

# **Chapter 3: COMPARING THE SELF-AGGREGATION POTENTIAL OF BOVINE SERUM ALBUMIN TO HUMAN SERUM ALBUMIN USING LASER LIGHT SCATTERING**

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## **3.1 Abstract**

Albumin is commonly deposited on a range of surfaces as a blocking protein and the structural rearrangement that follows can provide the driving force necessary for aggregation. A comparison of the self-aggregation potential between human serum (HSA) and bovine serum albumins (BSA) adsorbed onto polystyrene microspheres was made. A z-axis translating laser light scattering (ZATLLS) device recorded the sedimentation velocity of protein-bound particles. Once sedimentation velocity, solution density, and solution viscosity were measured, Stoke's law was used to infer the average aggregate size. Although HSA-coated and BSA-coated particles did not differ notably in sedimentation velocity, a significantly larger aggregate size was found for BSA-coated particles due to differences in desorbed protein found in solution viscosity and density. Since HSA-coated particles have lower self-association, it is the preferred surface coating relative to BSA.

### **3.2 Introduction**

Proteins control many cellular functions including binding, communication and trafficking, and apoptosis. If the protein structure rearranges, protein function can be compromised. Protein misfolding can be caused by primary structure mutations or other environmental stresses acting on the protein [1]. The aggregation of proteins marks a milestone in the progression of age-related diseases such as Alzheimer's, Parkinson's, and other amyloid diseases that lead to neuronal cell death [1-3]. In 2007 it was estimated that over five million Americans have Alzheimer's disease, while one million have been diagnosed with Parkinson's disease [4, 5]. Aggregation potential increases with protein age, chemical or thermal denaturing, or through the failure of chaperone molecules to guide proper conformation. Misfolded proteins can later assemble into larger insoluble clusters called plaques [1-3].

Protein aggregation has been measured using various techniques including both static (SLS) and dynamic (DLS) light scattering forms [6]. While spectrofluorimetry and analytical centrifugation are two established DLS techniques to measure the level of protein aggregation, our lab uses an alternative method called Z-axis translating laser light scattering (ZATLLS) [7, 8]. This device consists of a moving platform with an attached laser, detector, motor, and magnetic counter to determine the distance the platform is raised or lowered [2, 9]. The ZATLLS device has already been used to measure BSA aggregation when coated on polystyrene particles both alone and activated by transglutaminase in solution [2, 9].

Albumin is one of the most prevalent proteins found in both the bloodstream and cerebrospinal fluid [10, 11]. It functions as a transporter protein by binding to an assortment of endogenous and exogenous molecules [12, 13]. BSA and HSA are two of the more common species of serum albumins but albumin can be derived from a host of other species with subtle variations in amino acid sequence and length [11, 14, 15]. Both BSA and HSA are commonly used in experimental studies involving drug delivery, cell survival, and in the modeling or inhibition of aggregation [16-24]. BSA is also used frequently in blocking studies where it coats surfaces to prevent other proteins from attaching, such as in micropatterning, ELISA assays, or biochips [25-27]. However, BSA has displayed some level of aggregation when exposed to unfavorable exogenous

conditions or when activated by another molecule such as an enzyme [1, 6, 28]. The aggregation potential of HSA has not been reported and according to our knowledge, no comparison between these two albumins has been done.

BSA and HSA are very similar globular proteins that perform the same functions; however BSA has 582 amino acid residues while HSA has 585. There are also differences in the amino acid composition and charge between BSA, -17, and HSA, -15 [11, 14, 15]. These differences could affect conformation, which ultimately could regulate the driving force for aggregation of the protein. In this study, we evaluated whether BSA-coated polystyrene particles had more self-aggregation than HSA-coated particles to gauge whether the more widespread use of BSA in blocking studies is warranted.

