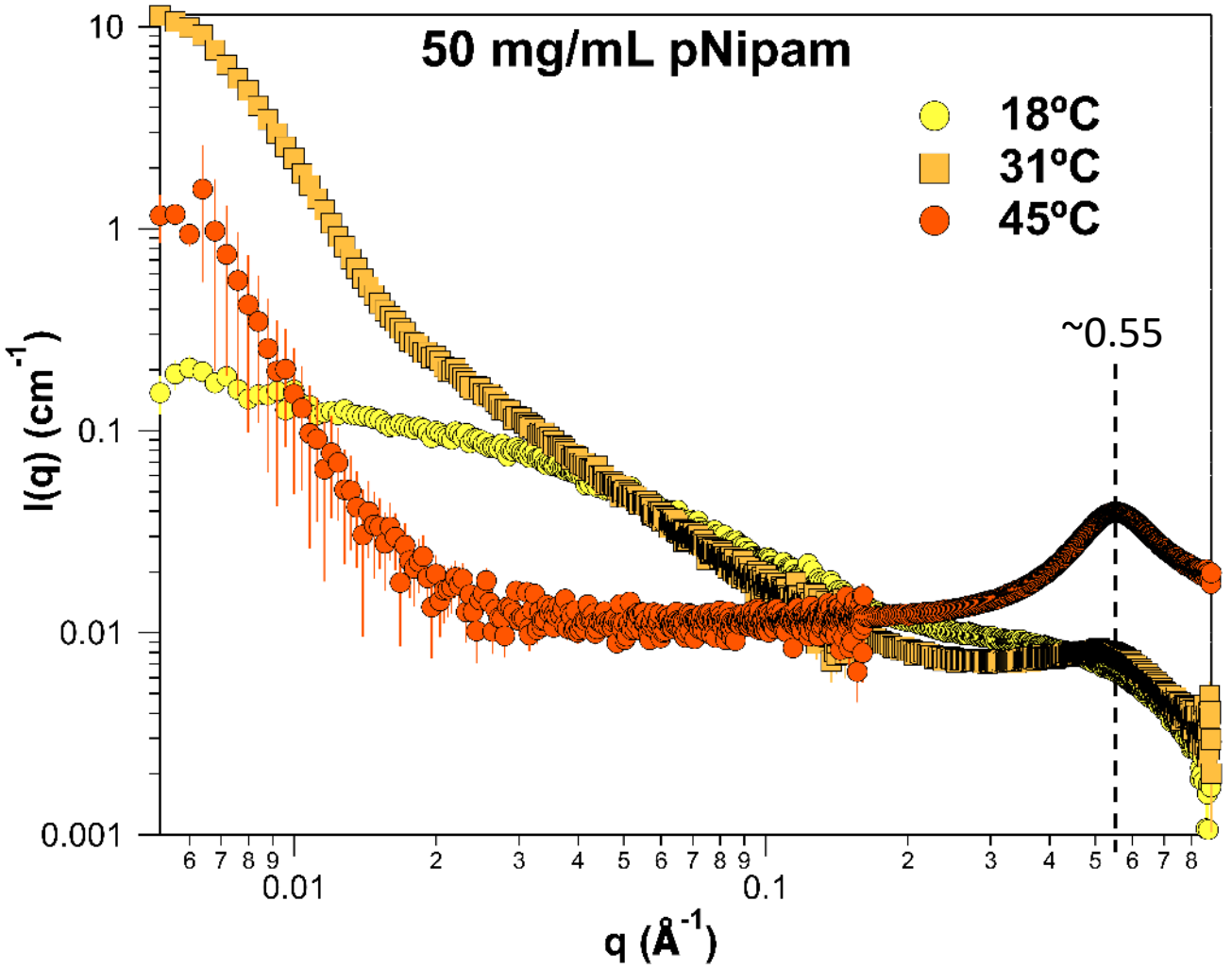


SAXS Data

Performed a temperature series to examine and verify the temperature our sample was transitioning at. Unfortunately, the series was interrupted.

We can see features emerge as temperature increases. Clearly visible at 31°C (just before the transition temperature).

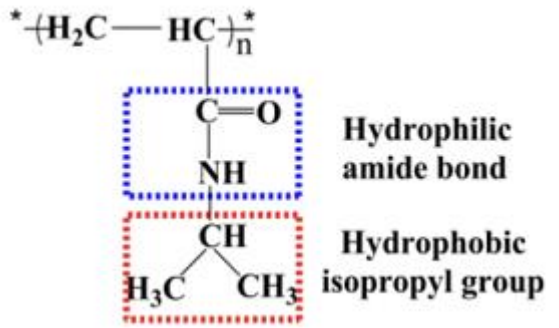


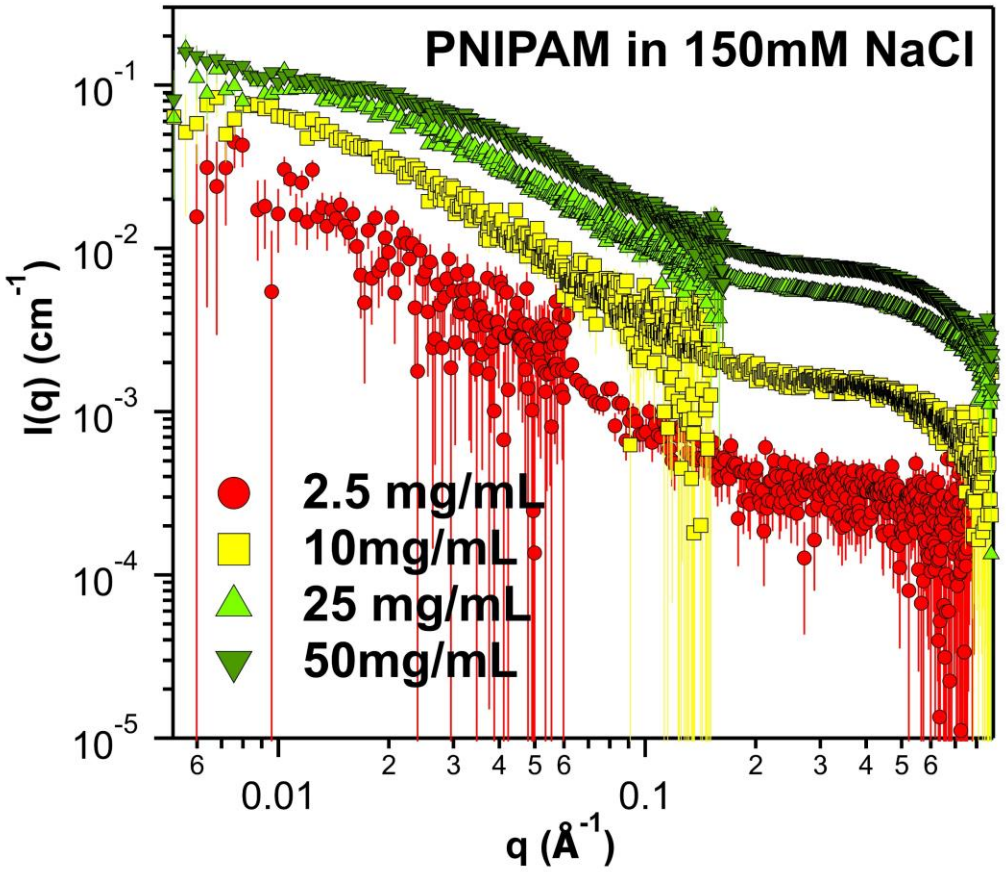


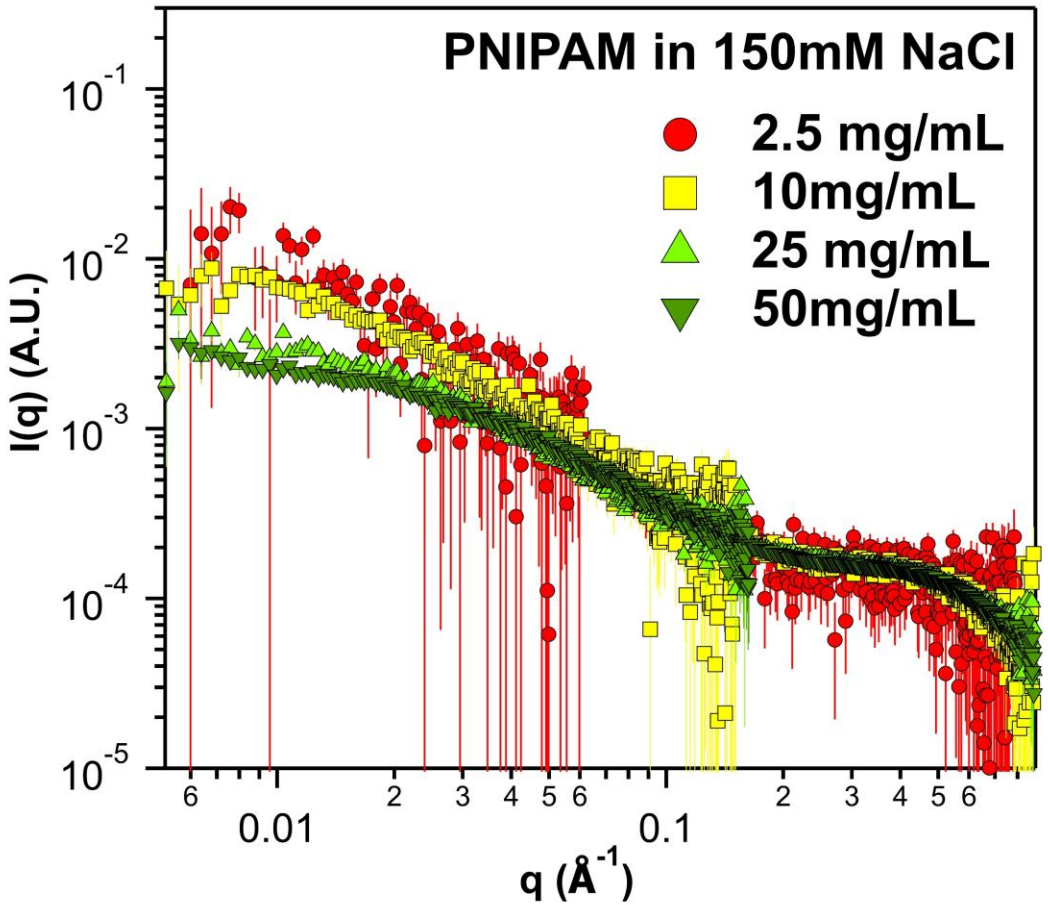
Macromolecules 2010 Jan 10; 43(4): 2009–2017. SAXS Investigation of the Effect of Temperature on the Multiscale Structure of a Macroporous Poly(*N*-isopropylacrylamide) Gel

SAXS Data

While uncharged, PNIPAM does bind to cations.

