Rapid Characterization of Polymers for Industrial and Pharmaceutical Applications using ARGEN™

Fluence Analytics

INTRODUCTION

Understanding the thermal, chemical and mechanical stability and physical properties of polymers is essential for development success. The case studies below outline the experimental methodologies and data analysis using ARGEN™ in order to characterize molecular weight as well as the effects of temperature, shear stress and solution conditions on the stability of an uncharacterized organic polymer (PS-A) and Bovine Serum Albumin (BSA). This data provides the pathway to expedite formulation development.

ARGEN™: SMART & RAPID THERAPEUTIC BIOPOLYMER DEVELOPMENT

ARGEN™ is a high throughput tool for rapid assessment of the stability and viability of therapeutic proteins, peptides, and biopolymers. The instrument uses a multi-stressor testing platform powered by static light scattering detection and intuitive data processing. These features enable teams to develop biologic formulations up to 16-fold faster.

HOW ARGEN™ WORKS

ARGEN™ utilizes fixed angle (90°), simultaneous multiple sample light scattering (SMSLS) technology which provides rapid, real-time, continuous data collection for characterizing qualitative and quantitative properties of target molecules. The device is equipped with 16 independently controlled sample cells, permitting the user to establish thermal, chemical, and mechanical (stirring) stress parameters on each sample concurrently. This allows for a highly flexible approach to experimental design.

ARGEN™ INTUITIVE CONTROL SOFTWARE

The ARGEN™ control software features an intuitive interface for all aspects of experimental design and independent control of each cell for parallel parameter adjustment and real time data processing.

EXPERIMENTAL METHODS

POLYMER CHARACTERIZATION

Two Polystyrene (PS) standards, 16,500 g/mol and 185,000 g/mol and an uncharacterized PS sample (PS-A) were serially diluted with tetrahydrofuran (THF) from 15.0 mg/mL to 0.166 mg/mL, generating an 8-point Debye plot for extrapolation of absolute molecular weight of each sample using the Debye equation:

$$\frac{K \cdot c_p}{I(\theta)} = \frac{1}{M_W}\left(1 + \frac{q^2\langle S^2\rangle_Z}{3}\right) + 2A_2 \cdot c_p$$

MONITORING POLYMER DEGRADATION

Samples of polystyrene (PS-A) were dissolved in THF at a [PS-A] = 10 mg/ml, placed into cuvettes and loaded into ARGEN™ sample cells. These samples were then thermally stressed at a temperature range from 30°C- 55°C for 15 hours to monitor degradation. A subsequent experiment was performed with stirring (mechanical stress) at 100 RPM and thermally stressed from 30°C-55°C for the entirety of the experiment. Finally, 3 samples of PS-A were dissolved in toluene (Tol), butyl acetate (BAc), or THF and allowed to degrade at 55°C with a stirring rate = 100 RPM for 10 hours.

MONITORING BIOPOLYMER (PROTEIN) DEGRADATION

Using Bovine Serum Albumin (BSA) as a model protein for aggregation and degradation, a [BSA] = 2 mg/mL sample was prepared in 50 mM phosphate. The pH of each solution was adjusted, providing a pH range = 1.54-5.85 (5 data points) with a final [BSA] = 1 mg/ml. Each sample was heated to 37°C for 5 days using the temperature control feature of the ARGEN™.

DATA INTERPRETATION AND ANALYSIS

POLYMER CHARACTERIZATION

Light scattering intensity was recorded for each dilution prior to data collection. Each dilution was analyzed`and monitored for at least 3 minutes to establish a stable baseline signal (**Figure 1 - inset**).

Debye analysis was used to determine the initial molecular weight of each sample required several