### 3.3 Materials and Methods

#### 3.3.1 ZATLLS

Polystyrene (PS) particles (poly(styrene with 2% divinylbenzene)) (37-74  $\mu\text{m}$ ) were purchased from PolySciences (Warrington, PA) and used as received. BSA (96% fatty acid free) and HSA (96% fatty acid free) were purchased from Sigma (St. Louis, MO) as powders and used as received. Twenty mL of a 0.1M borate buffer solution (pH 8.5) was added to 0.5 g of PS particles. The particles were vortexed for dispersion and centrifuged to recover them. Particles underwent protein physisorption by adding 15 mL of either a 10 mg/ml BSA-borate buffer solution or a 10mg/ml HSA-borate buffer solution and allowed to incubate overnight at room temperature. The solution was then re-centrifuged to isolate protein-bound PS particles. Next, 25 mL of a 16% (v/v) glycerol-water solution was added to the protein-coated particles and the sedimentation experiments were carried out [2, 9]. The density of the solution controlled the rate at which the particles settled in the dispersion.

A rectangular glass column containing the solution was fixed in the ZATLLS device (Figure 1) [9]. User defined values were inputted in LabVIEW (National Instruments, Austin, TX, USA) for both the time interval between each scan, approximately six minutes, and the scanning height. The voltage, recorded by the photodetector as a function of time and position, was measured for each scan as the dispersion clarified over

approximately three hours. The solution was decanted after each sedimentation experiment from which fluid density and viscosity specimens were collected and analyzed. [2, 9].

### 3.3.2 Viscosity and Density

An AR-G2 rheometer (TA Instruments, DE) with a 60 mm cone and plate geometry was used to measure the viscosity from the liquid saved at the end of each sedimentation experiment. The fluid density of the decanted solution was measured with a DA-300M density meter (Mettler-Toledo, Inc., Columbus, OH) [2, 9].

## 3.4 Results

Multiple sedimentation experiments of BSA- or HSA-coated particles were completed to yield a sample size of seven. Both the height and voltage values in the upward direction were recorded for every scan; therefore, a height of 16 cm corresponds to the top of the glass tube. The velocity for each run was found by tracking the clarification of the solution in the column (Figure 1). Sedimentation velocity voltage data for each height at every time interval was smoothed in Microsoft Excel. Four photodetector voltages, 0.33 V, 0.34 V, 0.35 V, and 0.36 V, were arbitrarily utilized as markers in the analysis. These horizontal marker lines allowed specific height values from figure 1 to be identified at each time and plotted for a height vs. time graph (Figure 2). To illustrate this process, shapes were placed on the second scan interval as it crossed each of the four isovoltage values. A smoothing routine in Microsoft Excel was employed to reduce noise by removing spurious measurements that fluctuated over a wide range of voltages between adjacent data points. Linear trend lines were then fitted to each of curves and the four corresponding slope values were averaged for a final sedimentation velocity for each experiment.

Using Stoke's Law,

$$D^2 = \frac{vb^3\eta}{4\Delta\rho g}$$

average aggregate size can be determined assuming spherical particles move in a creeping flow regime. The estimated aggregate diameter, D ( $\mu\text{m}$ ), is determined using the sedimentation velocity, v, a dimensionless laminar flow variable, b =24 in creeping flow, the viscosity,  $\eta$ , the density difference,  $\Delta\rho$ , between the solution from that of the particle (1.0500 g/cm<sup>3</sup>), and gravity, g [2, 9, 29]. Table 1 lists the average values for the solution density, solution viscosity, particle sedimentation velocity, and calculated average aggregate size excluding any experimental data that fell outside one standard deviation from the mean. Standard deviations of viscosity and density were calculated but are not shown since such small variations were found. A Welch's t-test was used to compare the significance of the average particle sizes of BSA (n=7) and HSA (n=7) which was deemed statistically significant at p < 0.01.

Viscosity of HSA and BSA at concentrations of 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 mg/ml, and 0 mg/ml in a 16% (v/v) glycerol-water solution were measured. The results were then plotted to form a calibration curve and a linear trend line was fitted to each albumin species (Figure 3). The resulting equation was used to determine the concentration of each albumin for the average measured viscosity. BSA-coated particle solutions had an average viscosity of 2.89 mPa-s, which is approximately 4.41 mg/ml BSA. HSA-coated particle solutions had a 2.00 mPa-s average viscosity corresponding to 1.41 mg/ml HSA. Therefore, more BSA desorbed from the particles during the experiment than HSA.

