

# Therapeutic protein characterization using light scattering techniques



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## Introduction

Proteins consist of polypeptide chains that are sensitive to various treatment conditions such as preparation, storage, buffer, etc. This application note describes dynamic and static light scattering characterization of a therapeutic antibody supplied in two forms: an untreated and a treated preparation. Information about the treatment process was not divulged.

## Experimental

Two untreated antibody samples were provided at a concentration of 7 mg/mL in ammonium phosphate buffer. Both samples were also supplied in a treated form at various lower concentrations. All samples were filtered through a 0.1  $\mu\text{m}$  Whatman Anotop filter (cat. 6809-1012) and then measured in the 12  $\mu\text{L}$  cuvette of the Zetasizer Nano.

A series of dilutions of the untreated samples was then prepared in the same buffer to measure and compare the molecular weight by static light scattering.

Finally, a melting point determination was run for both a pure and a treated sample. The melting point is the temperature at which a protein denatures. The Zetasizer Nano software allows automated temperature scans to determine the melting point temperature.

## Results

### Hydrodynamic Size

The dynamic light scattering measurements showed a significant difference between the treated and

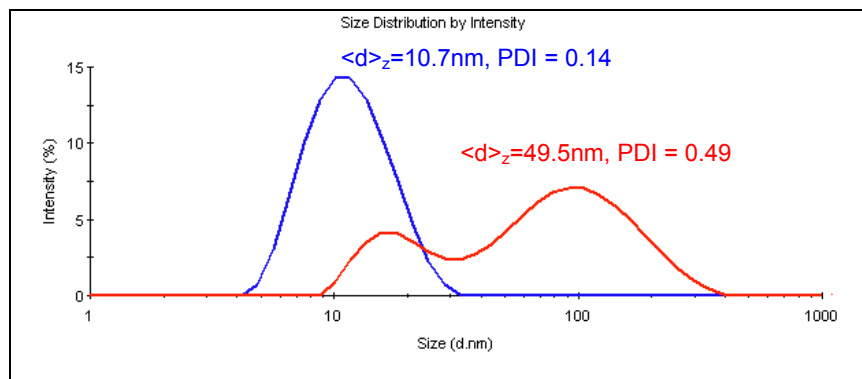


Figure 1: Size distribution from pure (blue) and treated (red) antibody sample

the pure samples. While the pure sample showed a typical z-average diameter  $\langle d \rangle$  of 11 nm, the treated sample had a z-average size of 50 nm, both with a polydispersity index (PDI) larger than 0.1 indicating more than a single species being present. This was confirmed by the size distribution analysis shown in Figure 1. The pure sample showed a single peak near the expected size, however the peak is broader than for a single species, indicating the presence of some fragments and oligomeric assemblies. The treated sample was clearly aggregated and significantly larger in size.

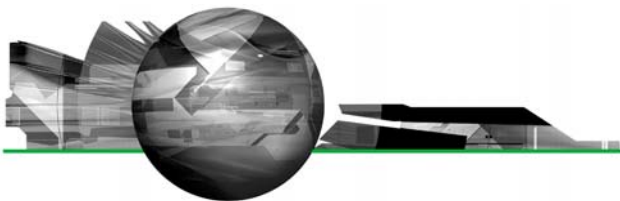
### Molecular Weight

This result was confirmed with static light scattering measurements. The expected molecular weight of this sample was about 145 kDa. However, there were antibody fragments present which had not completely been removed during the purification. As shown in Table 1, the pure antibody samples exhibited a MW slightly less than the expected value.

It is quite likely that this discrepancy originated from the presence of smaller Fab fragments. The molecular weight of the treated samples was significantly larger than the pure antibodies. This is in agreement with the measured size distribution: The treated sample contained a large amount of aggregates, and this gave rise to a large average molecular weight. The Zetasizer Nano software allows MW determinations even for samples larger than typical Rayleigh scatterers. For the treated samples a shape correction for spheres ( $R_G = 0.774 R_H$ ) was applied to the data. The effect on MW was less than 10%.

