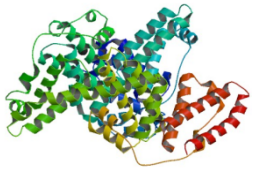

PROTEIN DENATURATION MONITORED WITH NON INVASIVE STATIC-MULTIPLE LIGHT SCATTERING

INTRODUCTION

Proteins are used in various fields such as food, pharmaceutics, biochemistry, biology and specially at high concentration. Medicine research is largely challenged with highly concentrated protein dispersion formulations, in particular for long-term delivery to reduce frequency of injections. Protein ability to remain well dispersed, avoid aggregation and keep its spatial configuration constant is driven by factors as temperature, salt concentration, or amino-acid addition. Protein aggregation is often studied by viscosity measurement, as protein denaturation leads to viscosity increase. DLS or zeta potential are also common methods but require heavy dilution.

In this note, we propose to monitor protein aggregation by measuring the mean diameter in concentrated media, with static multiple light scattering (SMLS). This technique analyses the dispersions in their native form without dilution and versus time. It is essential as size increase can modify end-use properties of products, and as dilution can affect particles size.

MATERIAL & METHOD

Materials

- Bovine Serum Albumin Protein (BSA) dispersed in water with concentration 10%wt and different amounts of an aminoacid histidine (from 3 to 20nM), analysed at 60°C
- BSA at different concentrations between 4 and 10% wt, analysed at 25°C

Measurement with Turbiscan

Turbiscan (880nm) is based on SMLS technology (Static Multiple Light Scattering) and enables to measure directly the mean spherical equivalent diameter (d).

With the signal intensity and knowing refractive index of continuous (n_f) and dispersed phase (n_p) and the particles concentration (φ) according to the Mie theory:

$$d = f(BS\ (or\ T), \varphi, n_p, n_f)$$

with BS for Backscattering Intensity and T for Transmission Intensity.

RESULTS

Case 1 - Temperature and histidine effects on the protein aggregation versus time

Temperature increase leads to proteins denaturation, which consists in modifying interactions, and leading to opaque samples due to size increase. Histidine, an amino-acid, is often used to protect therapeutical protein against denaturation.

The BSA protein was analyzed at 10%wt, the samples are quite transparent at room temperature, and go from transparent to opaque at 60°C, for low histidine amount as shown on the Figure 1. This figure displays the raw data obtained directly from the transmitted signal.

RAW DATA

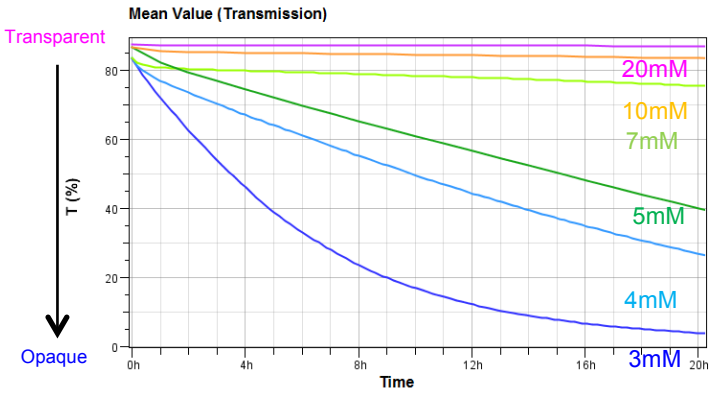

Figure 1: Transmission level (%) versus time for BSA 10% wt with different histidine amounts (mM) analysed at 60°C