Using ARGEN to Determine the Impact of Mechanical and Physical Stress

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The Stirring Problem:

There is intense interest among therapeutic protein researchers about factors which impact protein aggregation and stability. Aggregation of therapeutic proteins can render them biologically inactive or even induce an immunological response in patients. One of the well-known factors that can induce aggregation is stirring of samples. ARGEN provides the capability to directly probe contact stirring (i.e. magnetic stir bar) induced aggregation.

However, contact stirring is only one type of mechanical or physical stress that can occur during the production, purification and packaging of therapeutic proteins. Overhead stirring, capillary shear stress, filtration, peristaltic recirculation, etc. can all impact protein stability and induce aggregation. Since many or all of the listed mechanical and physical stressors can be present during therapeutic protein production, it is critically important to assess the impact of each stressor in a quantitative way.

So how does ARGEN provide the capability to examine other stressors besides contact stirring? Since ARGEN has 16 individual sample cells with fixed optical pathways that can all be operated independently, researchers can easily tailor experiments to match the conditions they wish to examine. With some basic laboratory equipment such as small peristaltic pumps, syringe pumps, tubing and filters, it is a simple matter to construct systems capable of probing the desired stressors using ARGEN.

Solving the Problem with ARGEN:

At PolyRMC, we are frequently tasked with researching the factors impacting protein aggregation, and ARGEN is an invaluable tool in this research. While thermal and contact stirring induced aggregation monitoring are built into the ARGEN platform, other examples of experiments that have been performed in our research group include:

- Non-contact stirring with overhead suspended magnetic stir bars controlled by the ARGEN software (3d-printed construction with inexpensive and readily available components, Figure 1)

- Examination of capillary shear through different tubing diameter and composition via reciprocating syringe pump (Figures 2 and 3)
- Non-contact stirring by overhead impellers controlled by stepper motors (motors and drivers are inexpensive and readily available)
- Recirculation of protein samples from sample cuvette via peristaltic pump (Figure 5)
- Recirculation of protein samples through differing filter materials via peristaltic pump
- Continuous extraction of protein samples from ARGEN through a secondary detector (MALS, fluorescence, UV, etc.) via syringe to provide complimentary analytical methods
- Continuous or discrete extraction of protein samples for GPC analysis with an autoinjector

Non-mechanical or physical stress applications of ARGEN:

- Direct titration in real time with salts, excipients, acids/bases, denaturation agents, etc.
- Generation of Debye plots (Figure 6)

Other experiments possible with ARGEN which PolyRMC has not examined:

- Continuous extraction and sample addition to an ARGEN cell from an externally shaken vessel
- Recirculation with external exposure to UV light or other radiation
- Recirculation with external exposure to ultrasound

Example Data:

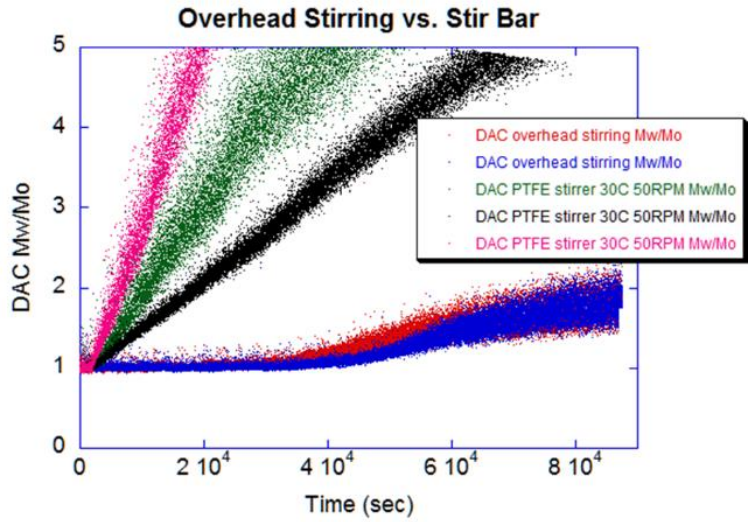

Figure 1: Non-contact stirring with overhead suspended magnetic stir bars controlled by the ARGEN software

Figure 1 shows the difference in aggregation rates (slope of Mw/Mo) between contact magnetic stirring and overhead non-contact stirring via stepper motor. Figure 1 clearly shows that changing the type of stirring stress applied to this sample of monoclonal antibody yields very different aggregation rates and profiles.

