

## ABSTRACT

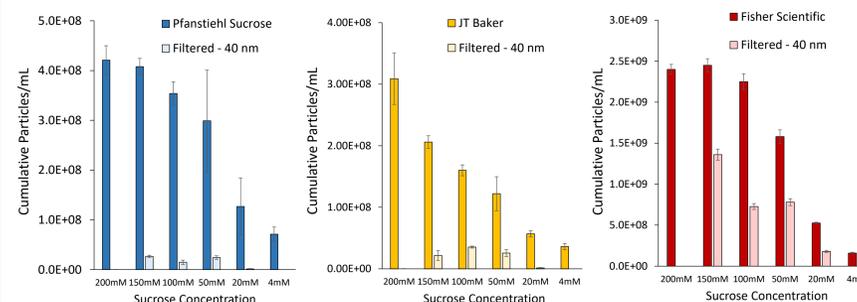
For decades, sucrose has been known to enhance the stability of proteins in aqueous solutions as well as serve as a protein stabilizer during freeze-drying processes and storage in dried formulations<sup>1</sup>. For example, it has been demonstrated that sucrose increases the denaturation midpoint of proteins<sup>2</sup>. Sucrose is present in many approved therapeutic protein formulations. Recently, it has been shown that there may be nanoparticle impurities ranging in size from 200-300 nanometers in diameter present in pharmaceutical-grade sucrose<sup>3</sup>. Our first aim in this current study was to quantify nanoparticles in various brands of sucrose. A second goal was to develop means by which to remove nanoparticles from sucrose solutions. Our last objective was to test the effects of sucrose with and without nanoparticles on aggregation of a model therapeutic protein during agitation stress. Intravenous immunoglobulin (IVIG) was chosen as the model therapeutic protein product. To quantify particle formation, fluid imaging microscopy (FlowCAM) and nanoparticle tracking analysis (Nanosight NS300) methodologies were used. Analysis demonstrated that ultracentrifugation did not effectively remove nanoparticles from sucrose solutions. In contrast, in 20 millimolar sucrose solutions, filtration with 40 nm viral filters removed at least a majority, if not all, of nanoparticles from solutions prepared from Pfanstiehl, JT Baker, and Fisher Scientific sucrose products. Agitation of IVIG in sucrose solutions with reduced nanoparticle concentrations resulted in a decrease in nanoparticles of IVIG formed during agitation by roughly half in Pfanstiehl and Fisher Scientific sucrose solutions. When looking at the larger microparticles indicative of protein aggregation, reduction of IVIG particles was observed in all cases. Filtered sucrose resulted in reduction of agitation-induced protein microparticles of 61%, 55%, and 63% for Pfanstiehl, JT Baker, and Fisher Scientific sucrose solutions, respectively. Additionally, within the different Pfanstiehl products, we were able to determine differences in beet-derived sucrose versus cane-derived sucrose. Nanoparticle analysis demonstrated IVIG in filtered beet-derived sucrose solutions to maintain the same particle levels before and after the induction of stress, signifying no increase in formation of protein nanoparticles. FlowCAM analysis determined filtering cane-derived sucrose seemed to have no effect on the reduction of IVIG microparticle generation while filtered beet-derived sucrose decreased agitation-induced protein microparticles by over half. Overall, nanoparticle impurities found in sucrose can be filtered using small, viral filtration systems. Agitation of IVIG in sucrose solutions containing nanoparticles resulted in much higher agitation-induced protein particle formation than in sucrose solutions from which nanoparticles had been reduced with filtration. Therefore, nanoparticles in sucrose solutions can stimulate protein particle formation.

## MATERIALS AND METHODS

IVIG was chosen as the model therapeutic protein product, was obtained commercially, and was stored at 4°C. Sucrose products were obtained commercially from Fisher Scientific, JT Baker, and Pfanstiehl. In addition, Pfanstiehl generously sent sucrose derived from sugar beets and sugarcane to determine possible differences in nanoparticle influences between two sucrose sources. Sucrose solutions were prepared in lab in filtered PBS at a concentration of 20 mM. In an attempt to remove particles from sucrose solutions, centrifugation was performed at 112,000g for 3 hours. Secondly, Optiscale™-40 small scale disposable capsule filters (paired with non-siliconized NORM-JECT 10 mL syringes) were used in an attempt to simply filter out particles from sucrose solutions. To determine the efficiency of the two purification methods, the techniques of nanoparticle tracking analysis (Nanosight NS300) and fluid imaging microscopy (FlowCAM) were used to determine nano- and microparticle concentrations and size distributions, respectively. To test effects of nanoparticles on aggregation of IVIG (1 mg/mL), solutions containing sucrose with and without particles were subjected to agitation stress by end-over-end rotations. Solutions were agitated at 10 RPM for 30 minutes at room temperature. Protein particles were quantified using Nanosight and FlowCAM instruments.