Sample	MW (kDa)	MW error (kDa)
San2-pure	129	+/- 21
San3-pure	139	+/- 8
San2-treated	1210	+/- 125
San3-treated	1270	+/- 65

Table 1: Molecular weight of pure and treated antibody samples, expected MW 145 kDa



## Melting Point

An automated temperature scan of the sample chamber allows observation of both the size and the scattering intensity as a function of temperature. The marked point where both the size and the intensity start to increase significantly is called the melting point. Here, denaturation of the molecules leads to massive aggregation, which manifests itself as both a larger size and a larger scattering intensity.

Figure 2 shows the melting point measurement for the pure antibody sample. There is a clear transition in the intensity plot at about 56°C. The treated sample, on the other hand, shows no transition at all: It appears as if the proprietary treatment has had a similar effect to temperature in denaturing the antibody.

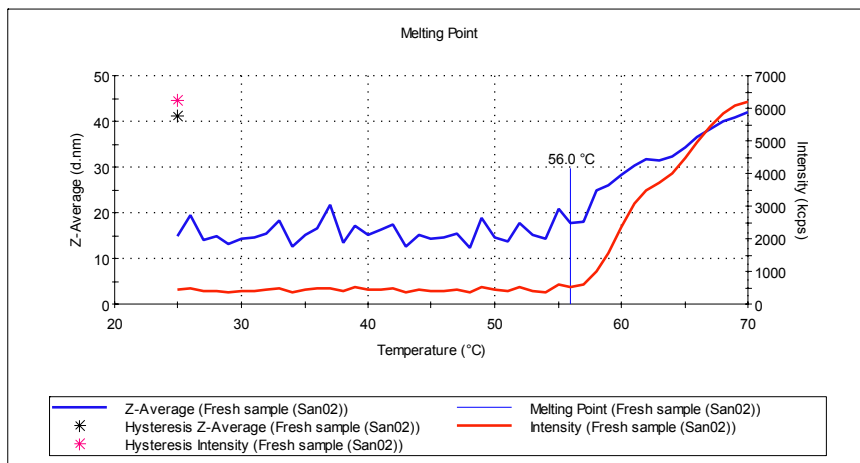


Figure 2: Melting point determination of the untreated sample.  $T_M = 56^\circ\text{C}$

## Zetasizer Nano System

The Zetasizer Nano system from Malvern Instruments is the first commercial instrument to include the hardware and software for combined dynamic, static, and electrophoretic light scattering measurements, providing the researcher with a wide range of sample properties, including the size, molecular weight, and zeta potential. The system was specifically designed to meet the low concentration and sample volume requirements typically associated with pharmaceutical and biomolecular applications, along with the high concentration requirements for colloidal applications.

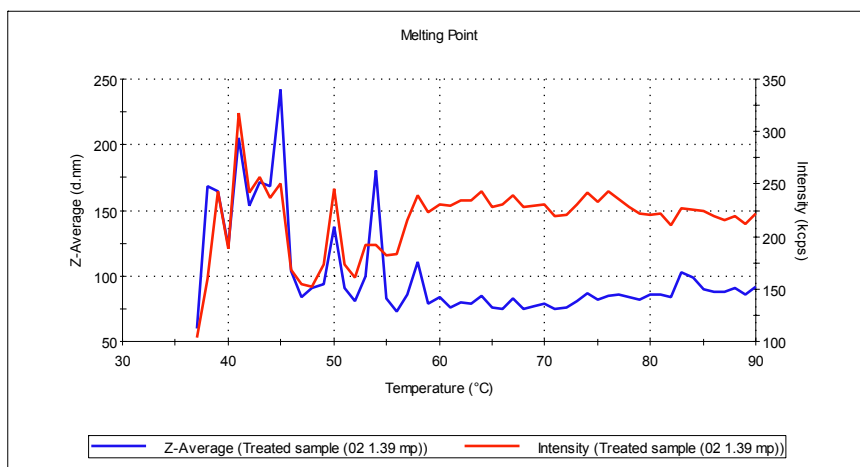


Figure 3: No melting point was found for the treated antibody sample.

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