Surface Properties are a Major Factor Controlling the Dispersibility of Dry Powder Protein Formulations Intended for Inhalation

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OBJECTIVES

To demonstrate the effects of surface properties on the dispersibility of dry powder protein formulations intended for inhalation.

INTRODUCTION

Significant efforts have been devoted to optimizing and enhancing the performance of dry powder aerosol formulations. However, information regarding the effect of physicochemical properties of the powders on aerosol performance remains scarce. Our research group has been developing dry powder formulations intended for pulmonary delivery utilizing Nektar's pulmonary delivery systems (PDS). In our experience spray dried powders with predominantly non polar surfaces are more dispersible than those with polar surfaces. To investigate the effect of surface properties on aerosol performance of spray dried powders we have used hemoglobin and myoglobin formulations as model compounds.

METHODS & MATERIALS

Surface Activity of Proteins

Surface tension of each solution prior to spray drying was measured using the Wilhelmy plate method using a Krüss model K 12 Tensiometer. Equilibrium adsorption parameters were determined by fitting the surface tension to the Szyszkowski Equation.

Spray Drying of the Formulations

Aqueous solutions of protein (hemoglobin or myoglobin) and trehalose containing 1% w/w solids were spray dried in a Büchi 190 spray dryer. The liquid feed rate was 5 ml/min, inlet temperature was maintained at 110°C and outlet temperature of 60°C, with an atomizer pressure of 60 psi and a vacuum of 90 bars.

Mathematical Modeling of Particle Formation during Spray Drying

Using an analytical description of the evaporation process given by Leong [1] and the parameters reported below the surface composition of the spray dried particles were estimated, using the a simple model where the surface concentration, C_s, can be expressed as a function of time and the initial conditions and the droplet radius R or diameter, d.

$$c_s = \frac{c_0 \exp\left(\frac{Pe}{2}\right)}{3\beta\left(1-\frac{\kappa t}{d_0^2}\right)^{\frac{1}{6}}} \qquad \beta = \int_0^1 R^2 \exp\left(\frac{Pe}{2}R^2\right) dR \qquad Pe = \frac{\kappa}{8D}$$

Parameters for simulation

Molecule	MW (Da)	Solubility* (mg/ml)	Diffusion Coefficient* (m^2/s)	Pe Peclet number	β
Hemoglobin	64500	8	6.9×10^{-11}	7.60	4.94
Myoglobin	17000	100	9.0×10^{-11}	5.80	2.45
Trehalose	342	-	5.0×10^{-10}	1.05	0.46

*in water at 25°C
Evaporation rate: $4.2 \cdot 10^{-9}$ m²/s; Initial droplet size: 9 µm; 1% w/w solids content in feed solution

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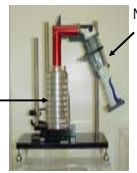
Aerosol Characterization

The aerosol performance of each powder was characterized by their *in vitro* emitted dose (ED) and their fine particle mass less than 3.3 µm (FPM<3.3µm), determined by using the PDS and an Andersen Cascade Impactor (ACI), respectively, under controlled ambient conditions (21°C and 40% RH).



$$ED = \frac{M_{filter}}{M_{blister}} \qquad FPM = \frac{M_{<3.3\mu m}}{M_{blister}} \times 100$$

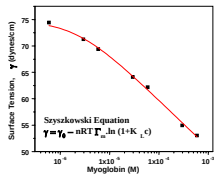
Powder Characterization

Particle morphology was evaluated using scanning electron microscopy (SEM). Powder surface composition was determined by X ray photoelectron spectroscopy (XPS). The specific surface area was measured from the experimentally determined N_2 BET adsorption isotherm. The true density of the powder was measured with a helium pycnometer. Surface energy was measured by inverse gas chromatography (iGC) at 30°C.

RESULTS

Surface Activity of Proteins in Aqueous Solution

Equilibrium surface tension isotherm of an aqueous solution of Myoglobin at 10 °C



The curve represents a mathematical fit to the Szyszkowski Equation.

Equilibrium adsorption parameters for Hemoglobin and Myoglobin at 25 °C

Protein	Adsorption Constant K_L (mol/cm³)	Surface Excess Γ_m (mol/cm²)
Hemoglobin	$(13.4 \pm 8.0) \times 10^6$	$(1.9 \pm 0.3) \times 10^{-6}$
Myoglobin	$(0.6 \pm 0.2) \times 10^6$	$(1.5 \pm 0.1) \times 10^{-6}$

- The adsorption parameters indicate that both proteins are surface active.
- Hemoglobin is more surface active than myoglobin.

