Thermodynamic Consequence of Aerosolizing Therapeutic Proteins in Solution

M. G Zeles-Hahn and C. S. Lengsfeld*
Department of Mechanical and Material Engineering
University of Denver
Denver, CO 80208 USA

Abstract

Proteins are increasingly sought after as potential treatments for multiple diseases. With the ease of delivery to the lungs, it is perceivable that therapeutic proteins could be atomized and delivered as such. However, proteins are fragile macromolecules that are susceptible to both physical and chemical degradation. We propose that there is a relationship between the enthalpy of melting for a protein and the aerosolized droplet's size and surface tension.

Maa [1] demonstrated that proteins degrade as a function of the surface to volume ratio utilizing standard pressure atomization techniques. More recent research utilizing electrospray of proteins does not correlate well with his original findings. The results from these studies are conflicting. Some proteins keep 100% activity after spraying while other lose activity depending on flow rates or concentration. After examining numerous protein properties that could affect stability such as: size, isoelectric point, type of protein, pH of buffer, melting temperature, and percent hydrophobic; a trend was seen to correlate with the denaturation temperature of the protein in solution. Melting enthalpy is directly correlated to the denaturation temperature. This relationship leads to the hypothesis that protein stability during all aerosolization processes is a thermodynamic issue. The energy on the surface of a drop is governed by Gibb's Free energy (G):

$$\Delta G = -S\Delta T + V\Delta P + \sum \mu dn + \sigma \Delta As \quad (1)$$

Assuming that the drop is at constant temperature, pressure and chemical composition, Equation 1 becomes:

$$\Delta G = \sigma \Delta As \quad (2)$$

The energy needed to unfold a protein is found in the melting temperature:

$$\Delta Hm = cp\Delta T \quad (3)$$

The melting enthalpy can be measured in a differential scanning calorimeter (DSC). The DSC operates under adiabatic conditions, relating Gibb's Free Energy directly to enthalpy

$$\Delta G = \Delta H - T\Delta S = \Delta H \text{ (adiabatic)} \quad (4)$$

We propose that if the energy contained with in the surface tension and change in surface area is greater than that of the melting enthalpy, the protein will denature

$$\Delta Hm < \sigma \Delta As \quad (5)$$

Applying Maa's data to the theory above, a trend can be seen. In using this data, three assumption were made 1) percent activity = 1 – percent aggregation, 2) surface tension doesn't vary significantly with concentration, and 3) melting enthalpy doesn't vary with concentration. Assumptions 1 and 2 are weak; a protein can denature without aggregation and surface tension does decrease with increasing concentration.

This relationship will be further explored by varying each of parameters of Equation 5 in depth. Changing the pH of a solution, the type of salt buffer, and the protein itself can vary the enthalpy of melting and surface tension. The size of the droplet is affected by the mechanism that creates the droplet, which in our case is the monodisperse piezoelectric drop generator. The drop generator can be easily manipulated to produce drops of different sizes. The ambition is to take the trial and error formulation development to an engineering foundation where calculations can be conducted prior to clinical trial to ensure stable molecular delivery.

Reference

[1] Maa, Y. F., P. A. T. Nguyen and S. W. Hsu, *Journal of Pharmaceutical Sciences* 87: 152-159 (1997).

Key words: Medical sprays, protein, droplet size, melting enthalpy