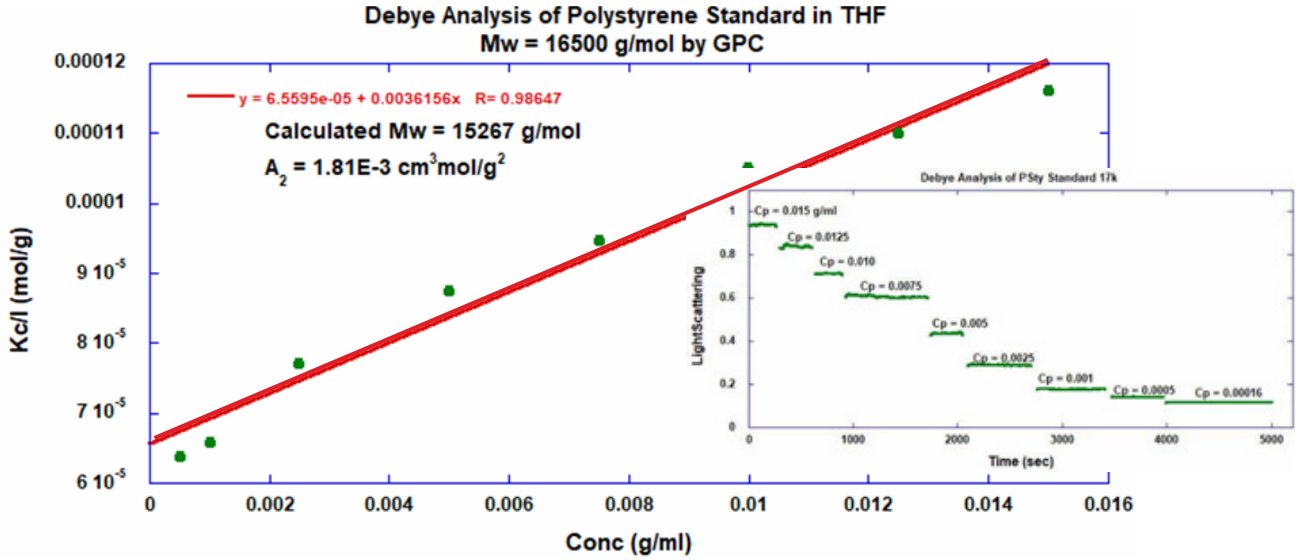

Figure 1: Debye analysis of a 16,500 g/mol polystyrene standard in THF. The data is fit using a linear least squares regression. The y-intercept yields the reciprocal of the molecular weight. [Inset] Baseline scattering intensities

assumptions. If $q^2\langle S^2\rangle_z \ll 1$ (true for most polymers) and the sample is diluted such that, then the equation can be simplified to:

$$\frac{K \cdot c_p}{I(\theta)} = \frac{1}{M_W}$$

When rearranged for single angle analysis at 90° and solving for weight averaged molecular weight the equation becomes:

$$M_W = \frac{I(90°)}{K \cdot c_p}$$

Using this equation and a linear fit of the dilution data (**Figure 1**), the 16,500 g/mol and 18,500 g/mol standards were calculated to have molecular weights of 15,300 g/mol, 193,000 g/mol, respectively. The unknown PS sample was determined to have a molecular weight of 5,500 g/mol (**Figure 2**).

POLYMER DEGRADATION

The degradation curves were normalized to the initial mass (of polymer) and normalized molecular weight was plotted vs. time. In the absence of

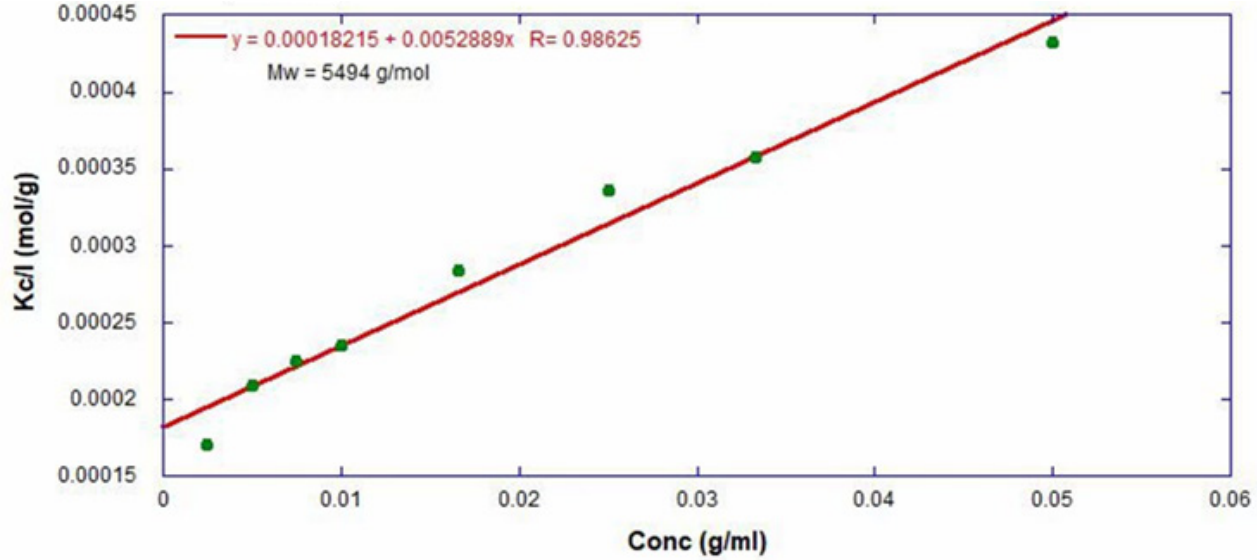

Figure 2: Debye analysis of a polystyrene sample with unknown molecular weight. The molecular weight was determined to be 5,500 g/mol.

