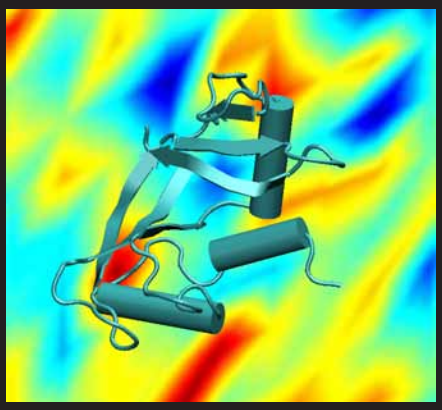
Protein Preservation

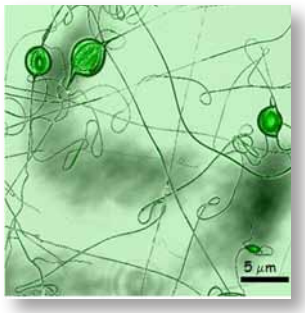
Objective

Our goal is to develop measurements for characterizing sugar-based glasses with respect to their ability to serve as preservation media for therapeutic proteins and cytokines, and to develop theoretical bases for those measurements. Methods developed will address critical needs of biopharmacuetical formulators, and will make possible sequestration of cytokines into, and their delivery from tissue scaffolds with minimal aggregation or chemical degradation.



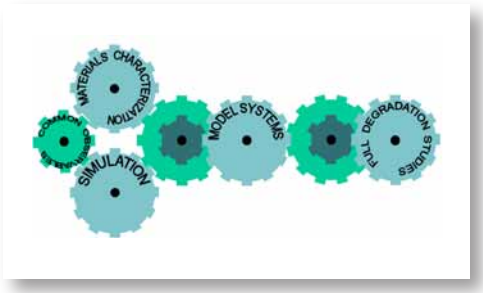
Impact and Customers

- We have demonstrated for the first time the central importance of high-frequency β relaxations to the stability of proteins in stabilizing sugar-based glass. This property had been completely overlooked heretofore.
- The characterization methods we have developed will make possible the safe incorporation of highly labile proteins into tissue scaffolds used in regenerative medicine.



- Biopharmaceutical drug sales accounted for $80B in 2007, and they show the highest rate of sales growth of any drug class. Roughly half of protein biopharmaceuticals are so unstable that they require sequestration in a sugar-based glass for storage. We are providing critically needed metrology as a basis for development of rational formulation approaches.
- Pharmaceutical companies, including Amgen, Pfizer, and MedImmune have adopted methods pioneered in our group at NIST for characterizing protein stabilizing materials.
- Our methods will increase the likelihood that national security needs for 10 year shelf life of dry-formulation vaccines (e.g. anthrax), which has thus far not been possible, can be met.

Approach



Stability of proteins in biopharmaceutical and drug delivery applications is critical. Although it is common practice in pharmaceutics to dry highly labile therapeutic proteins in sugar-based glass, and in regenerative medicine to mix proteins with synthetic polymers for delivery applications, there is only a basic understanding of the implications these operations have on proteins. Consequently, biopharmaceutical formulation of dry-state products is inefficient and risky, and proteins delivered in regenerative medicine applications are often degraded to an unacceptable extent. The primary roadblocks are: 1) lack of analytical tools for rapid formulation assessment, 2) extremely long testing cycle times, and 3) poor understanding of the preservation mechanisms.

We have assembled a team of NIST staff and University professors who are the top experts in the several distinct fields that relate to protein stabilization in glass. We are working jointly, using simulation, model studies, and biopharmaceutical degradation studies to determine the fundamental mechanisms of protein instability, and to develop precise measurement methods that yield information predictive of a formulation's ability to stabilize proteins. We are focusing on materials-based measurements that provide predictive information, and can be carried out in hours rather than years, as is currently the case.