## RESULTS (Nanoparticle Removal)

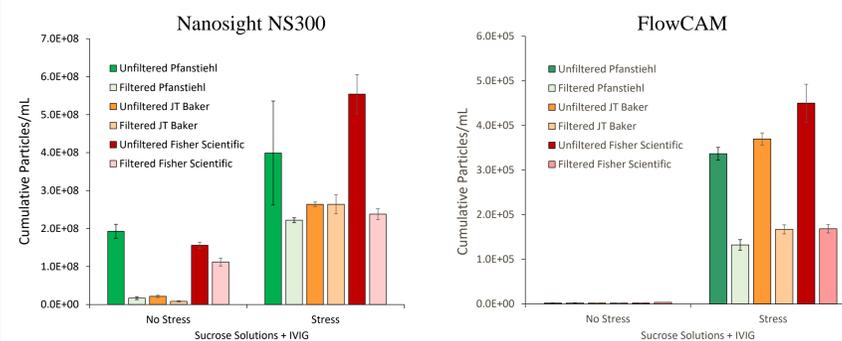
Initial nanoparticle analysis determined centrifugation at 112,000g for 3 hours did not effectively remove nanoparticles from sucrose solutions from all three manufacturers tested. Using a 40 nm viral filtration system removed 97%, 93%, and 61% of nanoparticles from solutions prepared from Pfanstiehl, JT Baker, and Fisher Scientific, respectively. This established filtered, or reduced, nanoparticle sucrose solutions for use in further experiments.



**Figure 1** Comparison of Nanoparticle Concentration before and after Filtration Using Optiscale™-40 Small Scale Disposable Capsule Filters Across Three Manufacturers via Nanoparticle Tracking Analysis (Nanosight NS300)

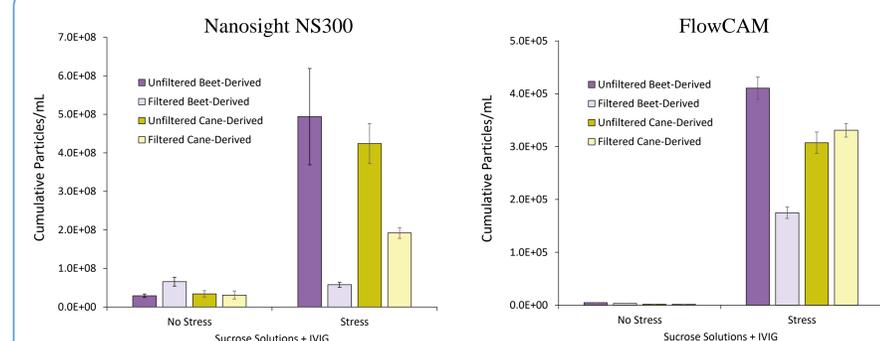
## RESULTS (IVIG Agitation Study)

Intravenous immunoglobulin (IVIG) agitated with reduced nanoparticle sucrose solutions resulted in a decrease of protein nanoparticle formation by 44%, 0%, and 57% in Pfanstiehl, JT Baker, and Fisher Scientific, respectively. More drastic reduction was observed in microparticle analysis on the order of 61%, 55%, and 63% with the three manufacturers, respectively.



**Figure 2** Comparison of Protein Nano- and Microparticle Generation before and after Agitation Stress in IVIG Paired with 20mM Sucrose of One of Three Manufacturers via Nanoparticle Tracking Analysis (Nanosight NS300) and Fluid Imaging Microscopy (FlowCAM)

Pfanstiehl, Inc. generously sent us sucrose products derived from both, sugar beets and sugarcane in order to determine if the sucrose source has an influence on any of the stated objectives of the current study. IVIG agitated in filtered beet-derived sucrose solutions maintained the same particle levels before and after the induction of stress, signifying no increase in formation of protein nanoparticles. FlowCAM analysis determined filtering cane-derived sucrose seemed to have no effect on the reduction of IVIG microparticle generation while beet-derived sucrose decreased agitation-induced protein microparticle production by 57%.



**Figure 3** Comparison of Protein Nano- and Microparticle Generation before and after Agitation Stress in IVIG Paired with Beet-Derived or Cane-Derived Sucrose (Pfanstiehl, Inc.) via Nanoparticle Tracking Analysis (Nanosight NS300) and Fluid Imaging Microscopy (FlowCAM)

## CONCLUSIONS

The monitoring of microparticles alone may not be sufficient in the optimization of safe formulations for therapeutic protein products. Nanoparticle impurities have been observed in sucrose of three tested manufacturers (including pharmaceutical-grade sucrose). These impurities can, to a degree, be filtered using small, viral filtration systems. Agitation of IVIG in filtered sucrose solutions resulted in significantly reduced agitation-induced protein particle formation when compared to IVIG in sucrose solutions containing nanoparticle impurities. A simple filtration step incorporated into industrial process development has the capability of reducing the influence of impurities of some excipients on the generation of immunogenic protein particle generation. It is the current protocol to monitor concentrations of only microparticles at the diameters of 10 micrometers and 25 micrometers. The data provided suggest an argument for tracking nanoparticles when developing and optimizing safe formulations for therapeutic protein products. This additional information may lead to the production of cleaner and safer protein products for patients.

## REFERENCES

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