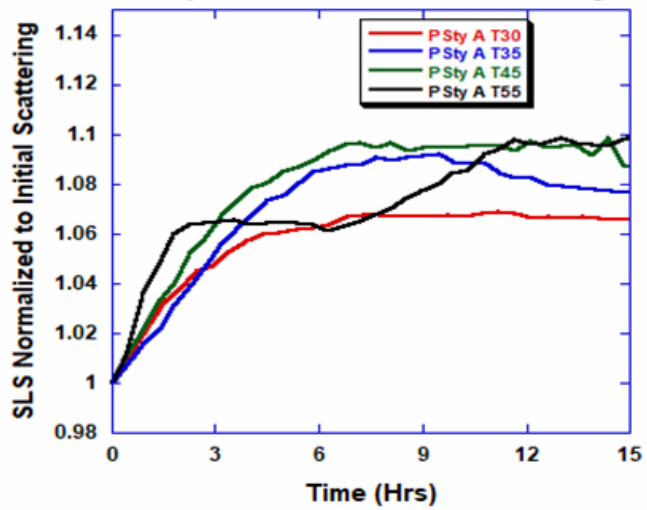

Figure 3: Dissolution profiles for PS-A at different temperatures with no stirring

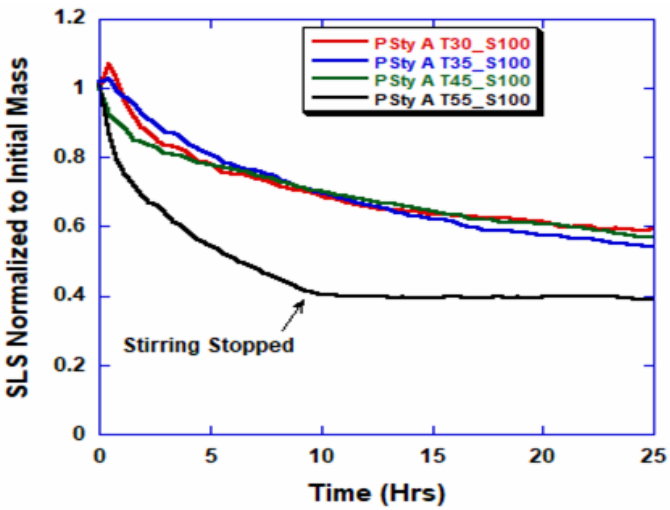

Figure 4: Degradation profiles for PS-A at different temperatures with stirring at 100 RPM

