Titre: " Protein characterization using the VASCO3 granulometer; illustration on Lysozyme size measurement"

Key words: Proteins, Lysozyme, Dynamic light scattering, particle size, granulometer, VASCO

Introduction:

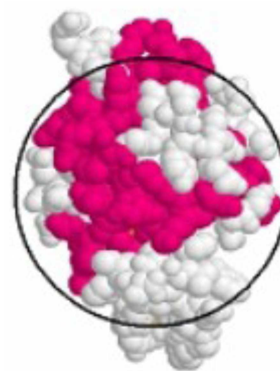
The size characterization of proteins and protein like particles in solutions is critical in many biological, medical and industrial applications; indeed, preparation and purification method, storage conditions and/or buffer choice can all influence the size and quality of proteins in a sample. Among the various experimental methods available today to measure the size of nano particles, Dynamic Light Scattering (DLS) is probably one of the fastest and easy to use method. Nevertheless, measuring very small particle with a poor refractive index contrast like proteins remains a challenge for many commercial DLS systems. In this note, we illustrate how the VASCO3 granulometer can be used in a very efficient and simple way to measure the size of proteins. As a concrete demonstrative example, we present some measurement on Lysozyme proteins.

Protein measurements with the VASCO3 granulometer

The VASCO™ particle size analyzer series offers an innovative solution to perform particle size measurements in a very broad range of applications from very concentrated samples (up to few tenths %) down to very diluted ones (concentration less than few parts per million) and very small particles. Thanks to its new generation of Avalanche Photo Detector named SP-APD for "Single Photon counting" APD used in combination with a high output power laser diode at 656 nm, this instrument provides an accurate and very sensitive particle size measurement even for low concentrations and weakly scattering particles. This makes the VASCO 3 a powerful tool for the analysis of challenging samples like protein dispersions. In this application note, we report a measurement of lysozyme dispersed at 1 mg/mL in PBS solution.

Sample preparation

For the sake of experimental demonstration, we have used lysozyme proteins of chicken egg white which have been extensively studied [1,2] due to their compact globular structure (see enclosed picture extracted from ref [2]) and stability over a wide range of solution conditions. These measured lysozymes have a molecular weight of 14.3 kDa and were purchased from Sigma-Aldrich (Ref 62970) [3].



This product is a powder which has been dialyzed and lyophilized. The preparation of the sample is made through several preparation steps:

First, the proteins are dispersed at 1 mg/mL in a PBS solution. This dispersion is then centrifugated at 6000 rpm for few minutes to remove residual micrometric impurities (especially dusts) and finally filtrated through a 0.02µm pore size filter of cellulose acetate.

Note that this step of "sample cleaning" is critical for protein analysis with Dynamic Light Scattering since this technique is quite sensitive to the presence of inhomogeneities suspended in the liquid. Thus, the light scattered by dusts or big aggregates in the analyzed volume can strongly interfere with the very weak light signal scattered by the proteins. Finally, 40µL of the protein dispersion is placed in the sample cell of the VASCO before being characterized.

Results

The light scattered by the sample is collected at an angle of 135° and send to the SP-APD with a single mode fiber before being processed by the VASCO proprietary designed correlator. With up to 1000 linear channels (DSP 16bits), a channel time selectable from 125 ns up to 30 ms, this correlator provides high accuracy and excellent time resolution even for fast events analysis. In combination with the SP-APD, the sample correlogram shown in figure 1 (purple spots) is obtained in less than 120 seconds.

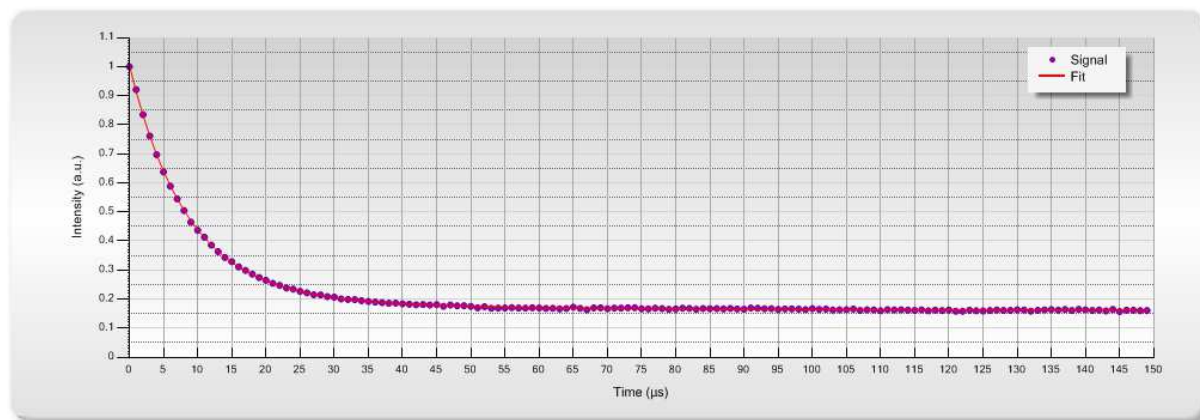


Figure 1 : *Correlogram obtained from the proteins dispersion, after 120 seconds of acquisition (purple spots) and fit of the Padé-Laplace algorithm (red curve). Correlator settings: time interval=1µs, number of channel =150*

The correlograms determined by the VASCO instrument are further processed by the NanoQ™ software with its unique “inversion algorithm” named Padé-Laplace algorithm. A first analysis on correlogram of Fig 1 indicates the presence of a single main population having **an average hydrodynamic diameter of 3.8 ±0.2 nm** which can be related to the presence of non-aggregated Lysozymes in PBS.

