Effect of agitation on protein aggregation in vials made from glass or plastics

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For several types of proteins, it has been demonstrated that potential for aggregation resulting from thermochromic aggregation is much lower when packaged in vials comprisingCrystal Zenith™ (CZ), a cyclic olefin polymer, as compared to vials comprising glass.

Background:

The increasing use of proteins as therapeutics has focused attention on the need to maintain the stability of these labile molecules during both storage and shipment. The physical degradation of therapeutic proteins can be furthered from aggregation and adhesion in primary containers, from chemical damage due to exposure to light, and from degradations including proteins, free radicals, and metal ions. In addition to loss of potency and valuable drug product, there is growing evidence that protein aggregates can be induced by an immune response to that can neutralize the effect of the drug (Dalesio et al.). Although the drug product is similar to an endogenous protein, the development of cross-reacting antibodies could lead to potentially life-threatening consequences for the patient. The trend in the pharmaceutical industry has been to package therapeutic proteins, particularly monoclonal antibodies at high concentration, in plastically clean, vials containing aqueous solutions. These primary drug products can be used for multiple applications including the administration of biologics.

The properties of aggregates formed by agitation in glass vials was also examined. Since PS80 was able to prevent aggregation in shaken vials, whether or not the surfactant could reverse preformed aggregates was tested. Figure 8 shows that when PS80 was added to a solution of aggregates of MA2 and incubated for one week, there was essentially no change in the turbidity of the solution, indicating that surfactant was unable to reverse aggregates of MA2. Solution of the aggregates into fresh buffer also failed to dissociate the aggregates (data not shown).

Materials and Methods

Vials:

Proleukin® 2 mL vials made of glass or the plastic Dailycry® Crystal Zenith cyclic olefin polymer. Stoppers were filled with Fluoroc® (a fluoropropyl), to minimize the effect of the elastomeric component on protein stability.

Proteins:

Rabbit IgG was obtained from Rockland Immunochemicals. Therapeutic proteins were purchased from a local pharmacy.

Buffers:

Proteins were dissolved or diluted into one of two buffers –

- PBS – 20 mM sodium phosphate 150 mM NaCl (pH 7.6)
- EP0 – 20 mM sodium phosphate 2.7 mM sodium citrate/100 mM NaCl (pH 7.6)

Methods:

- Aggregation: Visual inspection of particulate formation and turbidity of solutions were measured by measuring changes in absorbance at 350 nm before and after storage and/or agitation of samples in vials. Loss of protein due to aggregation was estimated by the decrease in absorbance of the solution at 280 nm.

- SE-HPLC: Protein was determined by the absorbance of the solution at 280 nm after centrifugation to remove insoluble aggregates. Insoluble material was pelleted by centrifugation (15,000 g for 15 min) and the supernatant was analyzed for protein concentration by SE-HPLC. The properties of aggregates formed by agitation in glass vials was also examined. Since PS80 was able to prevent aggregation in shaken vials, whether or not the surfactant could reverse preformed aggregates was tested. Figure 8 shows that when PS80 was added to a solution of aggregates of MA2 and incubated for one week, there was essentially no change in the turbidity of the solution, indicating that surfactant was unable to reverse aggregates of MA2. Solution of the aggregates into fresh buffer also failed to dissociate the aggregates (data not shown).

Conclusions:

- In general, proteins showed a reduced extent of aggregation in vials made of CZ compared to glass vials. The study design was focused on understanding the effects of agitation on the aggregation of therapeutic proteins.
- The application of this method can be used in evaluating vials for storage and administration of biologics.

References

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