PHARMACEUTICAL

Fast temperature screening of proteins

Introduction

Proteins are widely used in the pharmaceutical domain, especially as therapeutic agents. Each protein possess a specific denaturation temperature, which has to be determined prior to use, in order to prevent product loss during production, formulation and administration. This application note shows how RHEOLASER Crystal can be used for rapid screening for proteins, such as Bovine Serum Albumin (BSA, a model protein) dispersed in water and in phosphate buffer.

KEY BENEFITS

- FAST
- LOW SAMPLE VOLUME
- ACCURATE

Method

Samples of BSA with different concentrations (25-200mg.mL^{-1}) were prepared in pure water by dispersing the protein under stirring at 25°C for at least 1 hour. A phosphate buffer was used (Sigma-Aldrich PBS, pH 7.2) for tests with buffer. The samples were heated from 25°C to 90°C in a small volume cell, using less than 100 µL for each run. The heating rate was 2°C/min.

Bovine Serum Albumin in PBS

Figure 1 shows the Microdynamics (µD) and Microdynamics evolution (µDE) of a 75mg/mL BSA solution in PBS as a function of temperature.

µD is a measure of particle mobility in the sample, the higher this value is, the more the particles move in the sample. We can observe three different zones, due to sample changes during heating. These variation of µD can be assigned to a conformational change (I), denaturation (II) and gel formation (III).

In order to quantify the µD it can be expressed in µD evolution, which is the normalized integration of the peaks. RHEOSOFT Crystal allows the automated 1-click calculation of 4 characteristic temperatures. Mean transition temperature T50, on-set and off-set temperature T10 and T90, respectively, and the ΔT (T90-T10).

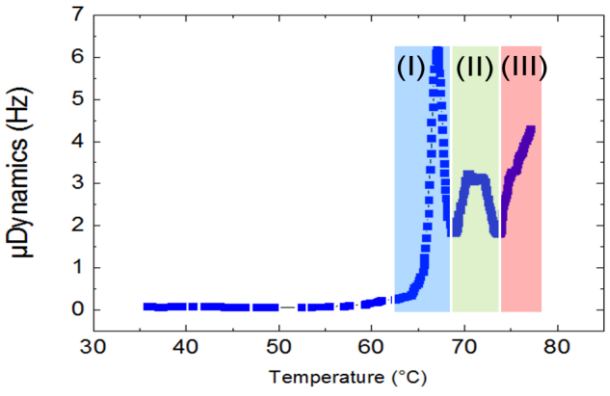

Fig. 1 - µDynamic as a function of temperature. 75mg.mL-1 BSA in PBS.

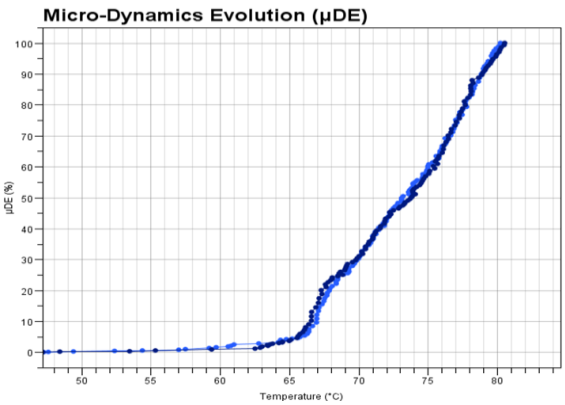

Fig. 2 - µDynamic evolution as a function of temperature. 75mg.mL-1 BSA in PBS.

Concentration effect

Figure 2 shows the µD evolution of different concentrations in PBS buffer. The characteristic temperatures T10 (temperature at µDE = 10%), was extracted and listed in table 1. It can be seen that the denaturation temperature decreases with increasing protein concentration.

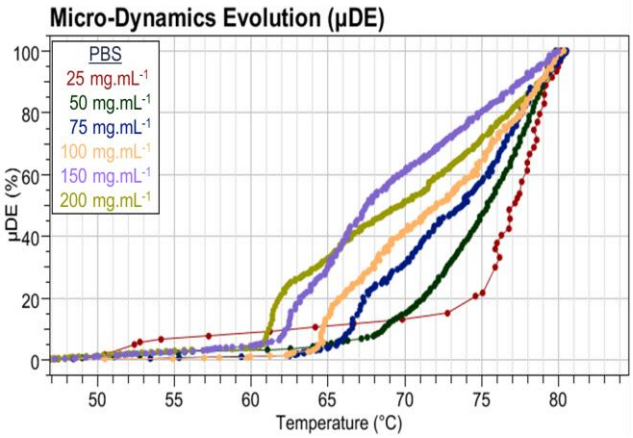

Fig. 3 - µDynamic evolution as a function of temperature for all BSA concentration in PBS.

Salt effect

BSA was also dispersed in water (without any salt). As seen in Fig. 3, the transition temperatures are much lower than in phosphate buffer. Accordingly to the literature, this is due to the absence of sodium ions, which possess a protective role in BSA denaturation, shifting the transition temperatures towards higher values when present in solution. The values are given in table 1 in comparison to those in PBS buffer.

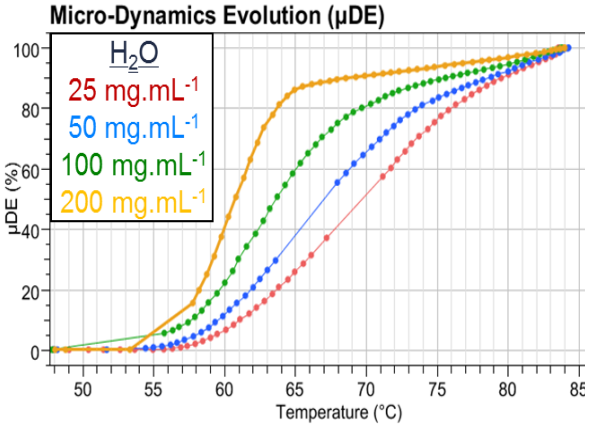

Fig. 4 - µDynamic evolution as a function of temperature in water only..

Tab. 1 : T10 temperature for all BSA concentration in PBS and H2O

Conc. (mg.mL-1)	PBS	H2O
25	77.3	69.9
50	72.2	67.8
75	68.5	-
100	66.2	64.6
150	63.1	-
200	61.9	61.1

CONCLUSION

RHEOLASER Crystal is a straightforward new technique to analyse rapidly the behaviour of proteins under heating. The combination of a precise temperature control with an optical method allows the determination of the microdynamics of proteins with heating rates up to 5°C/min, keeping an accurate determination of the denaturation process. Several mechanism (unfolding, denaturation and gelation) were observed.