Chapter 5: Conclusions and Future Work

5.1 Conclusions

The main purpose of this project was to assess the self-aggregation potential of albumin adsorbed on polystyrene microparticles. Sedimentation velocity of the protein-coated particles was measured using a DLS instrument called ZATLLS. At the end of each experiment, the solution was saved for viscosity and density measurements. Once values for sedimentation velocity, solution viscosity, and solution density were found, Stoke's law was utilized to infer the aggregate size. Results from chapter 2 show that BSA-coated polystyrene particles compared to non-coated ones show some level of aggregation. Also, that the higher solution viscosity measured caused the particles to fall gradually. This process and the ZATLLS device can be used to measure many protein reactions *in vitro*.

Next, two commonly used species of the blocking protein albumin were compared since small variations in amino acid constitution, protein length, and charge exist. Each albumin species was adsorbed onto polystyrene particles and the settling velocity was measured. Stoke's law was used to calculate the aggregate size and all experiments were averaged for a final value. Chapter 3 results display a difference in calculated average aggregate size between HSA-coated and BSA-coated polystyrene particles. It was found to be statistically significant with $p < 0.01$, though sedimentation velocities between the two were similar. Since BSA experiments had a larger average aggregate size and solution viscosity, calibration curves were used to determine the amount of desorbed albumin. HSA was shown to be more stable than BSA when adsorbed onto polystyrene surfaces and should be considered as a preferred blocking protein.

In chapter 4, BSA was denatured both reversibly and irreversibly before being coated onto polystyrene particles to compare the aggregation potentials. Solutions containing BSA were heated to 46ºC or 76ºC for reversible and irreversible structural changes respectively. The ZATLLS measured sedimentation velocities were nearly the same, but a large difference in average aggregate size was found. Reversibly denatured BSA average aggregate size was within ranges measured in previous work; however, irreversibly denatured BSA showed a much larger

level of aggregation. These results indicate that much less of the irreversibly denatured BSA stayed adsorbed onto the particles compared to the reversibly changed BSA.

5.2 Future Work

The ZATLLS system has been able to measure differences in sedimentation velocity between albumin-coated and non-coated polystyrene particles as seen in chapter 2. It would be interesting to use a different type of particle in the ZATLLS instrument, since protein adsorption is dependent on this feature. In chapter 3, a comparison between BSA and HSA aggregation after adsorption on polystyrene particles was done. It would be useful to compare the level of aggregation of multiple species of albumin at different concentration ranges. The differing concentrations of dissolved albumin can also be used to ascertain the range of the ZATLLS device. Conformational changes, both reversible and irreversible, were induced by temperature in BSA before adsorption as reported in chapter 4. Utilization of more temperatures would be beneficial in characterizing the stability of the albumin protein. This procedure can be modified so that other denaturing processes can be used and results can be compared to previous work to determine whether the denaturing method induces different degrees of aggregation. Although albumin was our target protein in this research, other proteins may also be used and characterized through this process.

Inhibition of protein aggregation is important in impeding the progression of neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's. Since albumin has already been shown to aggregate, it can be used as a model protein for these diseases. Aggregation inhibitory compounds, such as flavonoids, can be added to albumin solutions to establish the concentration range of the most effective ones. Once the most successful ones are determined, experiments utilizing the neurological disease proteins, peptides, or fragments can commence. The macromolecule will then be adsorbed onto particles and the sedimentation velocity measured so that a standard aggregate size can be calculated. Next, the inhibitory substances found from the albumin studies will be added and compared to the normal aggregate

size. These *in vitro* studies can ascertain the concentration of the most efficient inhibitory compounds and used as a starting point for *in vivo* analyses.