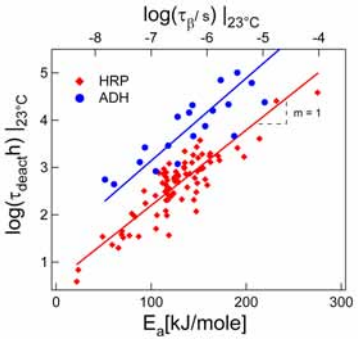
BIOMATERIALS

Accomplishments

Key Parameter

Administration of partially degraded biopharmaceuticals may result in anaphylactic shock or death. It is therefore critically important that the proteins in these products be stable against aggregation and chemical degradation. In many cases, the only hope of obtaining sufficient stability is to sequester the proteins in a sugar-based glass. In the two decades since this practice has become widespread there have been two hypotheses put forth regarding why sugars stabilize proteins, and formulators have used two basic metrics, inspired by these two hypotheses, to assess suitability of a sugar-glass for stabilizing proteins. It has been clear for some time, however, that these two metrics, glass viscosity (α relaxation), and protein conformation, are insufficient to predict protein stability.

Using neutron scattering, we were the first to demonstrate that a third metric, high-frequency (β) relaxation, is important to stability.

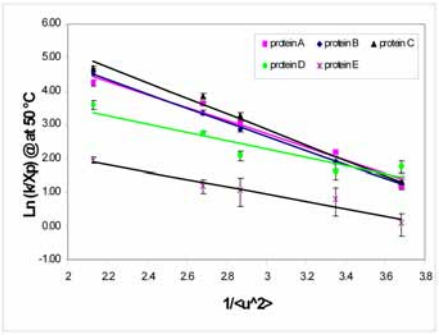


Stability follows β relaxation in >100 formulations

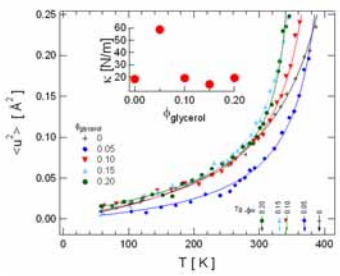
In further studies, using model proteins and dielectric spectroscopy, we demonstrated the central importance of β relaxation in protein stabilization. The Figure in the previous column shows results from protein stability testing in over 100 formulations. We find a direct, linear relationship between β relaxation times in the glasses and stability lifetimes of the proteins in those glasses.

Putting Things in Perspective

We have also performed studies on biopharmaceutical proteins to understand the relative importance of protein structure and relaxation dynamics in the glass. The Figure below shows results from aggregation studies of a series of cytokines and antibodies, performed in collaboration with colleagues at the University of Connecticut. In these studies, proteins were formulated with increasing levels of sucrose. As sucrose levels changed, so did α and β relaxation rates, as well as protein conformation. The aggregation rates correlate very well with β relaxation, and do not correlate with the other measures over the entire experimental parameter range. These results indicate that the traditional measures of formulation suitability, α relaxation and protein conformation, may be useful in some formulation regimes, but are not robust for all formulations.



β relaxation trumps protein conformation

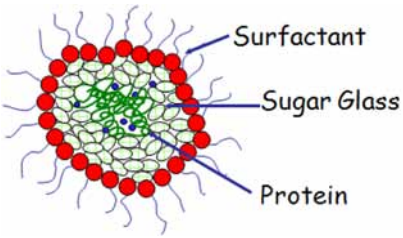


β relaxation can be suppressed by adding solvent

Drug Delivery For Regenerative Medicine

The environment in the body – warm and wet – poses a special challenge to protein stability in a polymeric or sugar-glass matrix. Small amounts of water can strongly influence (usually reduce) stability in a "dry" format. However, we know from neutron scattering that β relaxation can be suppressed and protein stability increased with small amounts of diluent, as in the Figure below, where glycerol was added to trehlose (sugar) glasses.

Sugar particles containing protein can be dispersed in solvents and incorporated into tissue-engineering scaffolds by first coating them with a surfactant as depicted below. Using β relaxation studies, we can tune the sugar glass composition for optimal performance while the scaffold is in the body.



Protein nanoparticles for drug delivery

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Publications

Psurek T, Soles CL, Page KA, Cicerone MT and Douglas JF *Quantifying Changes in the High-Frequency Dynamics of Mixtures by Dielectric Spectroscopy* J. Phys. Chem. B, (in press 2009)

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