Thermodynamic Consequence of Aerosolizing Therapeutic Proteins in Solution

M. G Zeles-Hahn, T. J. Anchordoquy, 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. Many publications have examined the atomization of protein solutions, yielding varied results. We propose that there is a relationship between the enthalpy of melting for a protein and the aerosolized droplet's size and surface tension. This relationship will be explored by varying each of the above parameters in depth. Changing the pH of a solution, the type of 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 a monodisperse piezoelectric drop generator. The ambition is to proved a clear relationship between measurable protein formulation properties and the smallest stable droplet size in a spray.

Introduction

Aerosol therapeutics are becoming a realistic option for treating chronic lung diseases and illnesses as well as vaccines for infectious diseases. This is partially due to ease of delivery of therapeutics to the lungs. Macromolecular delivery to the lung is particularly intriguing as it circumvents first pass metabolism issues. The literature indicates a wide range of aerosolization methods are being employed to deliver therapeutics to the lung with mixed results [1-4]. Observation of Maa et al. [5] associated protein stability with surface area to volume ratios. They noted a linear relationship between droplet diameter and protein aggregation. In addition, they believed that over time more protein would be drawn to the interface causing greater protein denaturation and aggregation. However, previous studies in our lab show no time dependant degradation. It has been shown previously that in order for a protein to remain stable its change in surface tension divided by change in surface area must be greater than zero, else the protein unfolds [6]. Based on this, we hypothesize that the energy input to the system in order to create the aerosol must be less then the energy within the protein in order for the molecule to remain in its native state.

Methods

Energy is required to break a column of liquid into droplets for atomization. This energy includes disrupting the surface tension of the liquid and increasing the surface area. The energy on the surface of a drop is governed by Gibb's Free energy (G):

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

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

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

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

$$\Delta H_m = c_p \Delta T \quad (3)$$

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

$$\Delta H_m < \sigma \Delta A_s \quad (5)$$

*Corresponding author, clengsfe@du.edu

Enzymatic assays are used to determine the structural stability and activity of each of the proteins during atomization. The assays are colorimetric in nature and were analyzed using a UV spectrophotometer. By observing the rate of product formation, the assay is able to show the activity of the enzyme and thus the structural stability. The rate of activity of the atomized sample is compared with the control to give a percent activity (Equation 7).

$$\%\text{Activity} = \frac{\text{SampleRate}}{\text{PositiveControlRate}} * 100 \tag{6}$$

The Thermodynamic stability is examined using a differential scanning calorimeter (DSC). The DSC works by heating a liquid sample over time and recording the changes in heat released from the sample compared with a blank. Each sample is typically scanned from 10°C to 100°C at a rate of 60°C/hour. The data recorded, heat capacity vs. temperature can be manipulated to give the enthalpy of the sample. As an enzyme denatures, heat is released. The peak of heat release is known melting temperature and the area under to peak is the melting enthalpy. The relationship between these variables is:

$$\Delta H_m = \int C_p * dT \tag{7}$$

A control buffer scan is subtracted from each enzyme scan to create a stable baseline.

Surface tension is the energy found at the interface of a liquid. For these experiments, it was measured with a tensiometer using the ring method. As a ring is lifted out of a liquid, there is a force pulling on the ring from the liquid. There is a maximum force on the ring just before the liquid breaks; this measured force is equal to the surface tension.

