

Protein Aggregation during Ultrasonic Nebulization

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Abstract

Therapeutic proteins represent an essential piece of a health management plan for diseases such as diabetes, cancer, hemophilia and myocardial infarction. These proteins, however, must be maintained in their correct, biologically active conformation throughout processing, transportation, and delivery. This requirement poses serious engineering challenges because of a proteins susceptibility to thermodynamic instabilities resulting from the weak bonds driving the tertiary structure of the molecule. A particularly problematic type of protein degradation is aggregation. Administration of aggregated proteins, a particularly problematic degradation form, can have dire consequences, including blocking a patient's responsiveness to therapy, inducing immunogenicity, and even anaphylactic shock and death. Normal shipping and delivery methodologies are suspected of causing protein aggregation. This work investigates the effect of ultrasonic nebulization on protein aggregation as a function of impurity level, gas-liquid surface to volume ratios, protein concentration, solution viscosity, and nebulization time. A 0.2M and pH of 4.2 Glycine buffer solution was utilized with IVIg protein at 0.5, 1, 5, and 10 mg/ml and 20°C. Protein aggregates were characterized using Microflow imaging. Transient cavitation and formation of radicals was monitored using classical iodine assays. Higher protein aggregation is observed in solutions that initially contain greater amounts of impurities or have a larger contact area with the gas interface. Monitoring of the formation of I₃⁻ from iodine as a function of nebulization time shows increasing production of radicals. All this supports the hypothesis that ultrasonic pressure waves in protein solutions cause transient cavitation which upon bubble implosion release hydroxyl radicals that can attack the protein in solution. Aggregate production does not continually increase with protein concentration, rather falls at higher concentrations. We have demonstrated this increase in viscosity inhibits cavitation by elevating the lowest pressure region based on a specified pressure drop.