### 3.5 Discussion

Coating PS particles with either BSA or HSA yielded the same average sedimentation velocity, although it would be misleading to conclude they have a similar clustering effect. BSA has already been shown to aggregate using both this system and with atomic force microscopy [2, 9, 30]. Each BSA-coated particle experiment displayed similar sedimentation characteristics as shown in figure 2a and HSA-coated particle experiments exhibited similar results to figure 2b. After sedimentation, solutions using BSA had a measured average viscosity almost 50% higher than similar solutions in which HSA-coated particles were used, 2.89 vs. 2.00 mPa-s. This difference causes BSA-coated particles to settle slower due to a larger viscous drag on formed agglomerates (Table 1).

HSA and BSA are considered “soft” proteins due to their tendency to adsorb onto most surfaces since conformational entropy increases [31]. Although these albumin molecules are remarkably similar, we observe a difference in self-aggregation potential. This dissimilarity could be due to differences in amino acid number and composition [11, 14, 15]. These variations affect both the 3-D arrangement and the charge of the protein [14, 32]. Also, conformational changes are generated when albumin is bound to a surface [12, 31]. Silica particles, alumina particles, and polystyrene surfaces have all been shown to alter the structure of BSA [33, 34]. The divergence in aggregation potential between HSA and BSA could be caused by differences in amino acid composition, charge of the protein, and structural changes induced by adsorption.

### **3.6 Conclusion**

All sedimentation velocity experiments performed displayed little variation between BSA- and HSA-coated polystyrene particles, but a significant difference is seen in average aggregate size. This difference is attributed to the higher viscosity and density measurements of the residual solution in the BSA-coated particle experiments. HSA is less likely to bind to itself compared to BSA therefore, leaving it available to bind other molecules. Since HSA undergoes less self-association, it warrants more consideration as the preferred blocking protein over BSA.

### 3.7 References

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### 3.8 Figures and Tables

**Table 1:** Average values are shown for the sedimentation velocity range, density, viscosity, and the calculated particle size for BSA-coated ( $n=7$ ) and HSA-coated particles ( $n=7$ ). Data that fell outside one standard deviation of the average particle size were excluded.

**Figure 1:** Single sedimentation graphs are shown to represent the BSA-coated polystyrene particles (a) and HSA-coated particles (b). The four horizontal lines on each graph represent the four randomly assigned isovoltage markers (.33, .34, .35, and .36 V) used to determine the height where each scan crossed. For example, black shapes encircle the height at which the highlighted fourth scan transverses these markers.

**Figure 2:** The height values in figure 2 and time are plotted to ascertain the sedimentation velocities. A representative single experiment is shown for the BSA-coated particles (a) and the HSA-coated ones (b) although similar results were obtained from each experiment.