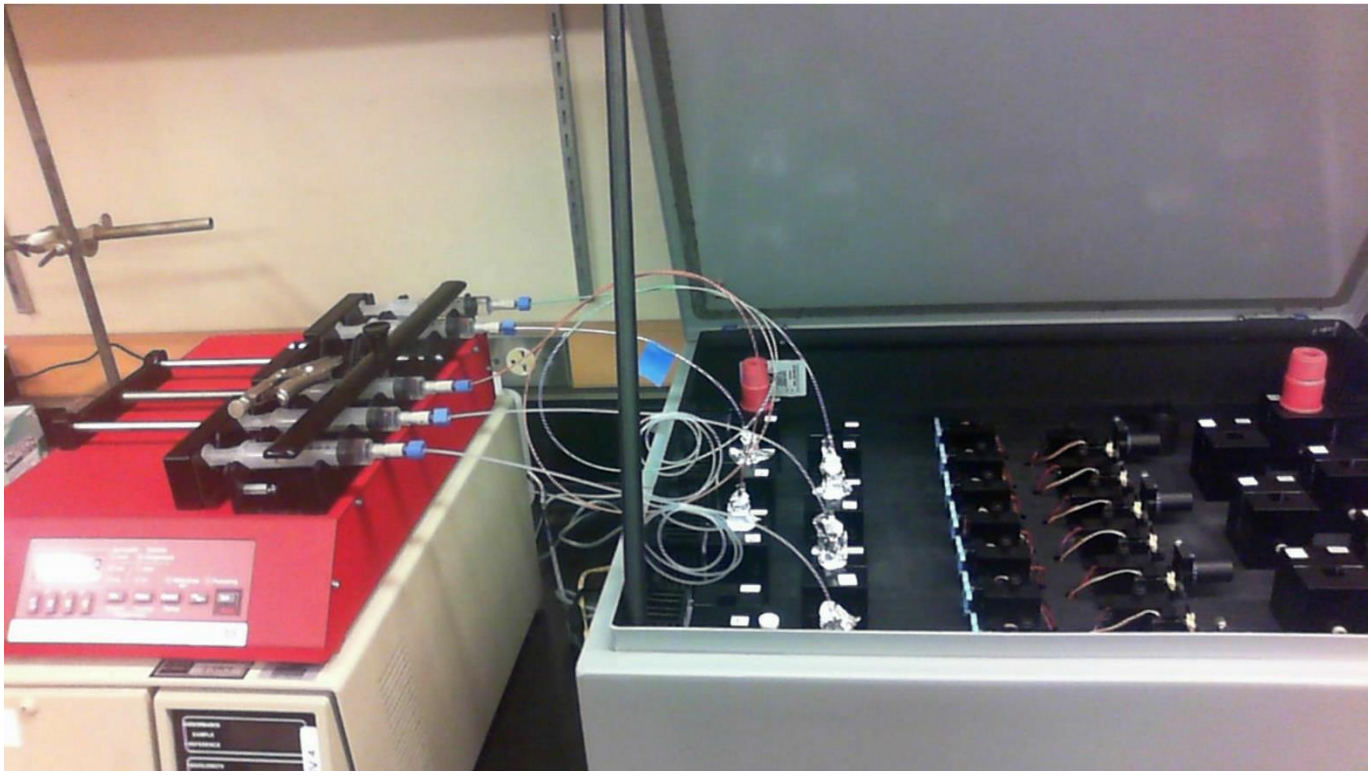

Figure 2: Shear experiment with syringe pump

Figure 2 is an experimental setup of a continuous shear experiment utilizing a syringe pump programmed to continuously alternate between inject and withdraw cycles at 1 ml/min to pass samples through capillaries and into sample cuvettes. An ARGEN prototype is depicted on the right in Figure 2.