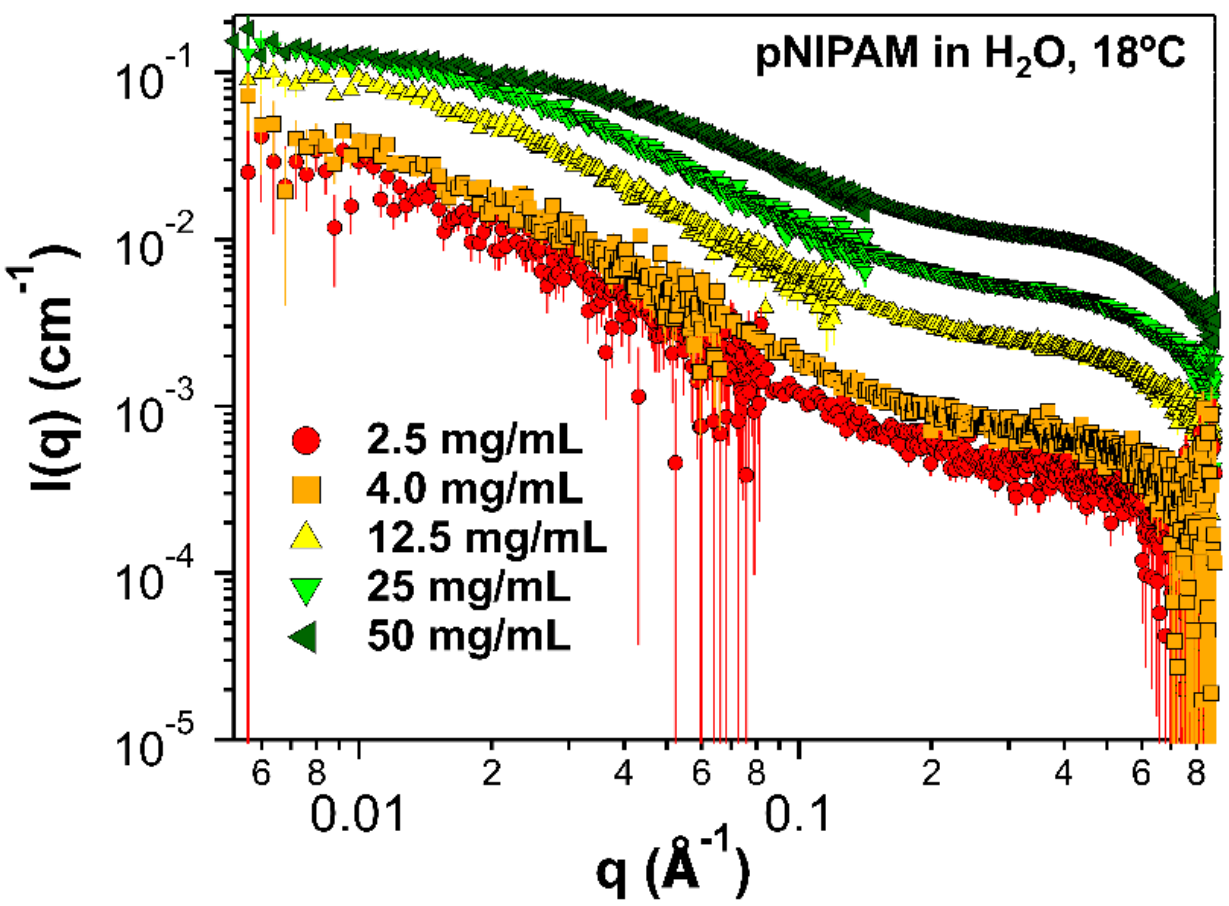


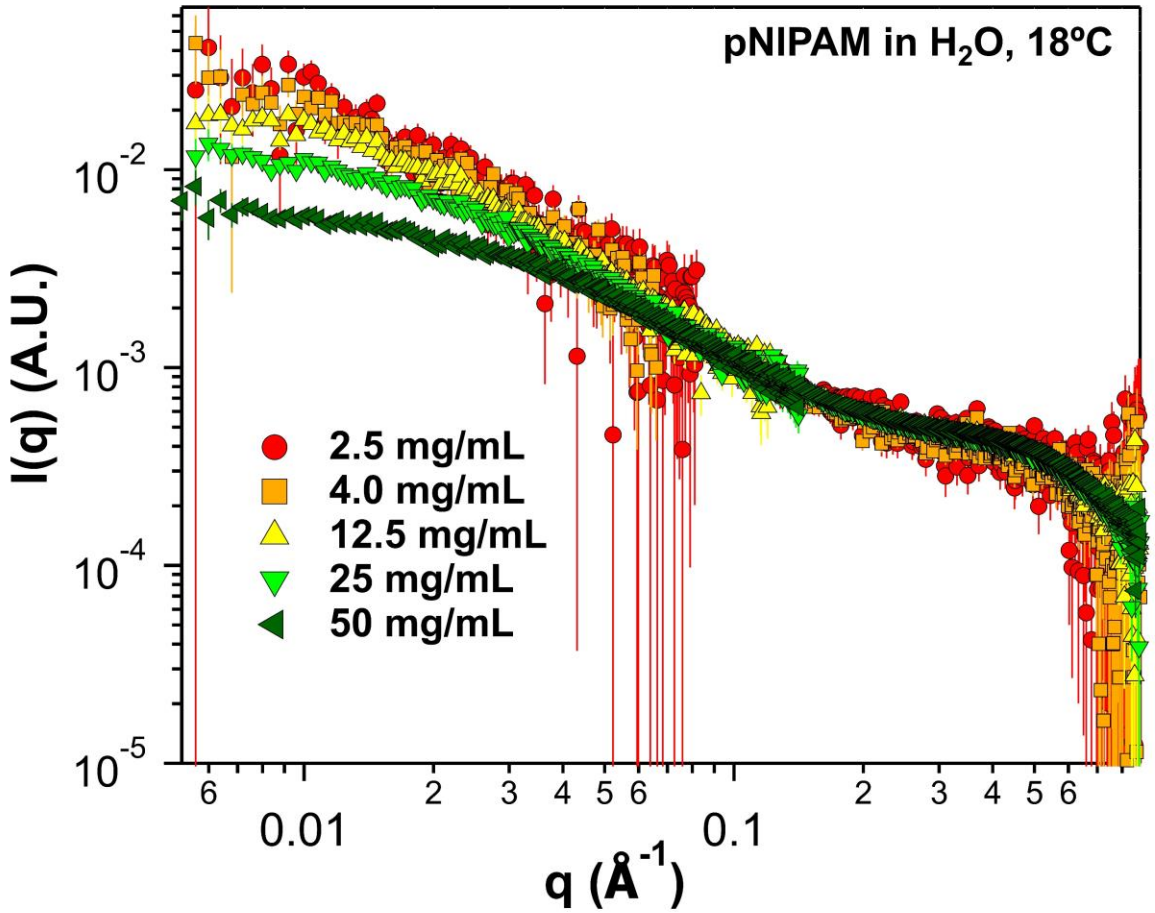




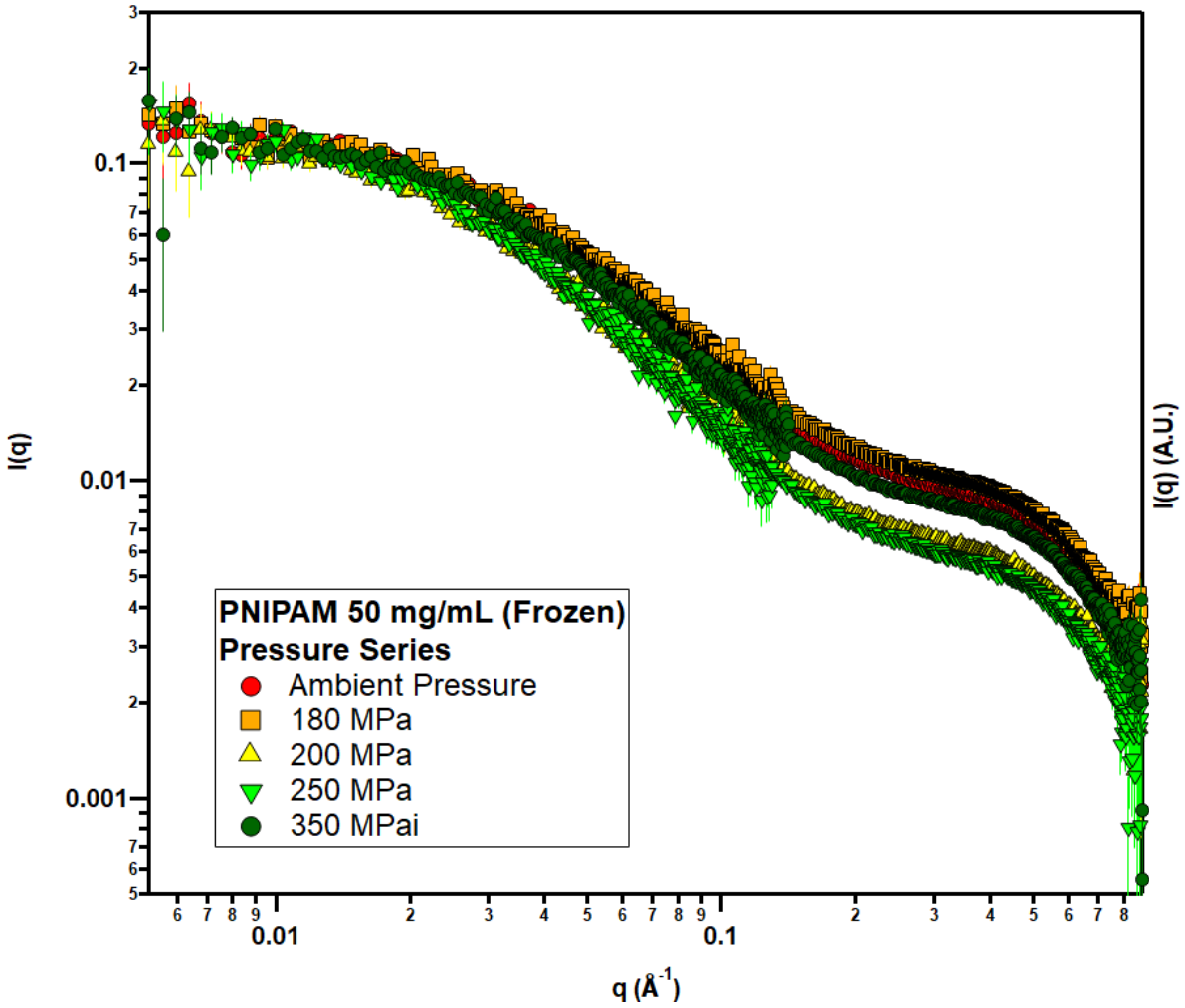
Upward curve at low q for 50mg/mL shows evidence of aggregation.