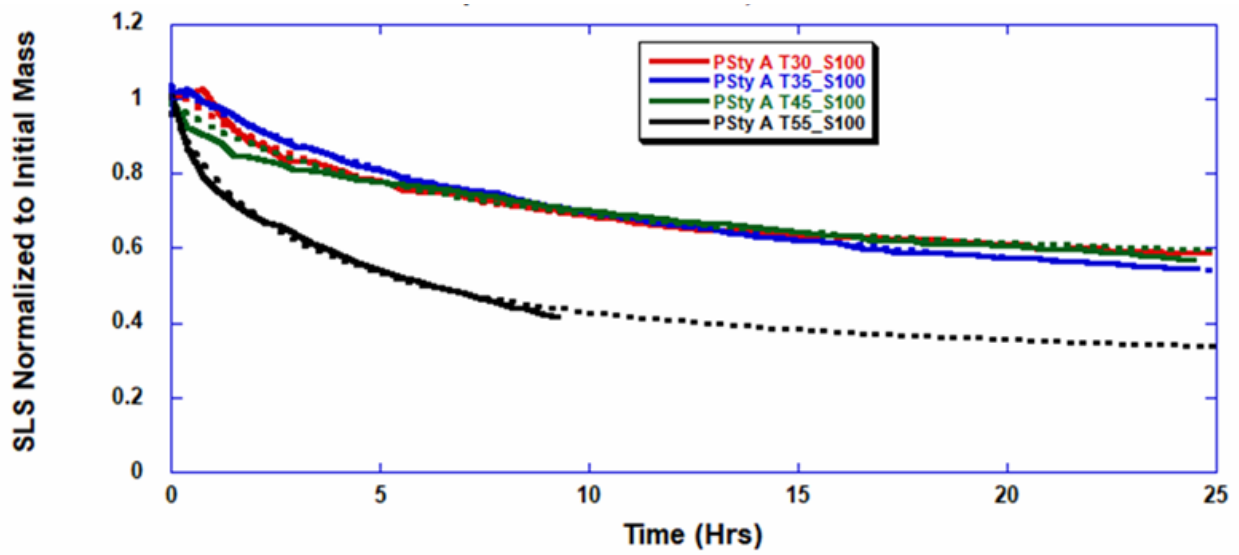

Figure 5: Polystyrene-A degradation at different temperatures with stirring at 100 RPM was fitted with a double exponential

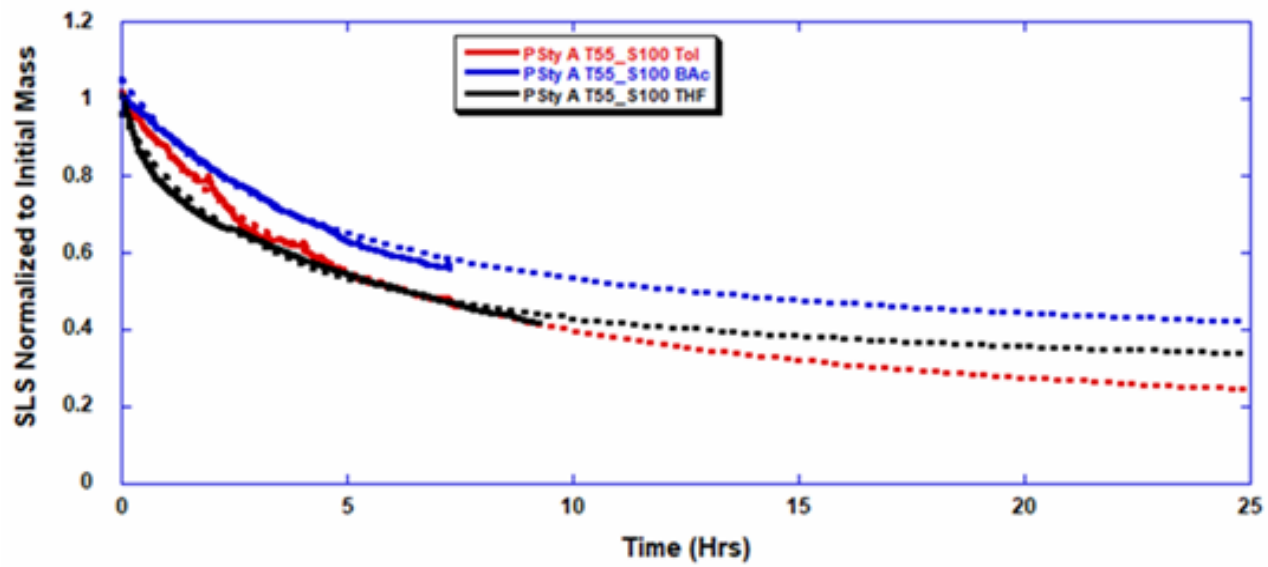

Figure 6: Polystyrene-A degradation in different solvents with stirring at 100 RPM was fitted with a double exponential

mechanical stress (stirring) (**Figure 3**), the polymer was soluble at all temperatures without any observed change in molecular weight and no indication of degradation. In the presence of mechanical (stirring) and temperature (heat) stresses, all samples were readily soluble within two minutes and degradation was observed immediately as indicated by the change in light scattering signal. Samples subjected to temperatures from 30°C - 45°C displayed a similar degradation profile (**Figure 4**).

At temperatures >55°C, the polymer dissolved rapidly (**Figure 3**). As previously noted, the removal of mechanical stress (stirring) halts degradation as evidenced by the change in light scattering intensity. Furthermore, the increase in degradation rate observed in the temperature range = 45°C to 55°C is indicative of a significant thermodynamic barrier related to the depolymerization of polystyrene (**Figure 5**).