In order to investigate further the size distribution profile, the same sample is analyzed using the statistic acquisition mode of NanoQ ™. This mode automatically cumulates results of several acquisitions on a single histogram and provides an accurate and exhaustive description of the sample dispersion without any assumption of the distribution form (Contrary to most of inversion algorithm assuming, for instance, perfect Gaussian distributions). The protein dispersion analysis is reported on figure 2.

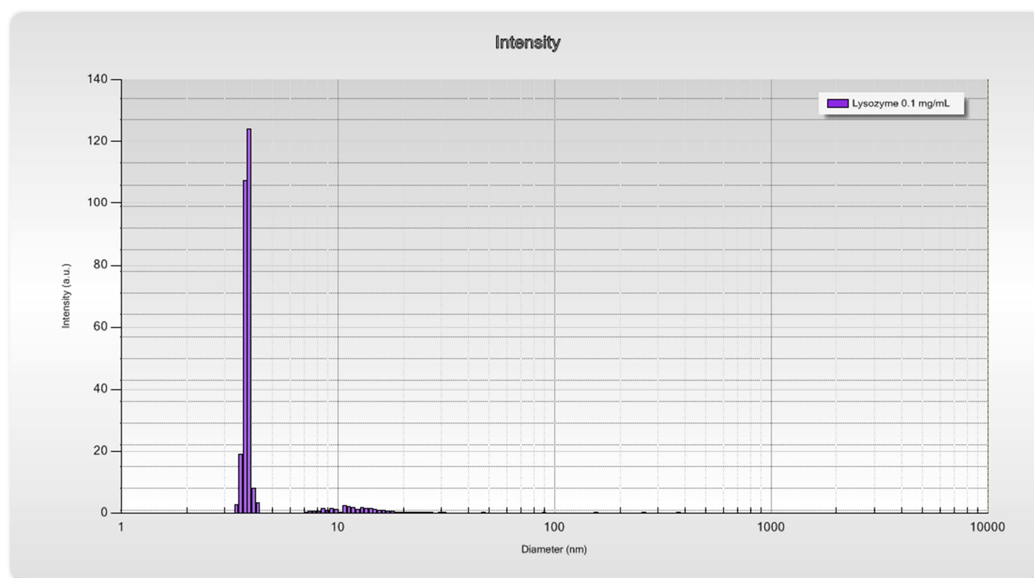


Figure 2 : Size histogram of the 1mg/mL proteins dispersion obtained with Padé-Laplace algorithm and the statistical acquisition mode. The horizontal axe shows the hydrodynamic diameter in nm and the vertical axe the amplitude in intensity (U.A.)

Figure 2 shows that on the histogram, the population of lysozyme appears like a peak around its average hydrodynamic diameter of 3.8 nm, with dispersion of detected particles only **between 3.4 and 4.3 nm**. The population of lysozyme is quite narrow around its average size which is consistent with the structure of a protein sample and from previous literature data.
However, from figure 2, one can see that the statistic analysis of the dispersion also reveals the presence of residual objects detected **between 7 and 27 nm**. These particles, probably aggregates or/and small impurities, have a very small intensity amplitude compared with the non-aggregated lysozyme population (< 2%), and they could have been easily unnoticed with an unique and basic analysis. Nevertheless, the multi acquisition has allowed to detect them with the VASCO showing the high level of performance of the combination SP-APD and Padé-Laplace algorithm and the relevance of the statistical analysis approach.

Conclusion

We have demonstrated the capability of the VASCO3 system to measure proteins like Lysozyme. The VASCO 3 analysis of the lysozyme dispersion, carefully prepared at 1mg/mL, indicates the presence of a main and monodisperse population having an average hydrodynamic diameter of 3.8 nm. The statistic analysis mode of NanoQ reveals also the existence of bigger residuals objects between 7 and 27 nm, being probably aggregates or/and small impurities.
The SP-APD and the high power laser diode of VASCO 3 provide a powerful tool to characterize low concentration proteins dispersion on a broad size range. The combination of our proprietary designed correlator and the NanoQ™ software advanced analysis allow also to make a thin and exhaustive description of the sample dispersion, a significant benefit to evaluate the degree of protein aggregation, for instance.

References:

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[2] Dejan Arzenšek, Dynamic light scattering and application to proteins in solutions, Seminar - 4th year, University of Ljubljana, May 19, 2010.

[3] http://www.sigmaaldrich.com/catalog, (Ref 62970)