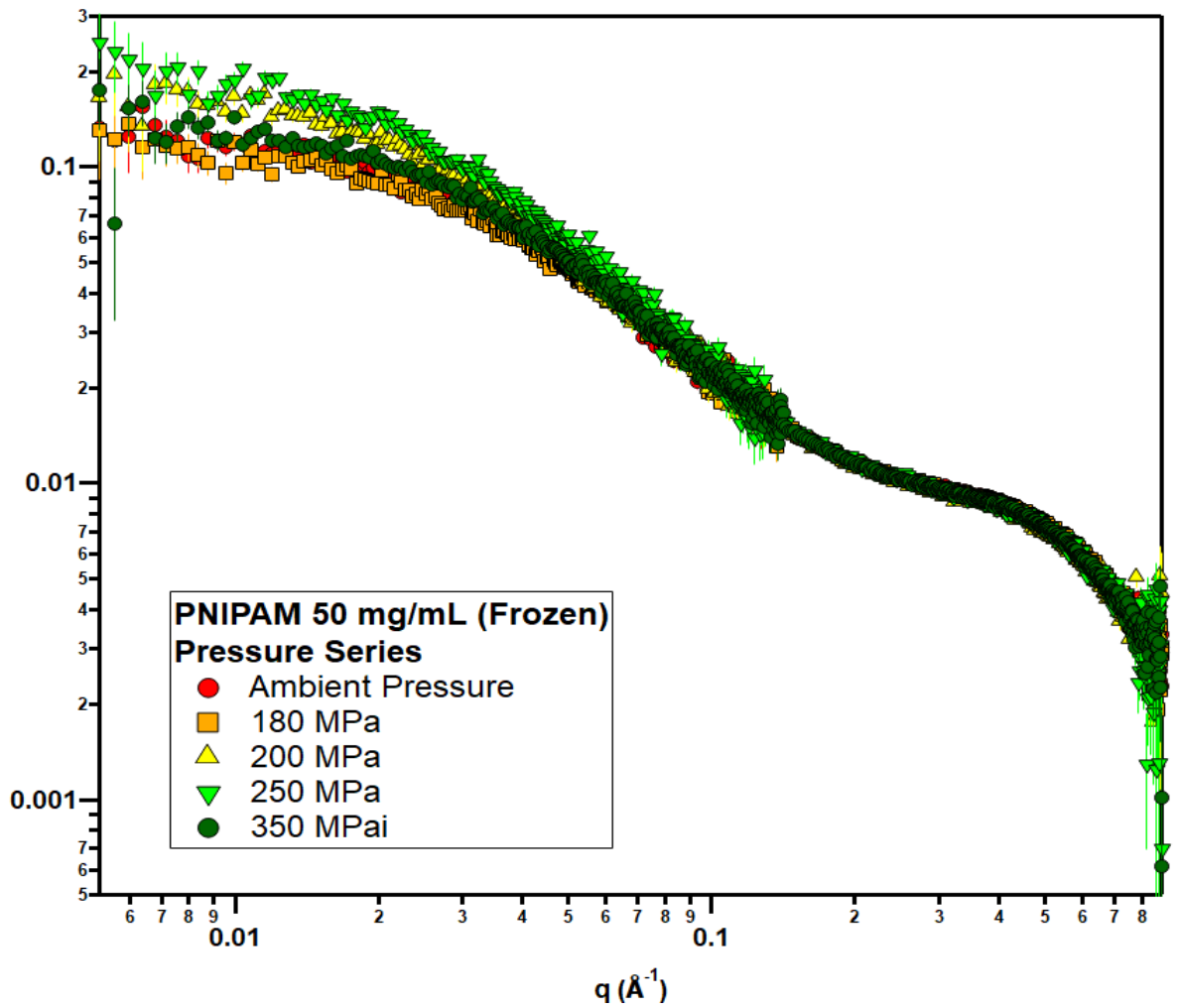
SAXS Data – No Salt Conc. Series



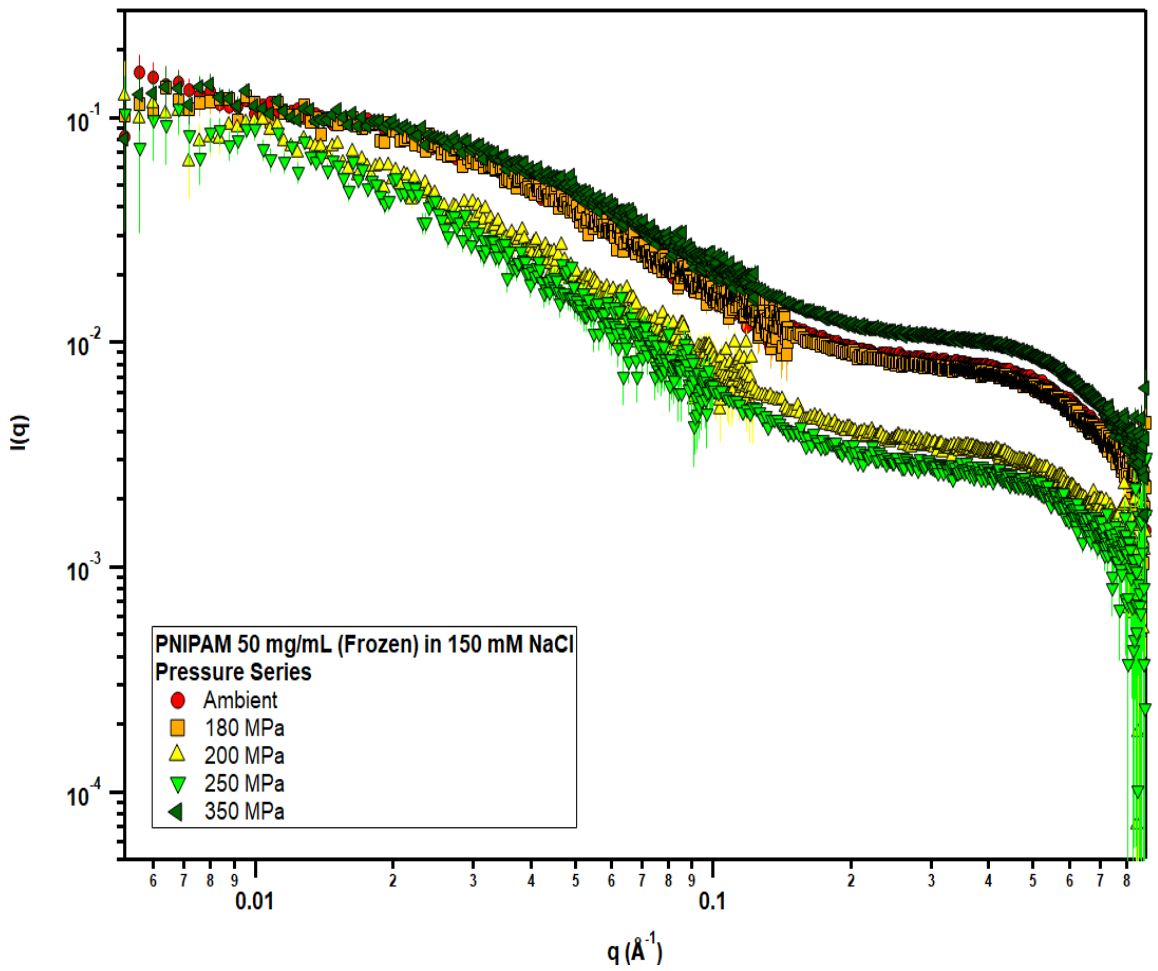


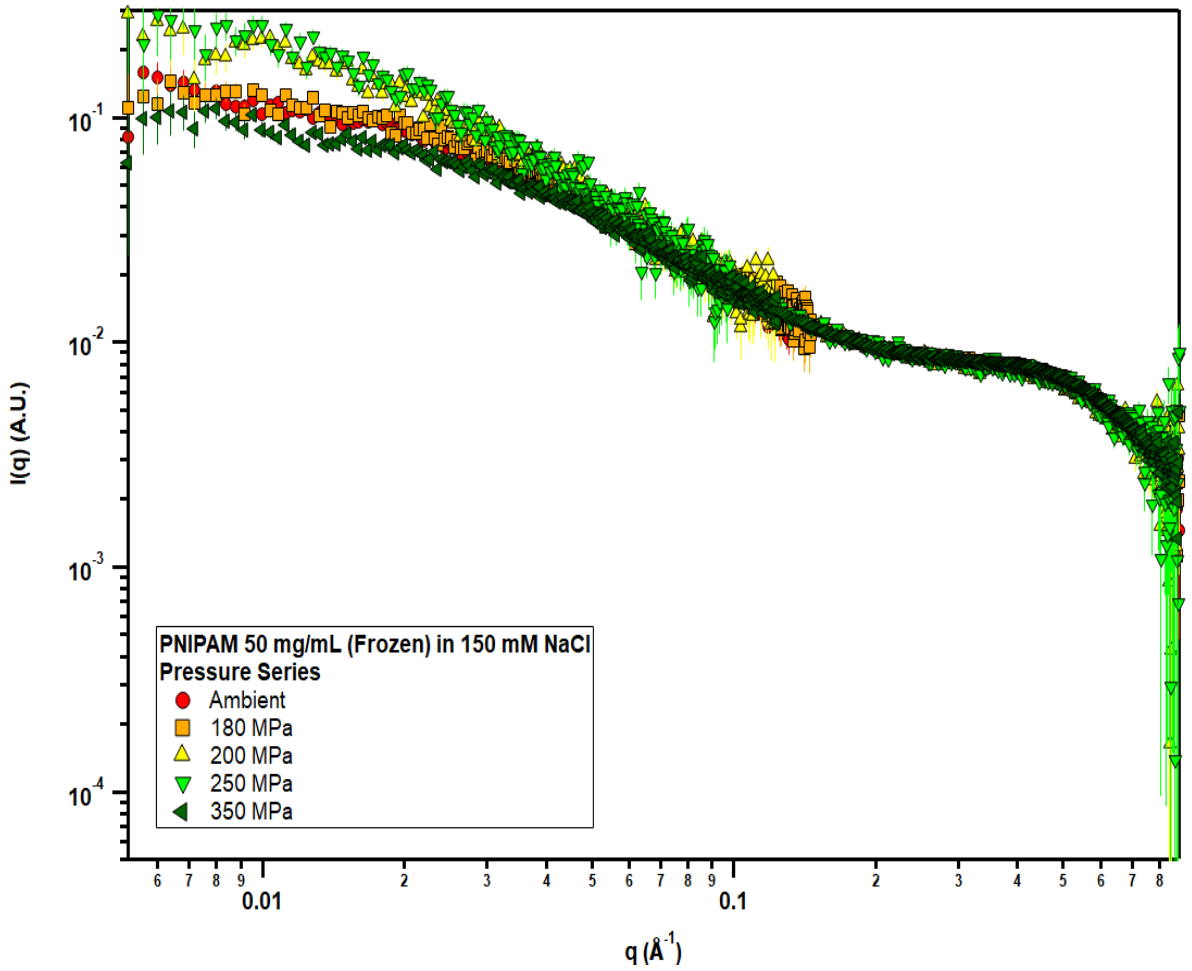
SAXS Data – Frozen Sample (No Salt)





SAXS Data – Frozen Samples (Salt)





The data above are for samples of the same concentration.

SAXS Data

Data for frozen samples exhibited perplexing behavior. Began to suspect freezing pNIPAM may be affecting data.