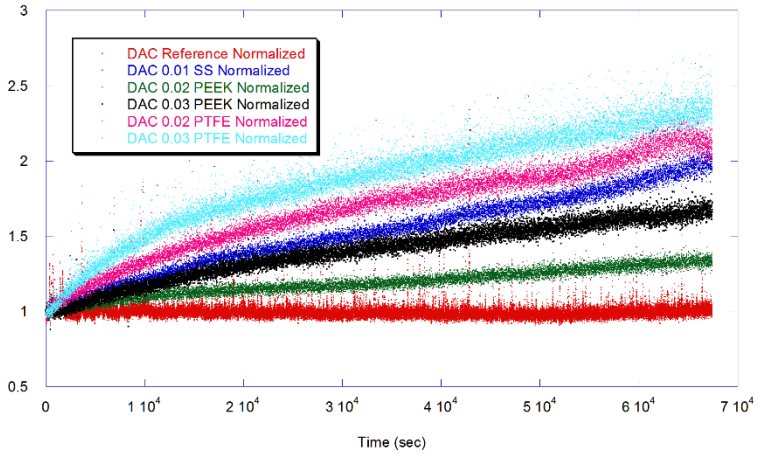

Figure 3: Differences in aggregation rates during capillary shear stress

Figure 3 shows the differences in aggregation rates observed during capillary shear stress of a monoclonal antibody. A mAb was continuously sheared through differing tubing diameters and materials at a flow rate of 1 ml/min via reciprocating syringe pump.

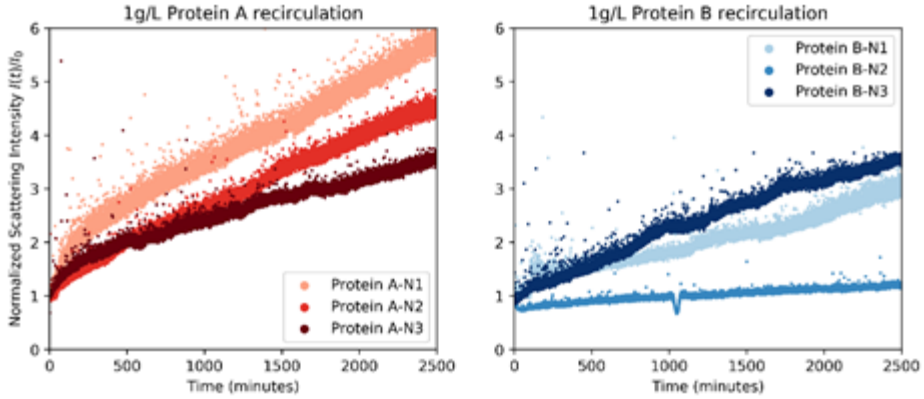

Figure 4: Aggregation induced by continuous peristaltic pump recirculation for two different therapeutic proteins.

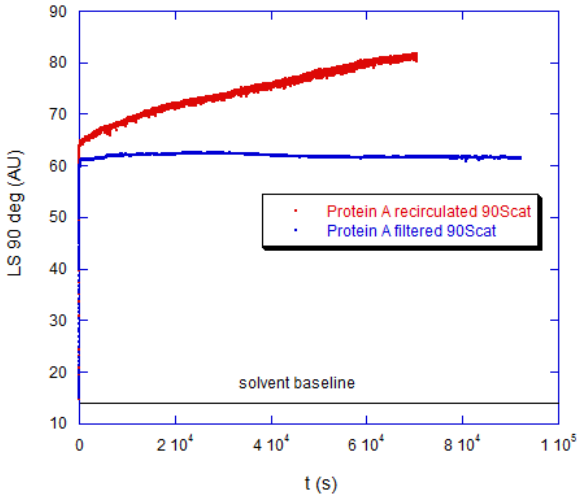

Figure 5: Recirculation of protein samples from sample cuvette via peristaltic pump

Figure 5 shows the use of a syringe filter place in inline during a peristaltic recirculation experiment (note, this data was acquired from a free standing 90° scattering cell but can also be obtained via ARGEN).

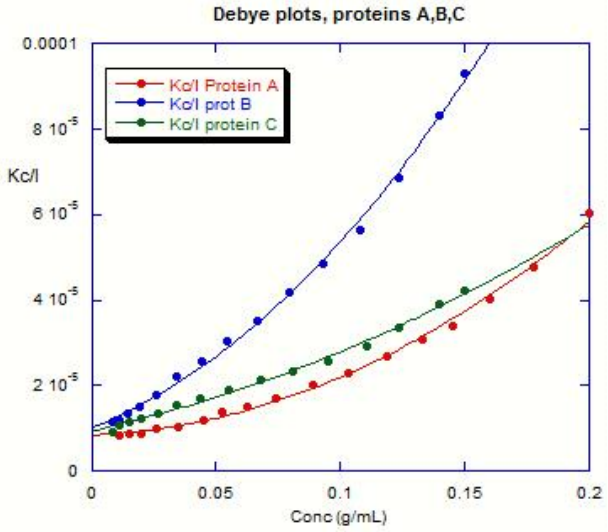

Figure 6: Debye plots

Figure 6 shows Debye plots generated by direct titration of three different protein samples in an ARGEN instrument.

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