PHARMACEUTICAL

Protein denaturation monitored with non-invasive SMLS

INTRODUCTION

Proteins are used in various fields such as food, pharmaceutics, biochemistry and biology, particularly at high concentration. Medicinal research is largely challenged with the formulation of highly concentrated protein dispersions, particularly for long term delivery in order to reduce the frequency of injections. The proteins ability to remain well dispersed, avoid aggregation and keep its spatial configuration constant is driven by factors such as temperature, salt concentration and amino acid addition. Protein aggregation is often studied by viscosity measurements as protein denaturation leads to an increase in viscosity. DLS and zeta potential measurements are also common methods but require heavy dilution.

KEY BENEFITS

VERSATILE

NO DILUTION

FAST AND ACCURATE

In this note, we propose to monitor protein aggregation by measuring the mean diameter in concentrated media with Static-Multiple Light Scattering (S-MLS). This technique analyses the dispersions in their native form versus time without dilution. This is beneficial as size increase can modify end use properties of such products.

MATERIALS

- Bovine Serum Albumin Protein (BSA) dispersed in water with concentration 10 wt. % and different amounts of an amino acid histidine (from 3 to 20nM), analyzed at 60°C

- BSA at different concentrations between 4 and 10 wt%, analyzed at 25°C

EXPERIMENTAL RESULTS

➢ Temperature and histidine effects on the protein aggregation versus time

Temperature increase leads to protein denaturation which consists of modifying intramolecular interactions, leading to size increase and opaque samples. Histidine, an amino-acid, is currently used to protect therapeutic proteins against denaturation.

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

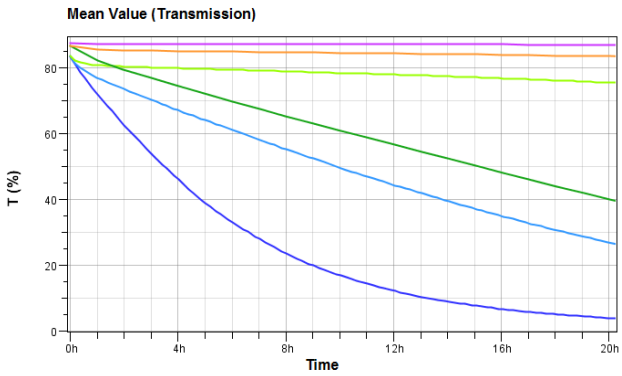

Figure 1: Transmission level (%) versus time for BSA 10%wt with different histidine amounts (nm) analysed at 60°C

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

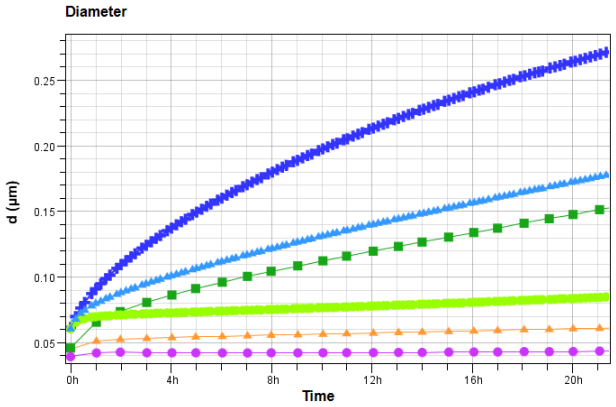

Figure 2: Mean size versus time (µm) for BSA 10 wt% with different histidine amounts (mM)

Increasing histidine concentration enables to keep diameters low and closer to their native state without denaturation, as shown on Figures 2 and 3.

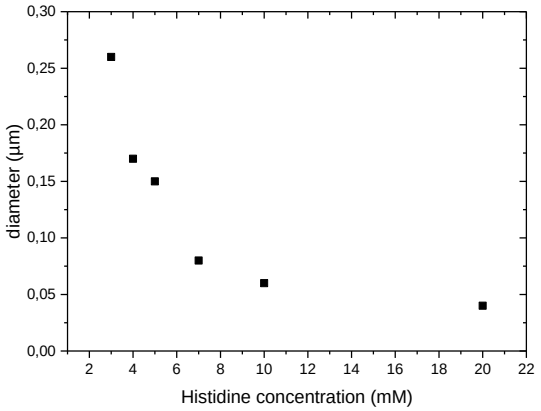

Figure 3: Mean size versus time (µm) for BSA 10 wt% vs histidine concentration (mM) after 20h measurement.

➢ Measuring protein size versus concentration after preparation

BSA mean diameter was also determined at different concentrations after preparation at 25°C, and comparisons to DLS measurements were also performed. The table hereunder gives the results of SMLS and DLS :

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

with ND = Non Diluted and D = Diluted

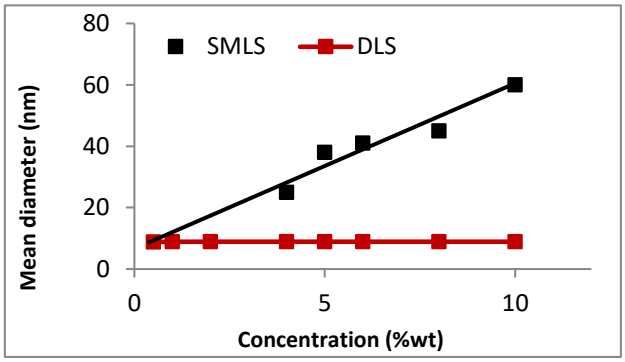

Figure 4: Mean size versus BSA concentration (%wt)

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 a quality measurement, does not detect this effect as dilution may break up agglomerates.

CONCLUSION

Turbiscan technology based on Static Multiple Light Scattering is proposed to measure mean particles size at one time or particle aggregation versus time or parameters, 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 in one click, without sample preparation or dilution, the mean particle size of particles, particularly for concentrated suspensions.

Turbiscan LAB also allows to compare samples in terms of physical stability.