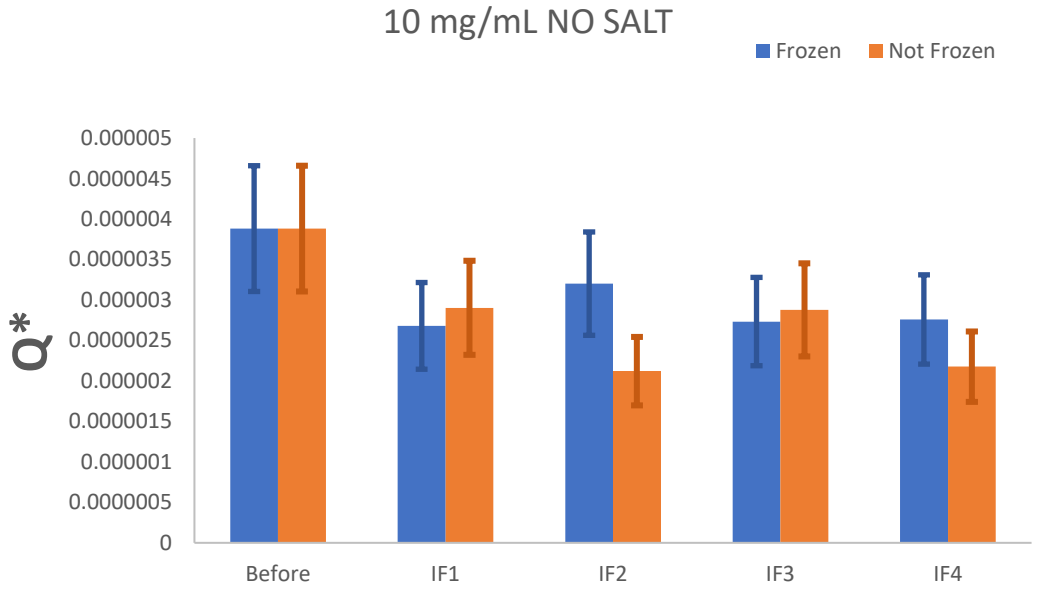
e.g. Were our frozen samples remaining homogenous when measured?

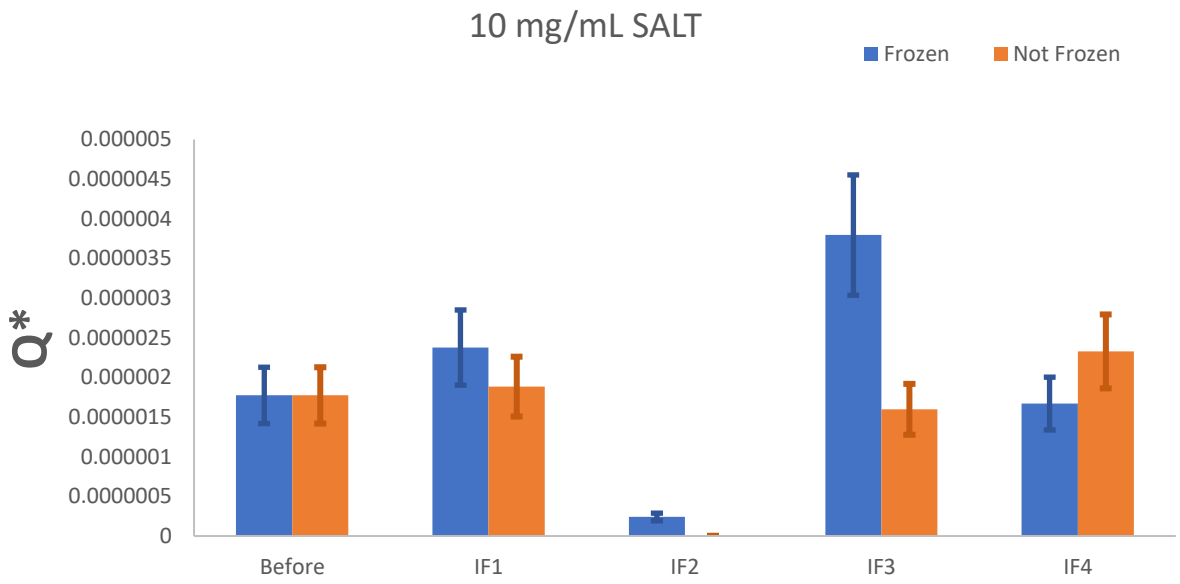
Was what we were seeing irreversible pressure effects that we “froze in”? These effects may relax / equilibrate over time and thus not be seen in the non-frozen samples.