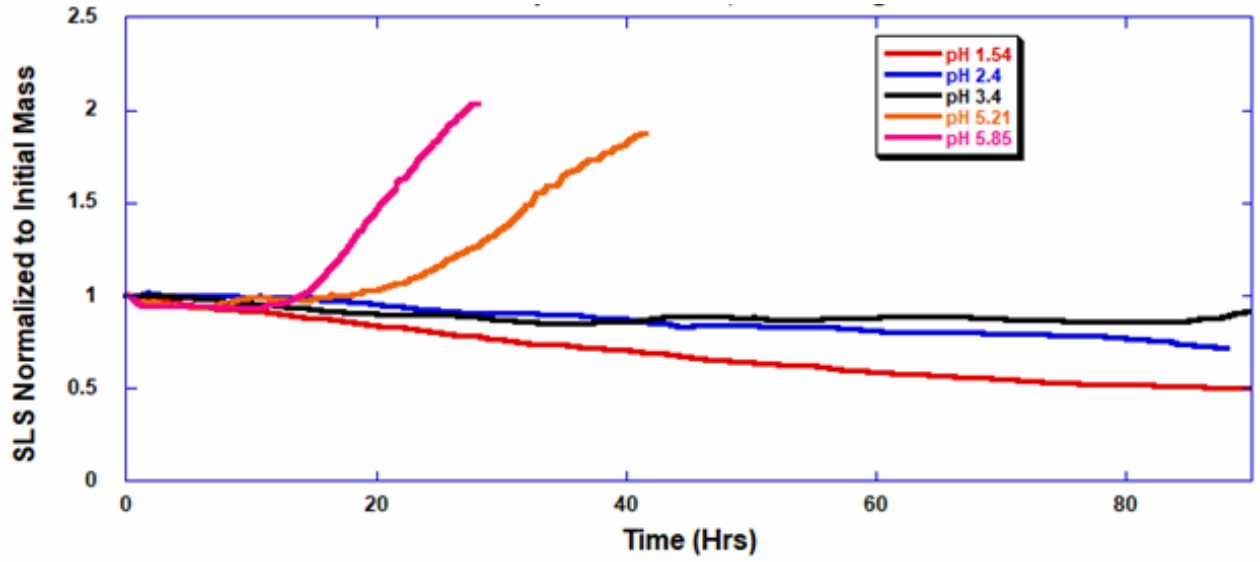

Figure 7: BSA degradation by acid hydrolysis at 27°C with no stirring

SOLVENT EFFECTS ON POLYMER STABILITY AND SOLUBILITY

THF and toluene displayed differential effects on PS-A stability and solubility. PS-A dissolved in THF degraded more rapidly while toluene proved to be a more robust solvent indicated by a more steady rate of solvolysis. Based on the double exponential fitting, polystyrene would be less stable in toluene than THF after 10 hours (**Figure 6**). This phenomena would require further investigation as these organic solvents are not known to participate in the electron transfer required to reduce the polyolefin bonds. Potential impurities in the sample or shear stress may be the root causes of PS-A degradation.

BIOPOLYMER DEGRADATION

The isoelectric point is the pH (of the solvent or buffer system) at which a biopolymer has no net charge. The isoelectric point of BSA is pH 4.8, and the protein is practically insoluble due to the lack of electrostatic repulsion at a pH 4.8 (buffer system). Upon acidification or basification of the solvent or buffer system containing BSA, the protein becomes either positively (acidic buffer) or negatively (basic buffer) charged, often resulting in an increase in colloidal stability and solubility via electrostatic repulsion.

Under thermal stress at pH 5.85 and 5.21 (BSA is negatively charged), the protein aggregates. At a pH ≤ 3.40, acid-catalyzed hydrolysis occurs resulting in protein fragmentation (creation of peptides) as observed by a decrease in the normalized molecular weight (**Figure 7**). Between pH 3.40 and pH 1.54, there is a four-fold increase in protein degradation rate due to an increase in the rate of acid-catalyzed hydrolysis.

CONCLUSION

ARGEN™ was used to successfully determine the molecular weight, elucidate the thermal, mechanical and chemical stability of an uncharacterized polymer and monitor the solution behavior of bovine serum albumin under several different pH conditions. The molecular weight of PS-A was elucidated by Debye analysis using data generated by the ARGEN™. Data collected on thermal, mechanical and chemical stressors provided vital information on polymer biopolymer stability. ARGEN™ proved to be a powerful tool with the capability to analyze the stability of both organic polymers and biopolymers in real time and provide the user with the data needed to expedite the development process and formulation development.