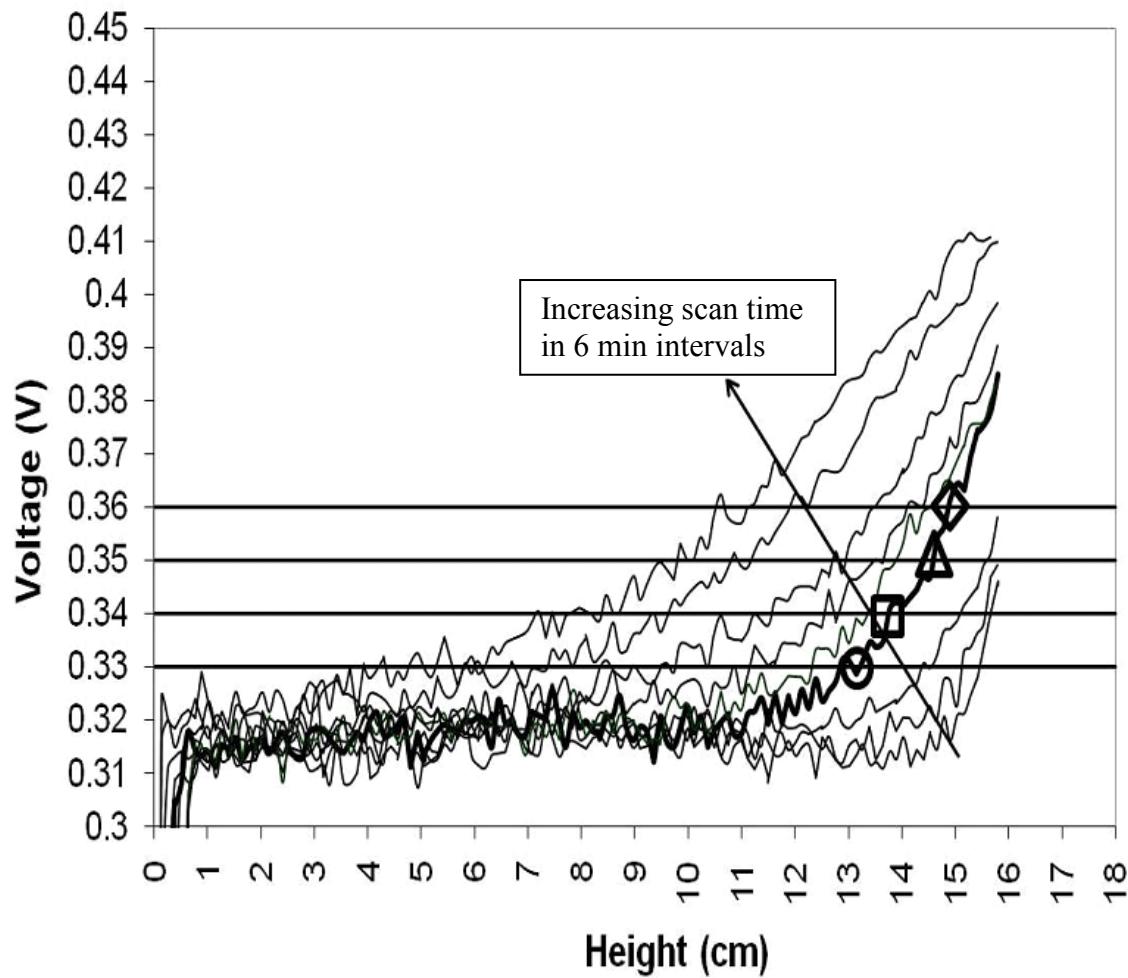
**Figure 3:** Calibration curves for BSA (a) and HSA (b) dissolved in 16% (v/v) glycerol-water solutions are shown. Measured average viscosity from BSA-coated and HSA-coated particle experiments were 2.89 mPa-s and 2.00 mPa-s respectively. The corresponding albumin concentration is 4.41 mg/ml for BSA-coated particle solutions and 1.41 mg/ml for HSA-coated particle solutions.

Table 1

	Sedimentation Velocity ( $\mu\text{m}/\text{s}$ )	Density ( $\text{g}/\text{cm}^3$ )	Viscosity ( $\text{mPa*s}$ )	Particle Size ( $\mu\text{m}$ )
BSA-coated particles (10 mg/ml)	$28.69 \pm 4.84$	1.0372	2.89	$107 \pm 14$
HSA-coated particles (10mg/ml)	$26.49 \pm 8.74$	1.0354	2.00	$81 \pm 9$

Figure 1

a)



b)