Or was there a systematic effect that was responsible, e.g. were our frozen samples remaining homogenous when measured?

SAXS Data

To investigate the homogeneity concern, calculated an invariant using ATSAS to probe potential differences in volume fraction of pNIPAM, but did not see an intelligible trend.

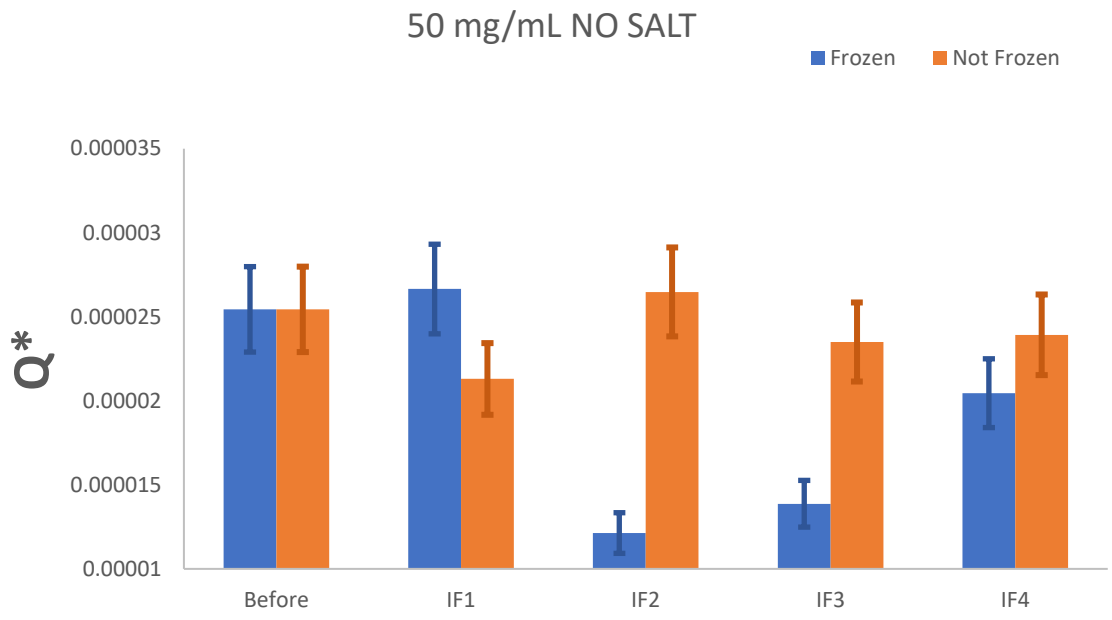


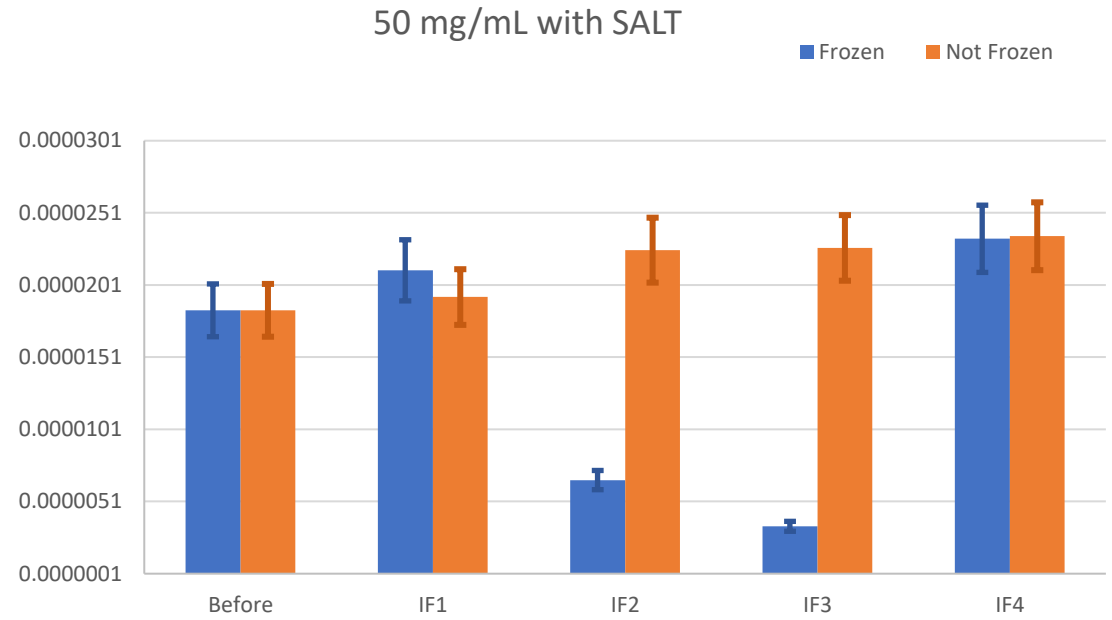


Extrapolation from ATSAS does not calculate errors bars, we conservatively assumed 20% error.

SAXS Data

Was able to calculate an invariant using ATSAS to probe potential differences in volume fraction of pNIPAM, but did not see an intelligible trend.





Extrapolation from ATSAS does not calculate errors bars, we conservatively assumed 20% error.

SAXS Data

Some samples seemed to have no difference within error, others did. This may be a consequence of different kinetics from incubations.

e.g. slow or fast equilibration times may be preserved or lost, respectively, by the time sample was frozen.

Standards – Identifying a Candidate Material

We're at NIST. Ask yourself: What makes an ideal reference / standard material?

- Responsive to pressure, temperature – both *in-* and ***ex-situ*** *in a controllable manner.*
- Stable on the order of ~weeks
- Readily available (affordable)
- Reliability + Robust
- Easy / Time Efficient to verify

Conclusions & Future Work:

- May be irreversible effects due to pressure, but *ex-situ* investigation inconclusive for now.
- Need to deconvolute freezing from aging effects
- Readily available (affordable)
- Sample is *very* sensitive to transition. Just handling sample can cause it to unintentionally transition.
- Pressurization procedure a bit difficult. Likely difficult to standardize with manual system.

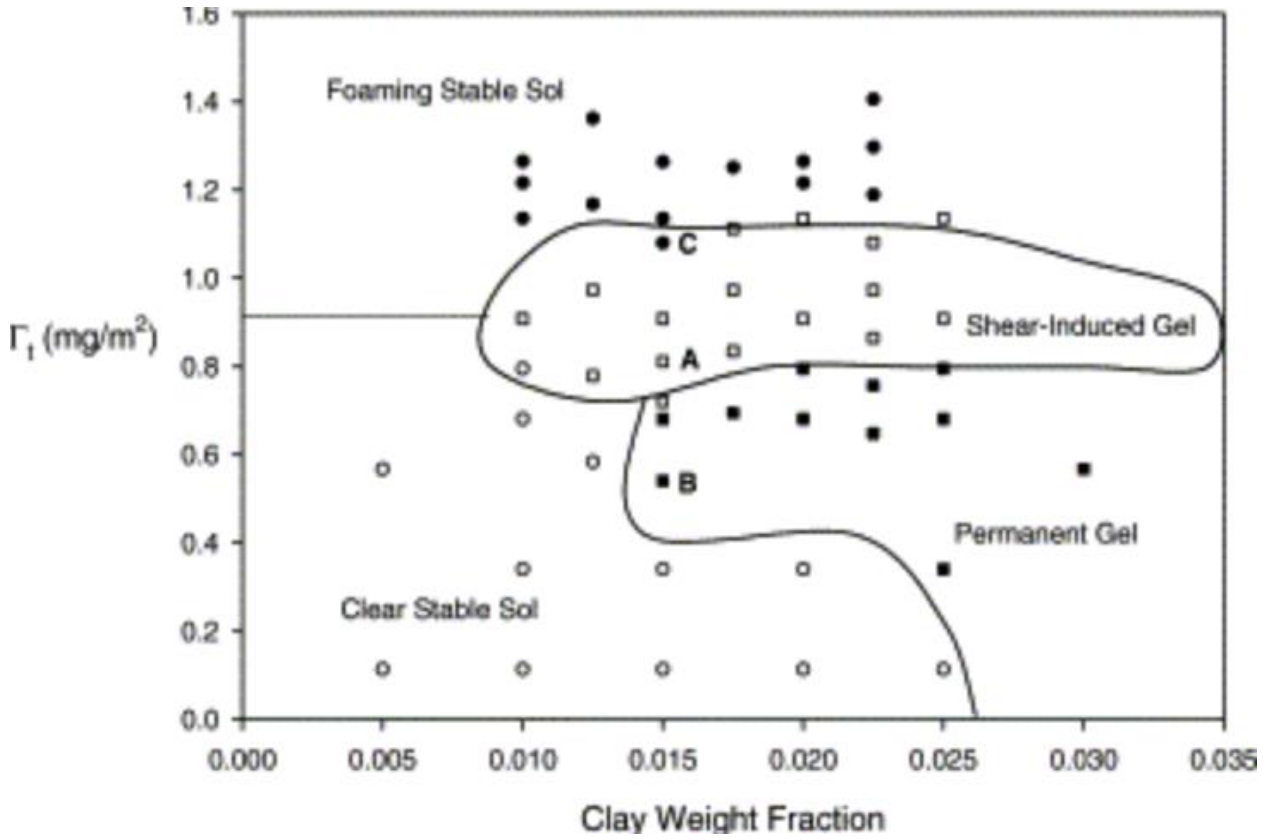

AND NOW FOR SOMETHING
COMPLETELY DIFFERENT

Laponite-PEO Shake Gels

When mixed in water laponite (a synthetic hectorite clay) and the polymer polyethylene oxide (PEO) for a shear induced, self-supporting gel that relaxes back to a liquid in a characteristic time.

