Biologics Aggregation Analysis

- Testing biotherapeutics in their original sealed container

Biotherapeutic medicines are important treatments for many chronic diseases, such as cancers, diabetes, and rheumatoid arthritis [1], and potential drug candidates for COVID-19 treatment and prevention. With a double-digit growth, biotherapeutics will represent more than 50% of the pharmaceutical pipeline in 2022. They include recombinant proteins, monoclonal antibodies, vaccines, blood, plasma and cell-gene therapy.

Therapeutic proteins, monoclonal antibodies and vaccines have a propensity for aggregation during manufacturing, shipping, and storage. This is of concern to both manufactures and regulatory agencies because can induce adverse immune responses in patients that may affect safety and efficacy [2].
The challenge in analysing protein aggregates lies in the unknown nature of the formed aggregates as well as the wide size range. It is often necessary the use of a combination of techniques [2]. Most of these techniques require opening the vial to test the content, an undesirable feature when analysing sterile finished products (vials or filled syringes) [3]. Assessment at the point-of-care is mostly done by visual inspection with a large associated error.

We present here a new simple and affordable method to analyse protein aggregates with a benchtop TD-NMR spectrometer.

Analysis of Protein Aggregation by TD-NMR: How does it work?

In water solution, the solute (protein) and the solvent are tumbling relative to different physical parameters (e.g. size and solution viscosity). This tumbling rate can be easily measured and interpreted by the Transverse Relaxation (T2) NMR property [4]. Moreover some protons from the protein, exchange with the surrounding water protons. As a consequence, the tumbling information of the protein (short T2 value, dotted line fig. 1) is transferred to the water signal (long T2 value, solid black line fig. 1) lowering the whole bulk water T2 measured value (red line in fig.1). If protein concentration and/or the size of the particle increases (e.g. protein aggregation), the lowering of the measured T2 value will be even more.

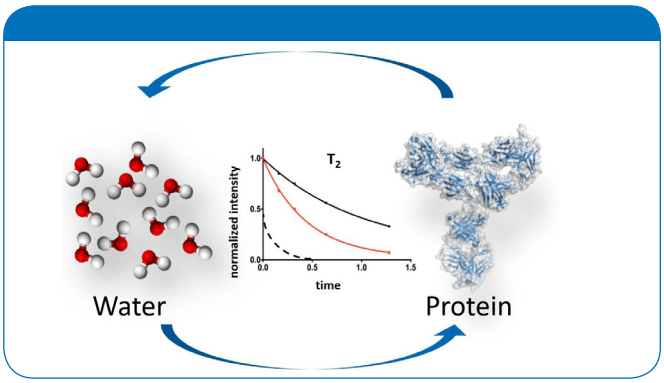

Fig 1. Water-protein proton chemical exchange and effect on the T2 value

Determination of level of aggregation by TD-NMR

R2 (R2=1/T2) is directly correlated to the increase of concentration (mg/ml) in a fresh Bovine Serum Albumin (BSA) buffer solution (fig 2). Linear fitting can be defined and used as model to extract information about concentration using the R2 TD-NMR value of different samples.
If a constant amount of protein is used, then R2 shows linear correlation with the level of aggregation (fig. 3). A "fresh BSA sample" (BSA @ 4°C for 2 hours) and "stressed BSA sample" (BSA @ 75°C for 2 hours) at the same concentration, were combined at different percent levels (i.e. 0, 25, 50, 75 and 100% of stressed sample). This approach enabled a controlled level of aggregation.
The same percent level of stressed samples was repeated for different protein concentration (i.e. 5, 10, 20, 30 and 40 mg/ml) to create different models. In conclusion, R2 can be used to quantify the amount of aggregation in each protein formulation.

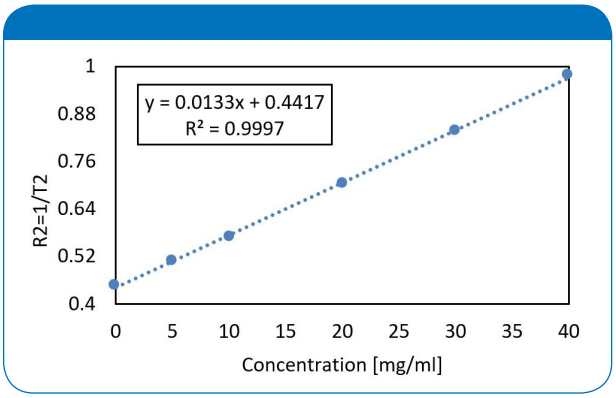

Fig 2. Linear correlation between R2=1/T2 and BSA water solution concentration.

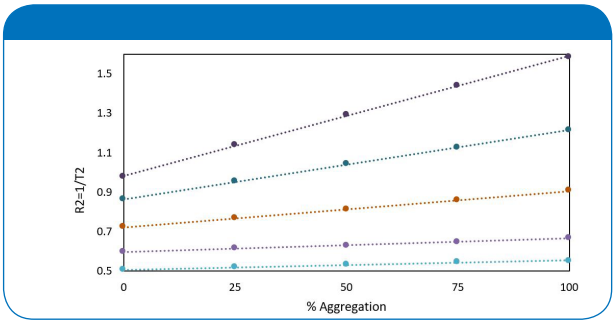

Fig 3. Linear correction between R2=1/T2 and different % of aggregated samples: 5 mg/ml; 10 mg/ml; 20 mg/ml; 30 mg/ml; 40 mg/ml
All the linear fitting squared value R2 were larger than 0.9965
All the measurements were done in five replicates with an error lower than 0.2%

Advantages

- Easy determination of concentration or aggregation of proteins
- Non-invasive and non-destructive technique
- No sample preparation required – allows measurement of protein in its native state
- Small footprint TD-NMR system (benchtop), cryogen-free
- Measurement of the pharmaceutical compound in its closed container packaging
- Equally well applicable to glass and plastic syringes, vials and ampoules
- Combined application with a single instrument on the same sample (e.g. contactless check weighing and aggregation)
- Robust method for development of new formulations
- Quick and easy measurement for quality check in bioproduction line

References

[1]https://www.who.int/medicines/access/biotherapeutics/en/ accessed 02Oct20
[2]Den Engelsman J., et al., Pharm Res. 2011, 28: 920–933
[3]Feng Y., Taraban M. B., Yu Y. B., Chem. Commun. 2015, 51: 6804–6807
[4]Abragam, A. (1961). Principles of Nuclear Magnetism. Clarendon Press. p. 15