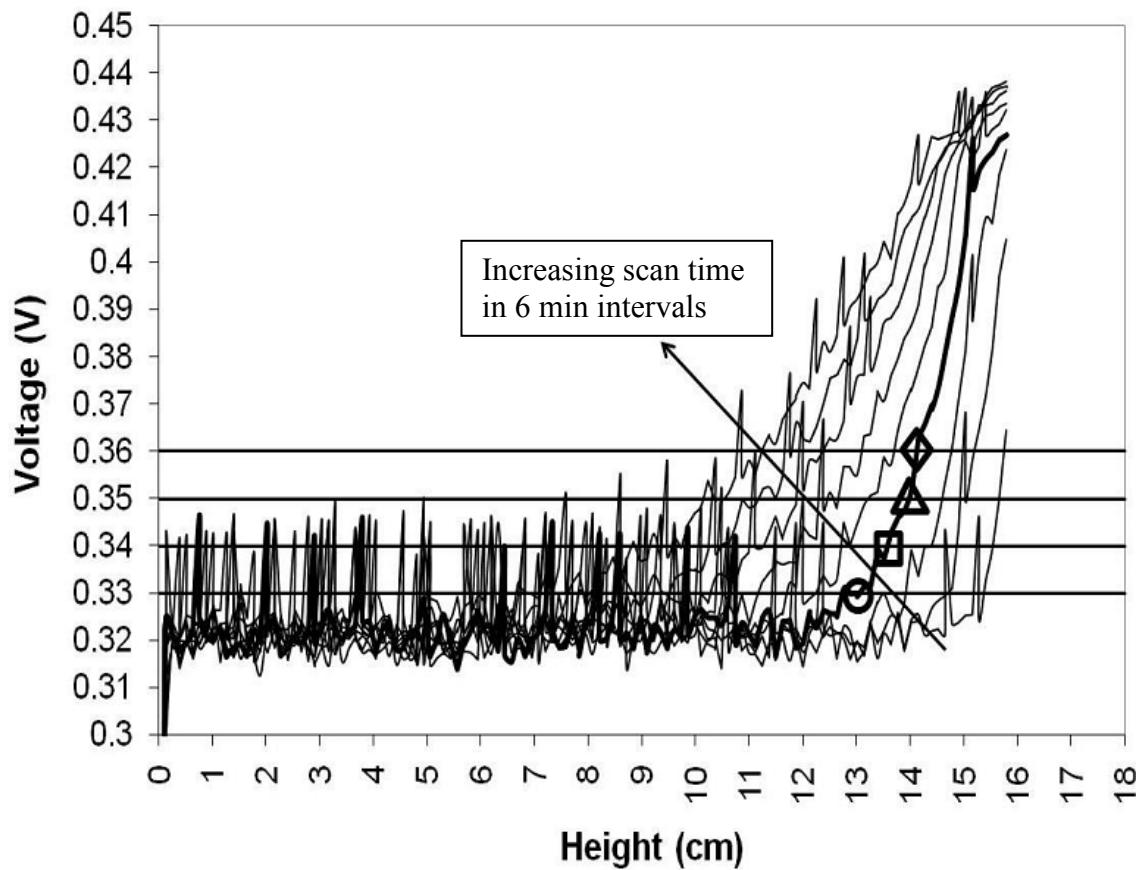
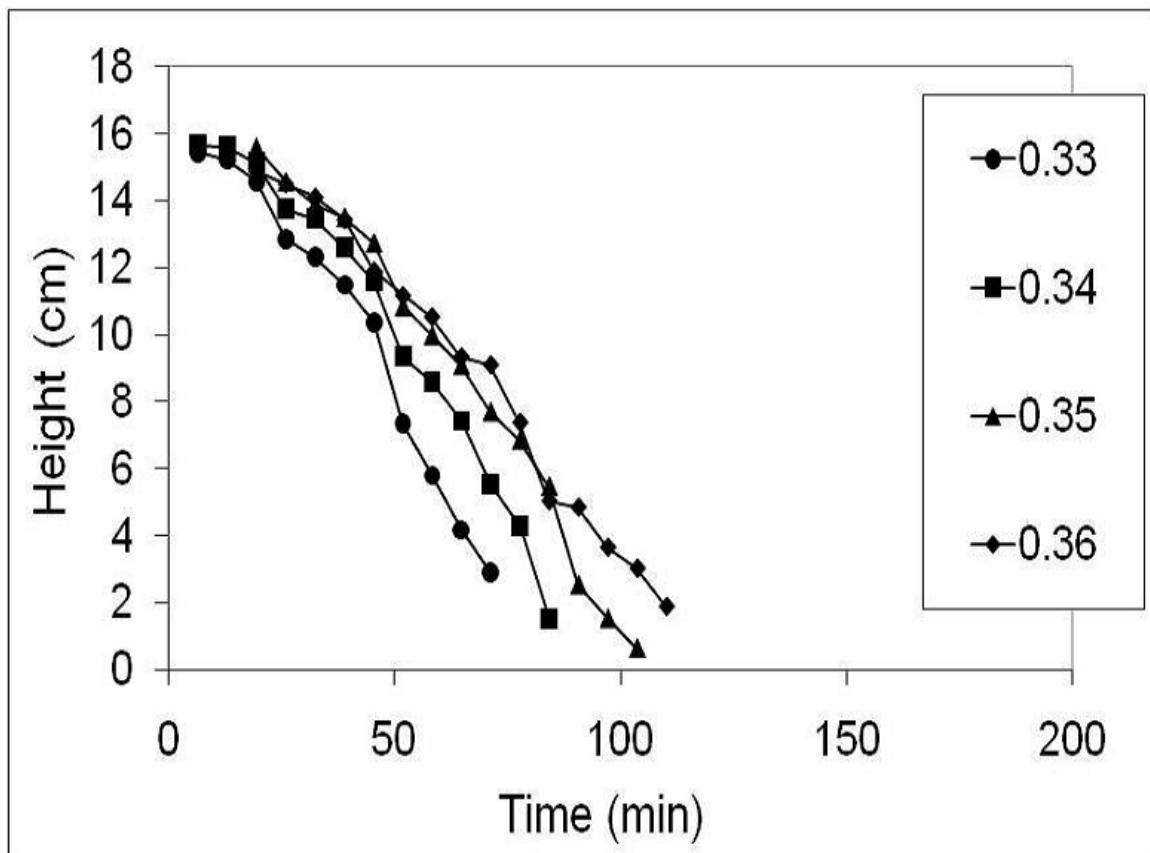