The composite shake-gel has a large number of applications ranging from cosmetics to mud-making material in drilling.

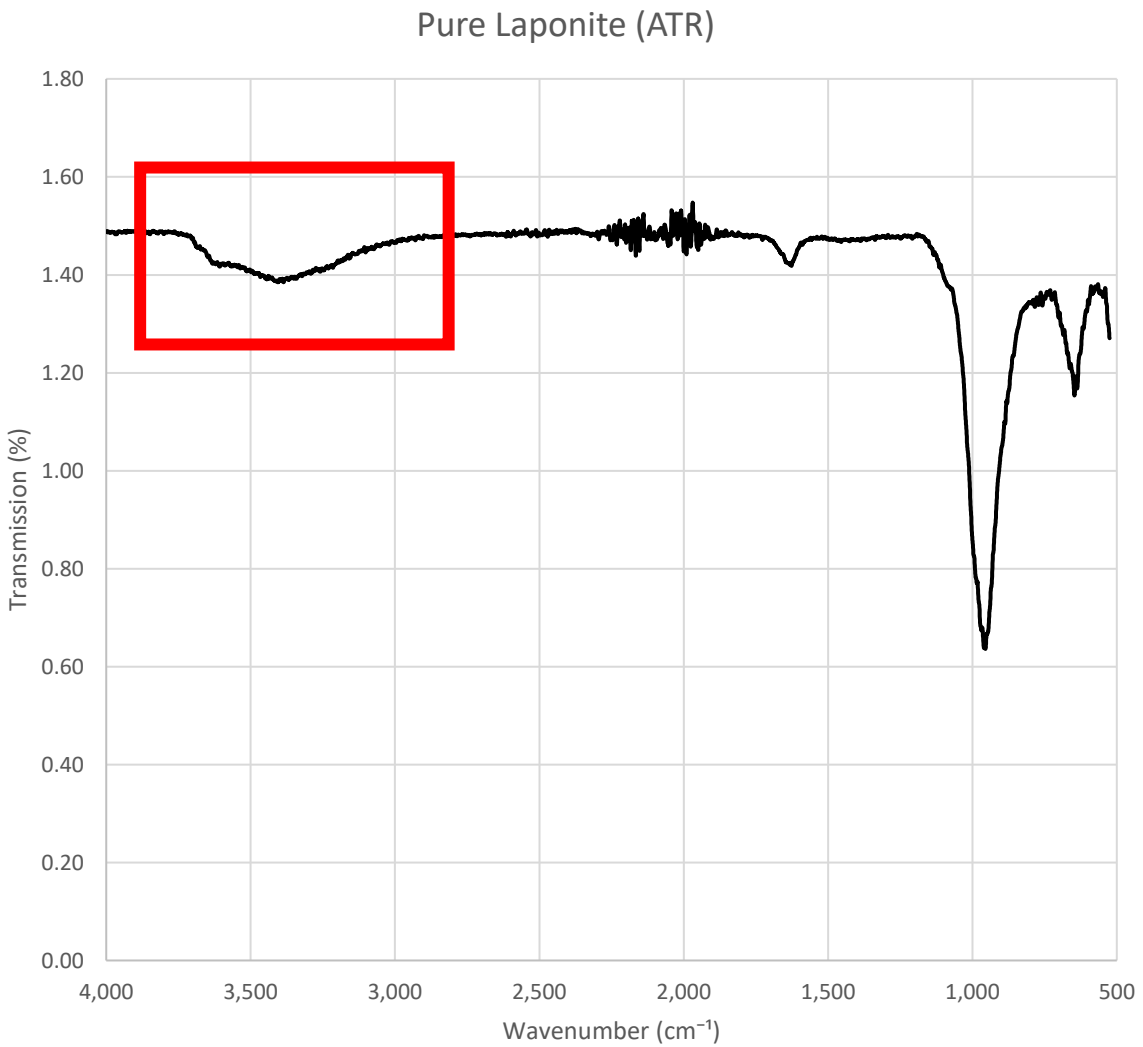
There is great interest in characterizing the behavior of these gels under pressure.

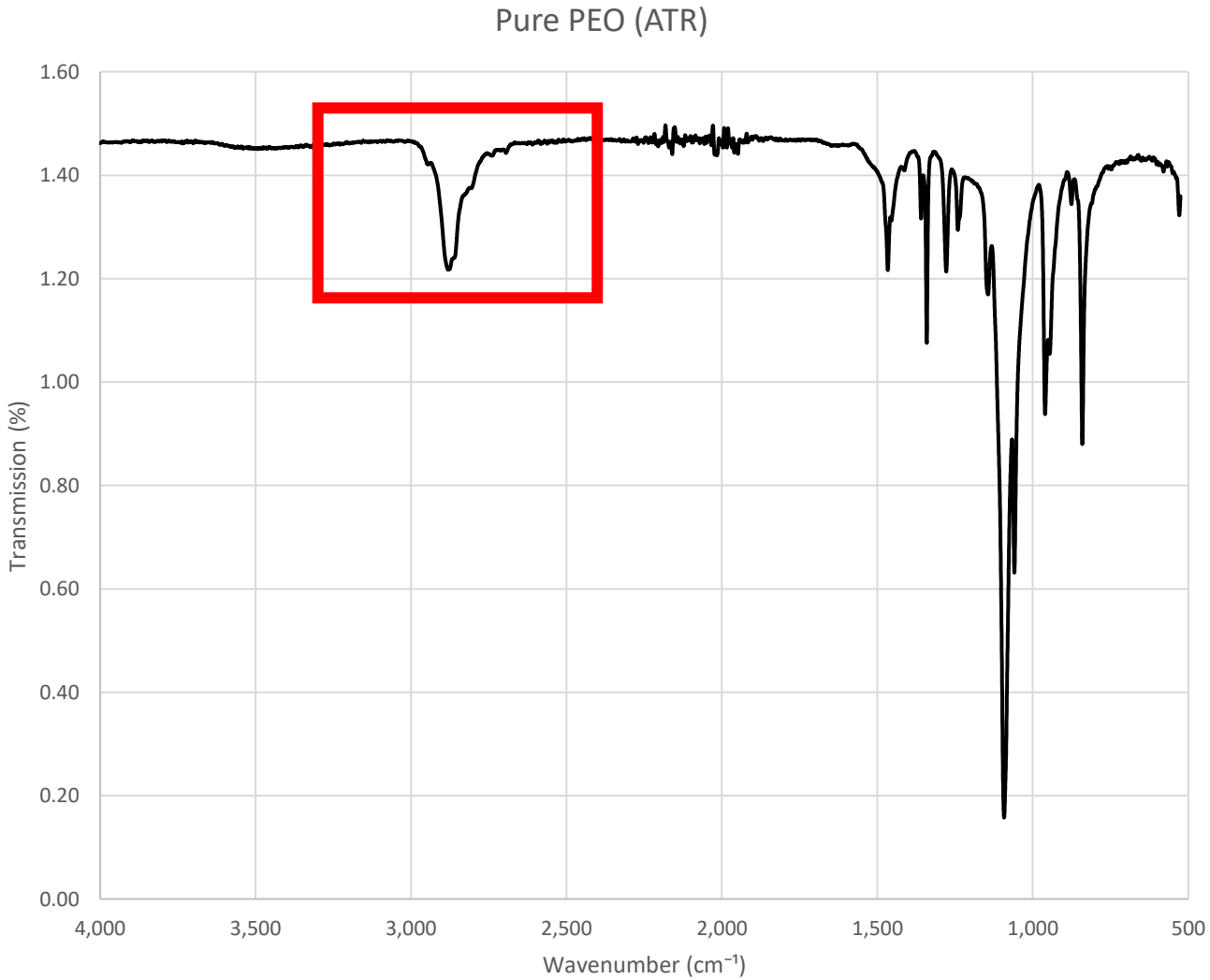


Colloids and Surfaces. 2004 Apr 12; 240(2004): 187–198. Reversible shear gelation of polymer-clay dispersions

FTIR References for Shake Gel Components

ATR FTIR spectra were obtained for the dry laponite and PEO samples.





= Region of interest.