Particle Formation Mechanism

We have developed a simple mathematical model to describe the particle formation mechanism during spray drying. Although the model does not account variations in droplet size and evaporation rate in a real spray drying situation, the model can be used to estimate the surface concentration. In addition the model predicts that the components solubility is the most important factor that determines both morphology and surface composition

Calculated Surface Protein Concentration

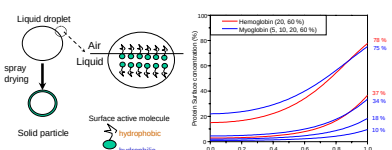
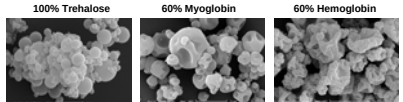


Figure 5. During spray drying (A), formulation components in solution preferentially orient at the surface due to their surface activity. Surface activity promotes the formation of a non-polar, low-energy surface. (B) Preferential surface enrichment is inversely proportional to their aqueous solubility.

Particle Morphology

100% Trehalose **60% Myoglobin** **60% Hemoglobin**

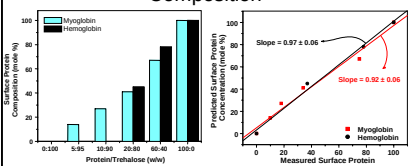


- Trehalose due to its large solubility forms spherical particles
- Myoglobin/trehalose formulations form smooth particles
- Hemoglobin/trehalose formulations form wrinkled particles due to the low solubility of hemoglobin

Qualitatively, the morphology differences observed for myoglobin and hemoglobin can be explained as follows: The less soluble protein will precipitate from the solution earlier during the spray drying process, i.e. the "skin" of the particle is formed early, when the droplets are still big, they then "deflate" and form the wrinkled morphology. In contrast, more soluble compounds form the skin later and result in more spherical particles because the size doesn't have to shrink as much after the skin in formed.

- The enrichment of protein concentration on the particle surface is dominated by the diffusivity and solubility of the protein and less by the surface activity of the protein.

Measured and Predicted Surface Composition

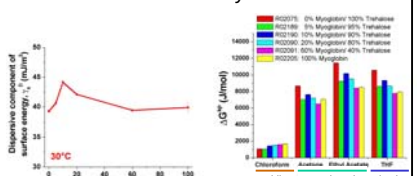


- In spray dried formulations of protein/trehalose mixtures, the protein preferentially accumulates at the particle surface.

Correlation between calculated surface protein concentration and measured protein concentration

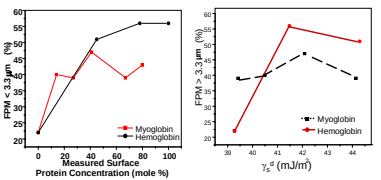
The surface protein concentration measured using XPS is correlated with that calculated based on the diffusivity and solubility of the formulation ingredients.

Surface Energy and Surface Polarity Measured by iGC



iGC analysis provides a measurement of the surface energy due to the non polar character of a solid surface based on interactions with non polar probes such as alkanes, which is referred to as dispersive surface energy, γ_s^d. Interactions with polar probes, classified based on their acid base properties, the powder surface provides a measurement of its polarity. γ_s^d of neat trehalose and neat protein are similar. The observed increase at 5 20% myoglobin formulations may be related only to the amount of globular protein on the particle surface. Polar probes show that the acidic/basic character of the surface is more sensitive to changes of the surface composition.

Surface composition contributes to aerosol performance



Dependence of Fine particle fraction < 3.3 µm on surface protein concentration.

The powders containing hemoglobin and myoglobin performed better than the powder containing 100% trehalose. The performance correlates with the surface protein concentration. Hemoglobin containing powders performed better than those containing myoglobin. This along with the higher surface activity of hemoglobin compared to myoglobin suggests that aerosol performance is related to the surface activity of the protein.

Surface active molecules orient at the air/liquid interface during drying and the non polar portions enrich the surface as suggested by the correlation of the fine particle fraction and the dispersive surface energy.

- Aerosol performance is dependent on the surface protein concentration
- Aerosol performance is related to the surface activity of the protein in the formulation
- Aerosol performance is also related to surface energy of the particles' surface measured by iGC

CONCLUSIONS

- ***Hemoglobin and Myoglobin are surface active***
- ***Hemoglobin is more surface active than myoglobin***
- ***The morphology and the surface enrichment of spray-dried protein powders is dominated by the solubility and diffusion coefficient of the protein molecule***
- ***Aerosol performance of such powders dependens on the surface activity of the protein in the formulation and its surface concentration***
- ***Control of the surface properties of the particles determines the performance of dry powder aerosols***

REFERENCE

1. Leong, K. H. (1987). *J Aerosol Sci* 18, 511.

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