Figure 2

a)



b)

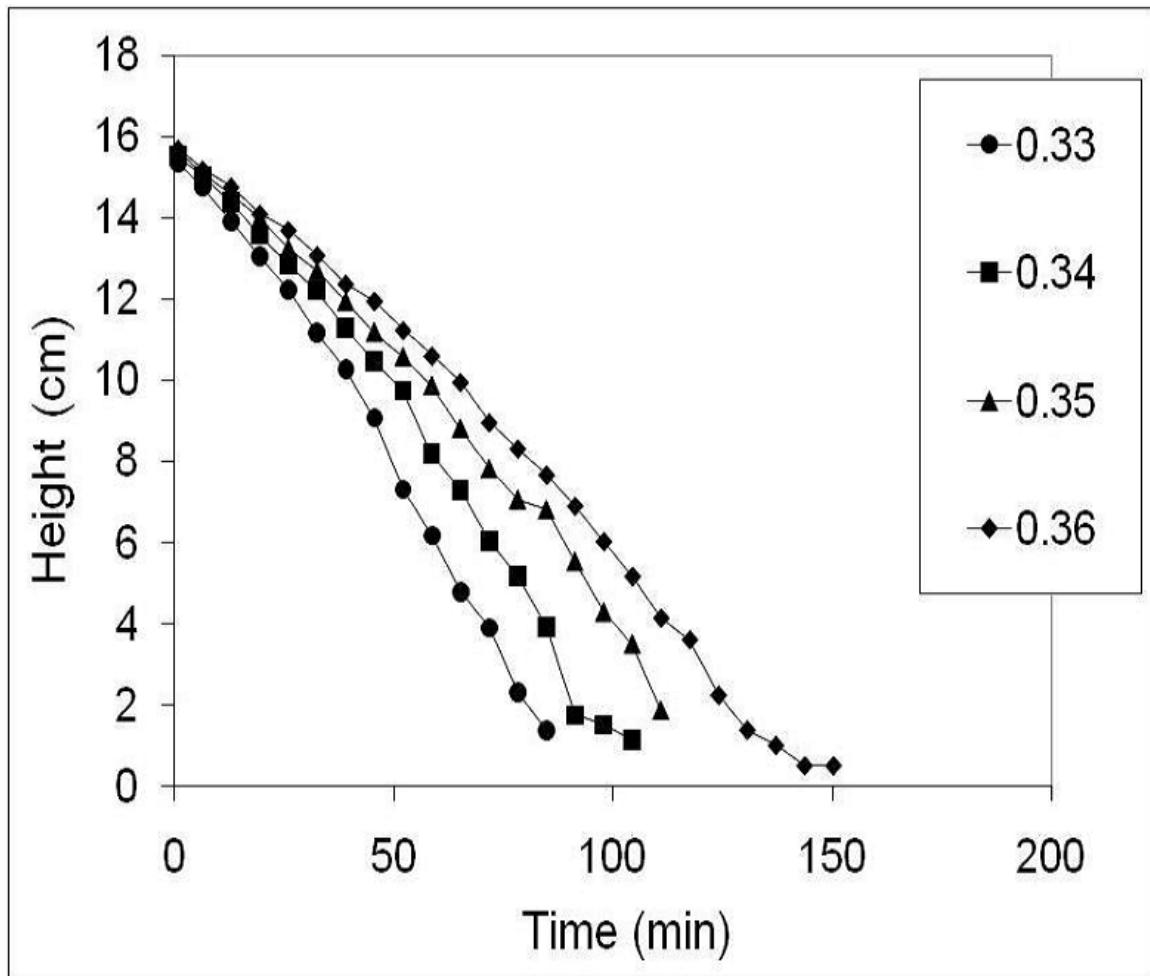