In-situ Pressure FTIR Experimental Design

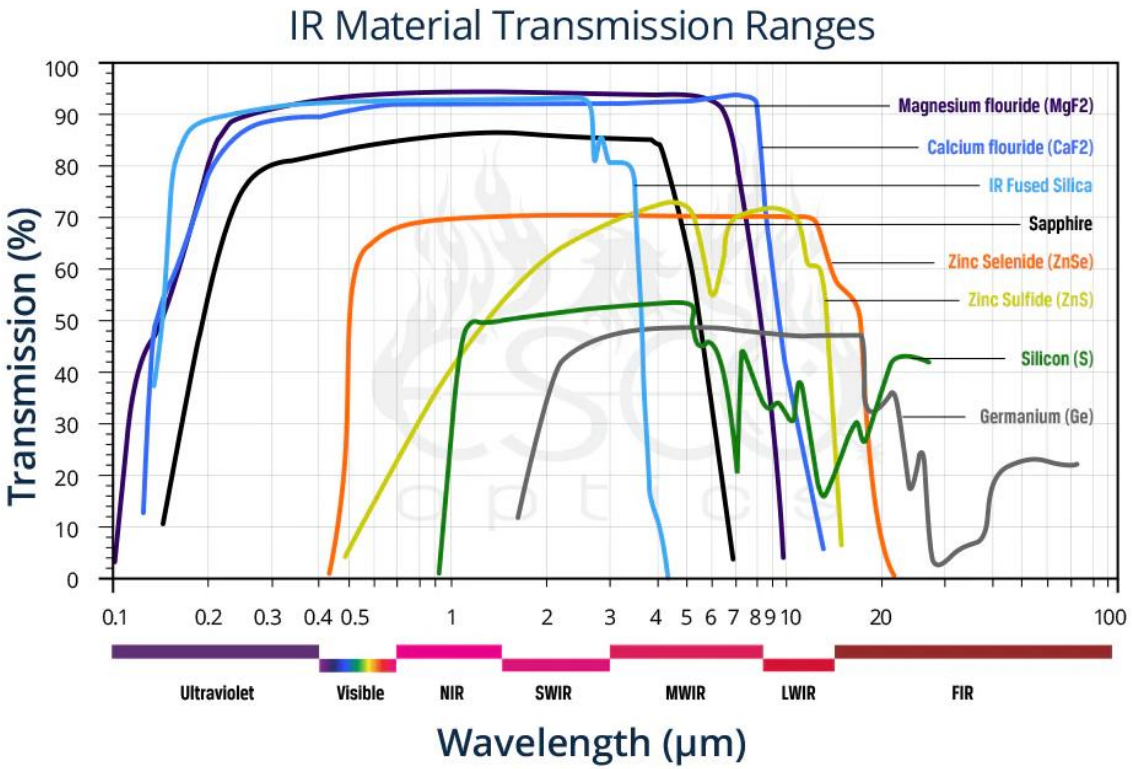
Want to investigate FTIR spectrum of laponite-PEO complex (liquid phase) and laponite-PEO complex (gelled) at both ambient and elevated pressure.

Required developing a Juscelino-rigged setup of placing SANS pressure cell into the FTIR instrument.

Testing of SANS cell in the FTIR raised some issues:

-Sapphire window cut off fingerprint region

-Signal of remaining peaks hard to see (sample volume seemed to saturate signal)

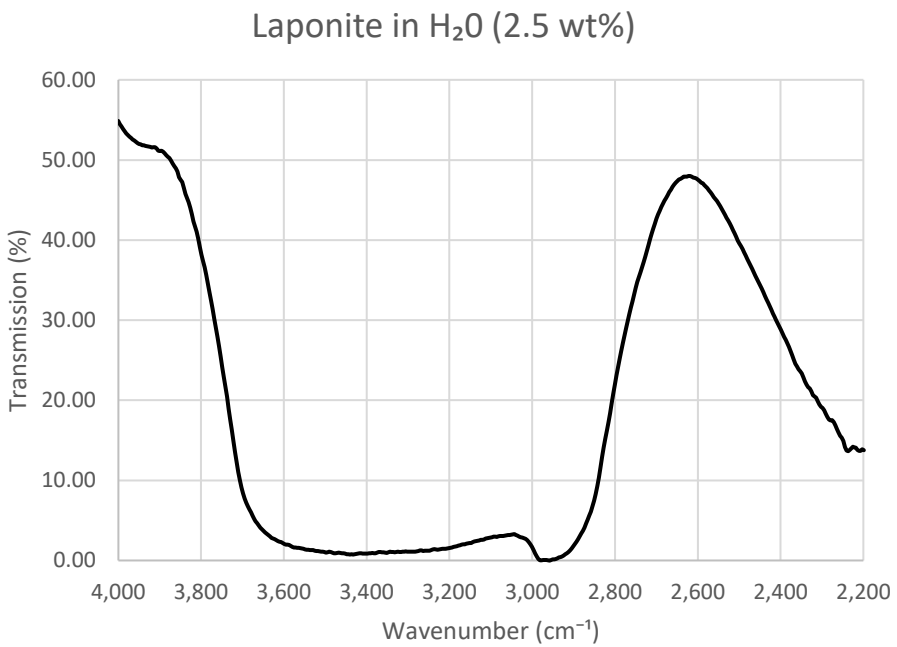


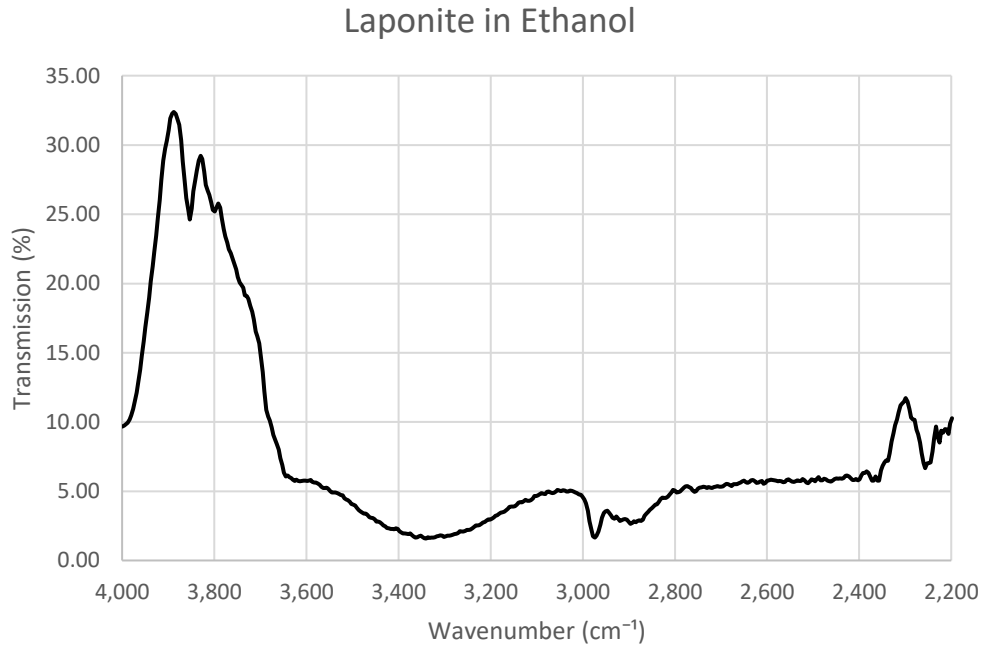
-Attempted to use a reference (polystyrene and Teflon cutouts) in cell to monitor shifts of Lap/PEO peaks. Further complicated visualization of data

https://escooptics.com/blogs/news/what-optical-materials-work-best-in-the-ir-infrared

In-situ Pressure FTIR Experimental Design

Shifted to using a sapphire cutout to hold a small volume of sample against the sapphire window.





Ran into same over saturation problem even with minute sample volumes (no more than was required to spread a film across area exposed to beam).

Came to conclusion sample had too much water. Suggested by a strong OH feature that shifted when examining sample dissolved in ethanol and PEG with no water.

*Axes are a bit off. Result of grouping files in OMNIC before saving the .csv files. Don't put too much stock on the relative values of the transmission axes.

A Change of Plans

Inability to make out features for the mixture of Laponite and PEO in water prompted us to shift to examining pNIPAM as a crossover between the two projects.

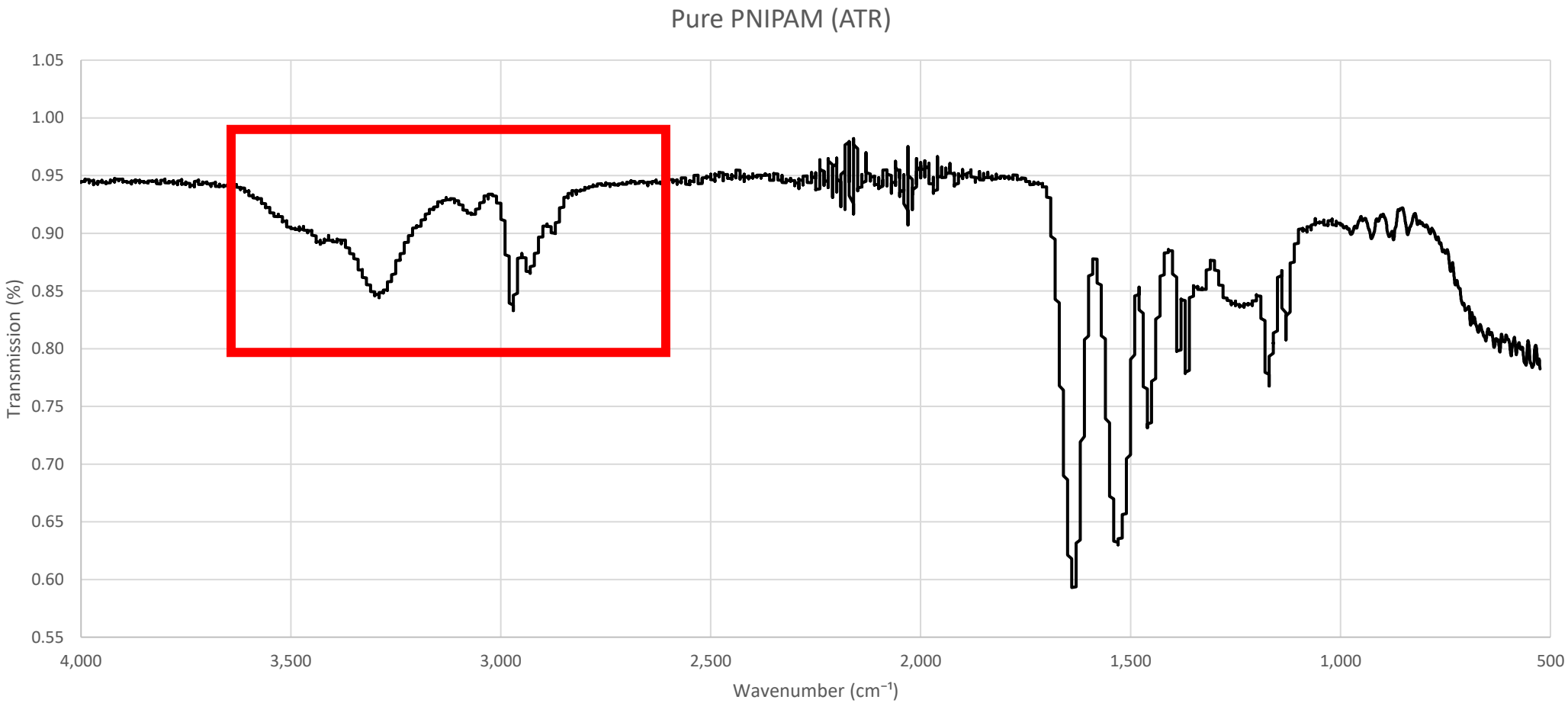
Some work has been done investigating pNIPAM under pressure. As previously mentioned, some work has been done on examining pNIPAM under high pressure.