Preliminary Results

Data found in Maa et al. [5] was plotted initially to determine if Equation 5 might be valid (Figure 1). A few assumptions had to be made in order to plot the data. First, it was assumed that the percent activity of the protein is inversely related to the percent of aggregation. Maa was interested only in the amount of aggregation of the protein. It is known that if a protein aggregates, it is no longer active. However, a protein can unfold and be inactive without aggregation. Based on this assumption the values used for percent activity might be artificially high. The second assumption was to assume that surface tension does not change with concentration of the protein. It has been shown elsewhere that as the protein concentration increases, the surface tension decreases [7]. The surface tension in Maa's paper was measured at a high concentration than that used during atomization. Therefore, the surface tension used in Figure 1 will be smaller than the true value. The last assumption was to agree that melting enthalpy does not change with protein concentration. This is a realistic assumption when the protein used goes through a reversible unfolding process and it can be scaled to the concentration used in the study. Given these assumptions, the true values would shift the point to the left and down. Regardless, the trend is pretty clear. As the drop decreases in size, the activity of the protein also decreases. It is surprising that even with these assumptions the critical transition value appears to be centered around ten, an order of magnitude higher than simple theory would predict.

Future

Maa's data provides confidence in out theory, but more data needs to be collected to fully develop an all-inclusive equation. We need to understand if each protein has a particular transition value or if it can be roughly considered universal. The theory also needs to be tested over a much broader range rather than simple droplet size modification. This relationship will be further explored by changing the pH of a solution, the type of buffer, and the protein itself that attribute to 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 (Figure 2) 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.

Nomenclature

A *Area*
C_p Heat capacity
G Gibb's Free Energy

H Enthaply
P Pressure
S Entropy
T Temperature
U Internal Energy
V Volume

σ Surface Tension

Subscripts
s *Surface*
m *Melting*

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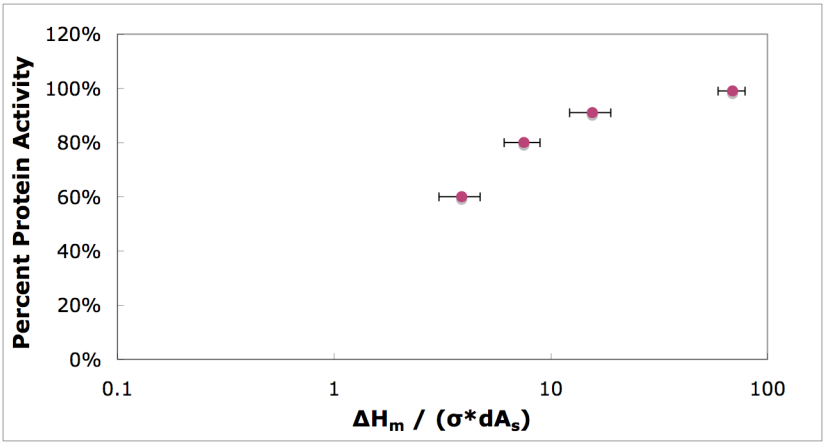

Figure 1: Graph of protein data from Maa et al [5]. The plot shows that as the melting enthalpy of the protein decreases the activity of the protein decreases as well. See paper for assumptions used for plotting the data.

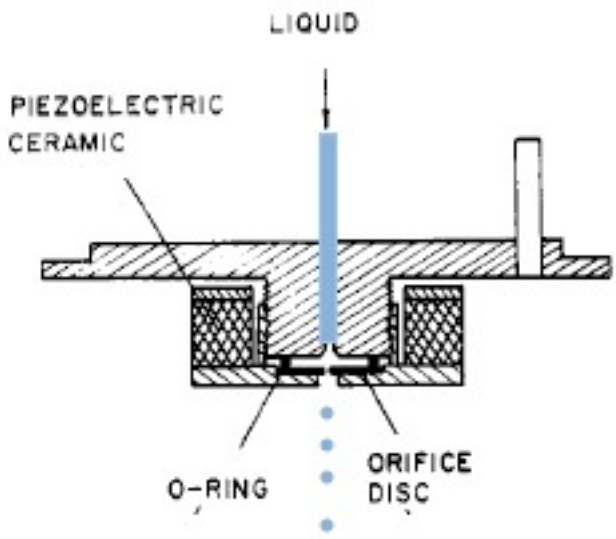

Figure 2: Diagram of the drop generator. It uses a piezoelectric to create monodisperse drops.