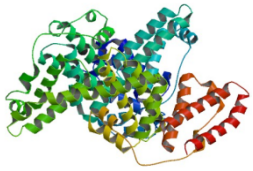

PROTEIN DENATURATION MONITORED WITH NON INVASIVE STATIC-MULTIPLE LIGHT SCATTERING

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

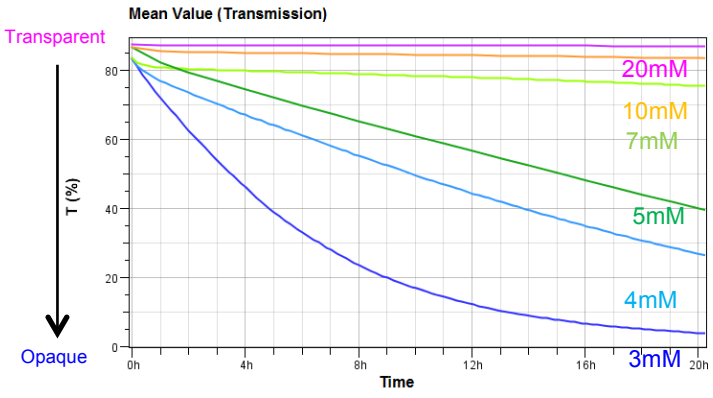

Figure 1: Transmission level (%) versus time for BSA 10% wt with different histidine amounts (mM) analysed at 60°C

The following figure shows the diameter evolution of BSA dispersions with various histidine concentrations versus ageing time, obtained with no dilution.

MEAN DIAMETER

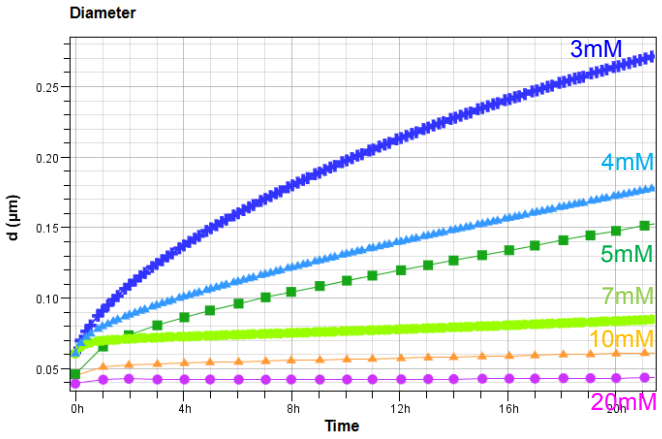

Figure 2: Mean size versus time (µm) for BSA 10% wt with different histidine amounts (mM)

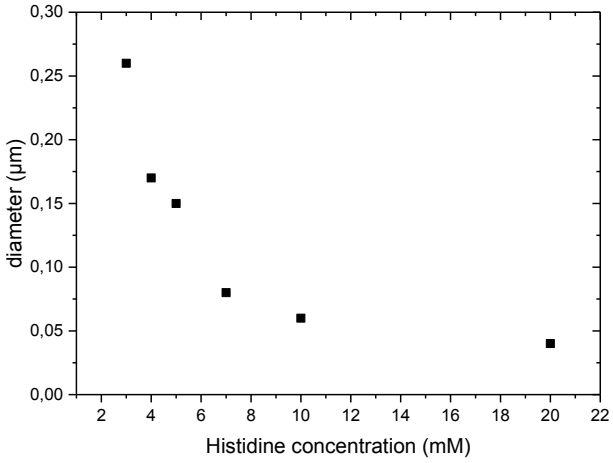

Figure 3: Mean size (µm) for BSA 10% wt versus histidine concentration (mM) after 20 hours measurement

Increasing histidine concentration enables to keep the diameter lower, and closer to the native state without denaturation, as shown on Figures 2 and 3.

Case 2 - Measuring protein size vs concentration after preparation

BSA mean diameter was also determined with SMLS at different concentrations right after preparation at 25°C, and DLS measurements were also performed to compare both techniques.

The table hereunder gives the results of SMLS and DLS:

BSA	DLS		SMLS	
concentration	state	Diameter	state	diameter
1%	ND	8 nm	ND	NA
4%	D	8 nm	ND	25 nm
10%	D	8 nm	ND	60 nm

(ND = Non Diluted and D = Diluted)

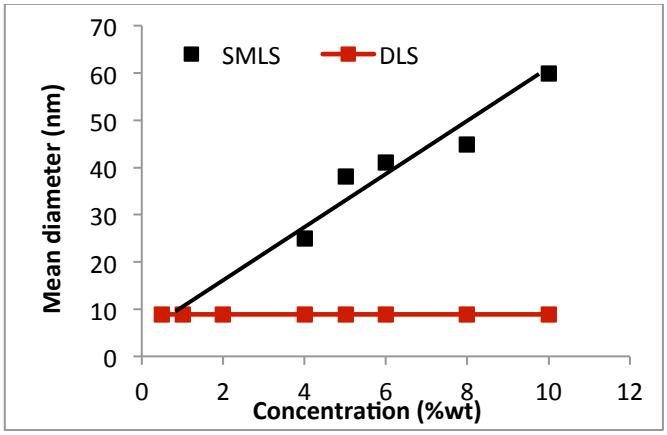

Figure 4: Mean size versus BSA concentration (%wt)

The results obtained with SMLS show that increasing BSA concentration increases particle size due to protein-protein interactions, such as electrostatic attraction.

DLS measurement, which needs heavy dilution of the samples for good quality measurement, does not detect this concentration effect.

Dilution for the measurement may break agglomerates and redisperse proteins.

SUMMARY

Turbiscan technology based on Static Multiple Light Scattering is proposed to measure mean particles size at a given time or particle aggregation versus time or any parameter, in a large range of concentration between 0.0001 and 95%, for sizes between 10 nm and 1000 µm.

This technique has the advantage to measure the mean particles size in one-click, without sample preparation or dilution, particularly for concentrated suspensions.

Turbiscan LAB also allows comparing samples in terms of physical stability.