Non-ionic polymers like PNIPAM find applications in diverse areas, and its response to T and P can be modulated by, e.g., co-polymerization or cross-linking:

- microfluidic chips
- nanoswitches
- smart biomaterials (injectable hydrogels)
- tissue engineering
- smart transport

A Change of Plans

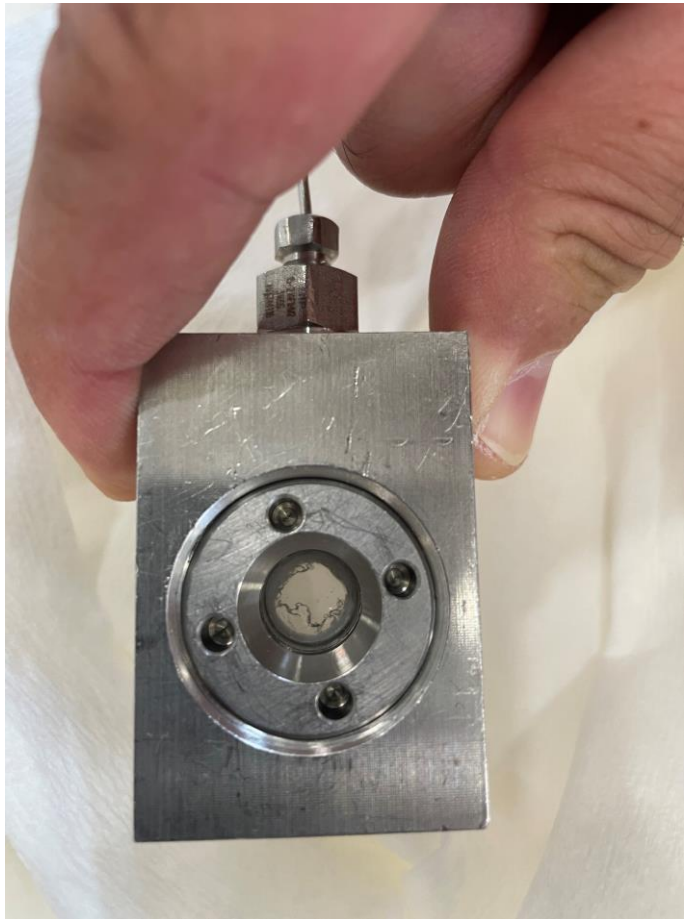
pNIPAM ATR Reference:



= Region of interest.

In-situ Pressure FTIR Experimental Design

HP SANS cell modified with sapphire slide cover cut-out was loaded with a sample of extremely high concentration pNIPAM to reduce water content.



Resulting gel was essentially a “taffy-like” consistency.

Gel was pressed against window of SANS cell and sapphire cutout to form a film.

System was pressurized using helium gas up for 100 bar and up to 300 bar using the syringe pump system.

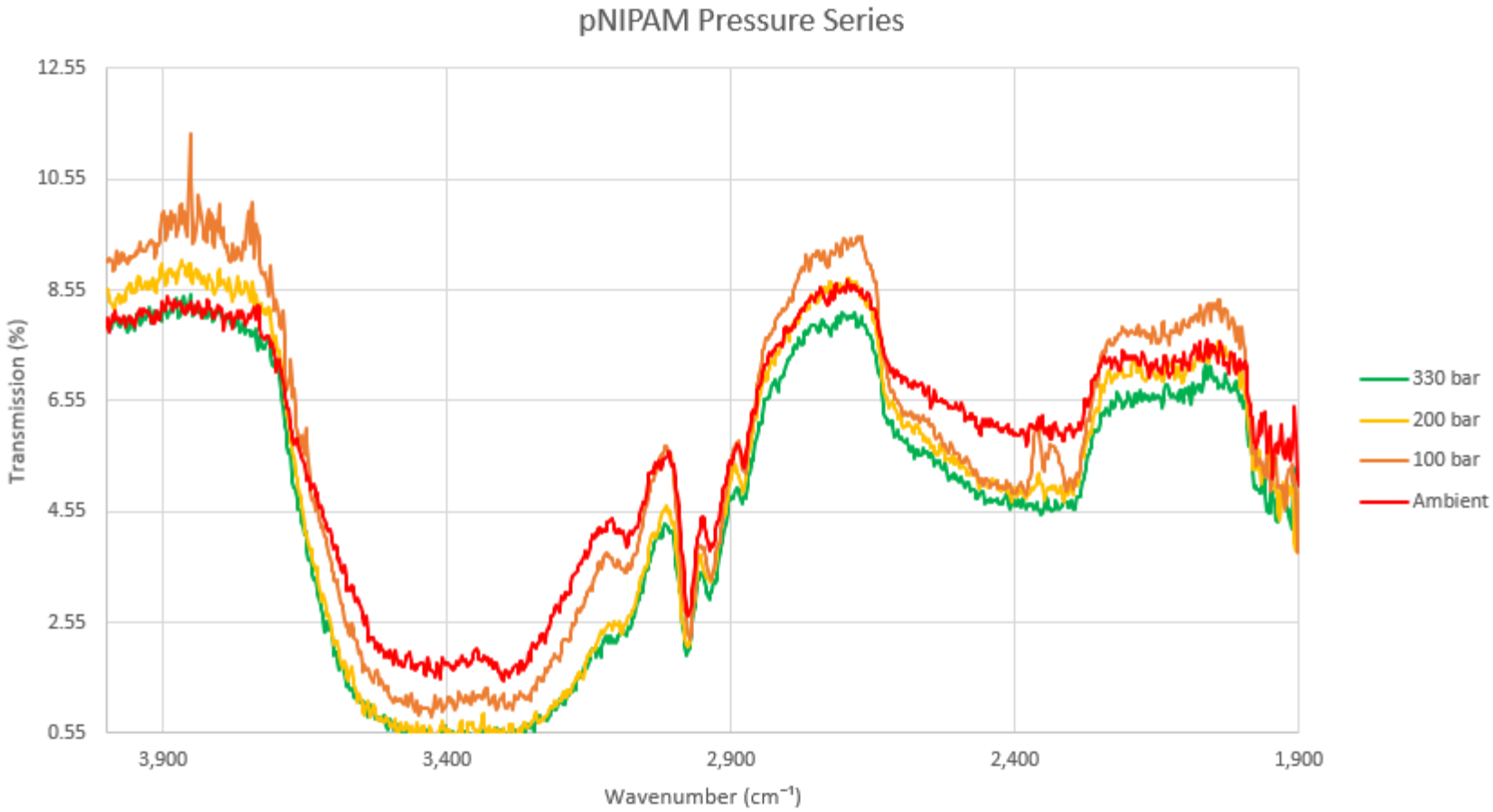
pNIPAM (*in-situ*) Pressurized FTIR

No meaningful shift was observed up to ~300 bar.

There could be effects occurring in the fingerprint region beyond sapphire window cutoff.

May need higher pressures.

→Neutrons! Don't have to worry about the IR cutoff from sapphire.



What Will I take Back to the Classroom?

1. Mindset of troubleshooting and tinkering is essential! Ran into many different problems throughout the summer project
   - SAXS issues regarding temperature automation
   - UV vis on the fritz
   - Many iterations of HP FTIR sample environment

2. Summer School Format
   - Rotation of experiments in groups was an effective strategy I can try in my classes

Acknowledgements

Some well deserved “Thank-you”s are in order.

- Yamali Hernandez for welcoming me back to another year in the RET
- Alex Grishaev for assistance and access to SAXS beam at IBBR
- Donna Kaltayer for her routine assistance in the lab spaces
- Juscelino Leão for the large swathes of time he devoted into trying lots of “crazy ideas” and innovating many solutions!

- Susana Teixeira for another year of being a fantastic mentor and friend

Thank you!


CAR 4
Card Reader Required to Access First Floor
IN CASE OF FIRE USE STAIRWAY DO NOT USE ELEVATORS
ELEVATOR
Integrated Safety Culture Traits &
Integrated Safety Culture
NBSR Fuel Element Assembly