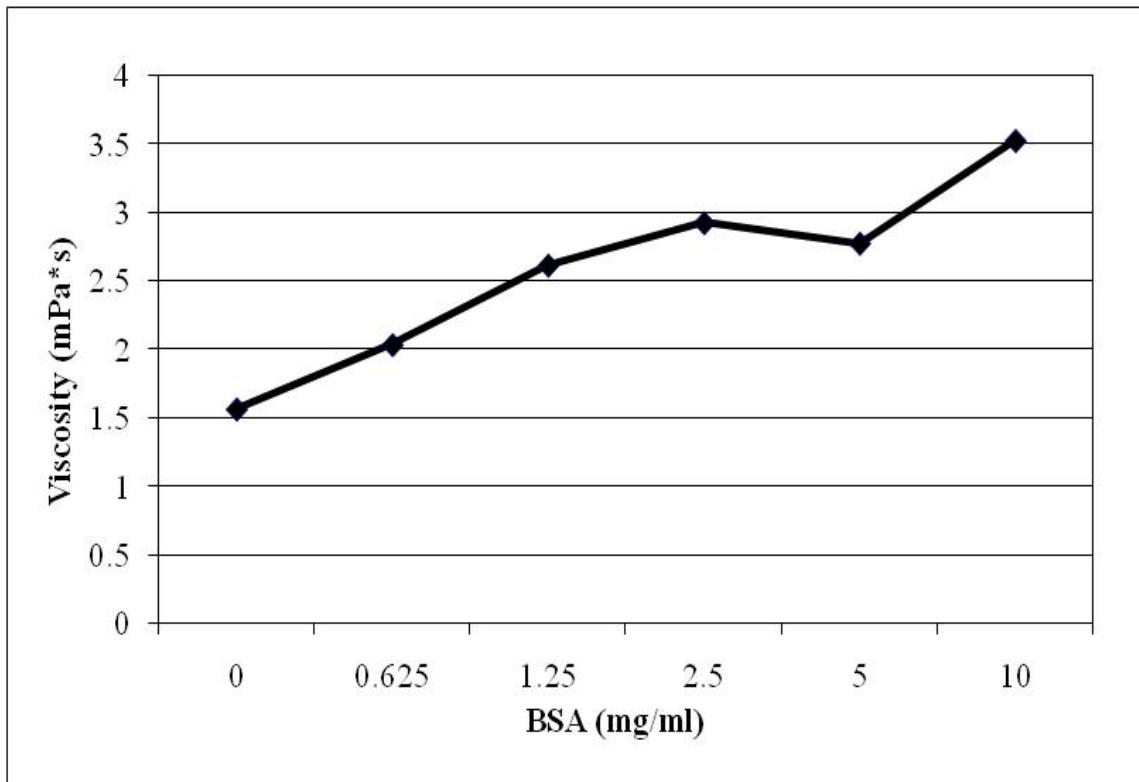


Figure 3

a)



b)

