Characterization of injectables Proteins and Active Pharmaceutical Ingredients with In situ DLS Instrumentation

Authors: *David Jacob, Sylvain Boj- Cordouan Technologies*

Key Words*: Protein aggregation, Biopharmaceutical API, Injectable, In situ, contactless measurements, Dynamic Light Scattering (DLS), Nano-Particle sizer, VASCO Kin*

During the development of pharmaceutical injectable products, including vaccines, manufacturers require a constant assessment of proteins and active ingredients to ensure the product quality. Proteins, in particular, are fragile and can easily be modified under specific conditions, such as temperature and pressure.

Measurement and Analytics of Protein and API during process

For many biopharmaceutical products, particularly injectables, active principle ingredients (API) stability and protein aggregation are factors that ultimately impact both the quality and usability of the final product. Aggregation of proteins for example can occur at any stage in the therapeutic protein's lifecycle, including at protein expression, refolding, purification, sterilization, shipping, storage, and delivery. While different manufacturing processes have been implicated in increasing the risk of protein aggregation and API degradation, the true mechanism by which these degradations occur still remains unknown in many cases.

What is known is that chemical degradation of a protein-based product is increased in the presence of viral or microbial contaminants and certain storage conditions. Minimization of these and other risk factors is key to prevent – or at least mitigate – protein degradation in biopharmaceuticals.

Many researchers today are working toward improving in situ monitoring of therapeutic protein degradation and denaturation, both during production and storage. Additionally, stricter health regulations are being developed to ensure appropriate control of biopharmaceutical products to ensure protein stability throughout the stages of the biopharmaceutical lifecycle.

Measurement Techniques of in Biopharmaceutical Products characterization

Multi-angle static light scattering (MALS) represents one measurement technique that can be utilized to examine the presence of protein aggregates, especially at an early stage. Some investigators combine MALS with different separating methods, like asymmetrical-flow field-flow fractionation, analytical ultracentrifugation, and size-exclusion chromatography (SEC).

Batch dynamic light scattering (DLS) is another measurement and analytical technique that can provide a characterization of protein aggregates. The DLS method is often used when the dilution or shear encountered in SEC creates disassociation of the protein aggregates, or when assessing aggregation of proteins under different temperatures and other environmental conditions. The DLS technique enables accurate measurements of particle sizes from one nanometer to up to a few microns in a minute.

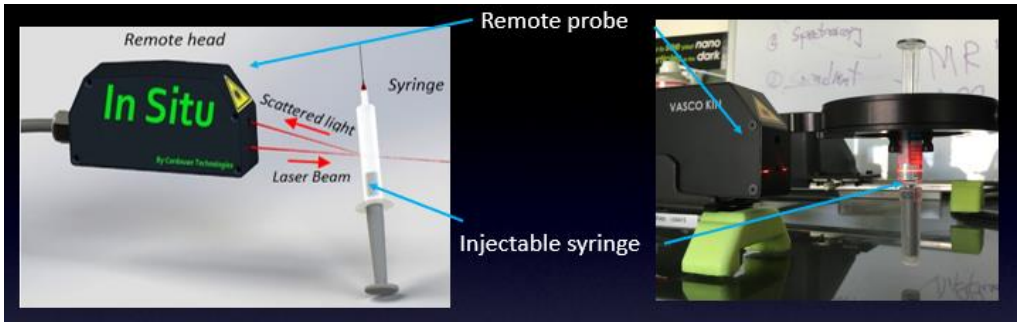
These methods, while efficient, often require handling and preparation of the sample before and during the measurement. This handling, in turn, can alter the sample aggregation. Therefore, researchers must use a technique that allows for direct analysis of samples in the storage medium, such as in a hermetically sealed syringe or vial.

In Situ Contactless Measurement with VASCO KIN™

A new possibility to control the modification of proteins and API in various containers like syringes is now available through an **in situ contactless** DLS measurement system, the **VASCO KIN™.** This innovative instrument developed by Cordouan Technologies is a new-generation time-resolved modality for accurate kinetic analyses which can assist in the characterization of protein and active pharmaceutical ingredients. The **VASCO KIN™** allows for real-time monitoring of nanoparticle synthesis, agglomeration, and the stability of suspensions; Also, it features an in situ and contactless remote optical head, which significantly reduces the risk of contamination. The in-situ head is robust and compact, results in rapid measurement, and features an embedded visible alignment laser to assist with easy installation and accurate positioning.

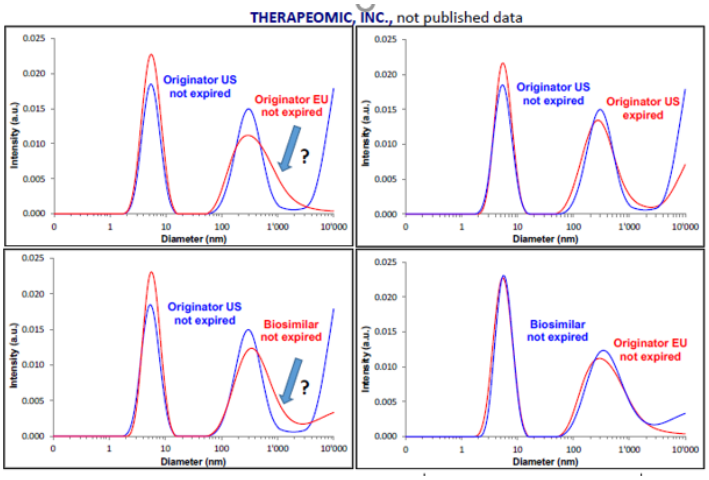
Additionally, the VASCO KIN provides the user with a single and continuous measurement. The tool gives direct access to all characterization data of a reaction, including the size distribution, scattered intensity, and correlograms, among other data. The VASCO KIN contains a ultrafine spectrum - frequency stabilized laser and high sensitivity artifact-free avalanche photodiode detector, which provides high measurement accuracy and accommodates very low scattering samples (ie, very low concentration and/or down to 1 nm size particles). Also, the instrument has a dedicated PC that includes software correlation and a complete and dedicated user-friendly NanoKin® software.

The VASCO KIN uses a completely mobile DLS system and its novel Optical Fiber Remote Probe (OFRP) to overcome this issue. This probe is robust and features an optimized optomechanical assembly designed for both contactless and direct measurements of the sample. It also lacks the need for any sample batching procedure. The OFRP, which is connected to an Optical Unit, injects a laser beam into the protein sample and gathers scattered light in a backward direction at a 170° angle.



Thus the **VASCO KIN™** is the ideal tool for deeper insight into protein and API modifications under a variety of storage conditions like syringes and hermetically sealed vials.

Recent investigations used the VASCO KIN instrument for contactless particle size measurements in commercial injectables like flu vaccine and proteins-based products. An example of such investigation is shown below with the particle size distribution measurement comparison between an originator and a biosimilar injectables from EU and US, either expired or not expired. Such results illustrate in a clear manner that thanks to the VASCO Kin measurement, it is possible to compare and discriminate in an accurate way injectable product by their origin or detect modification over their shelve lifetime. These findings clearly demonstrated the efficacy and utility of the VASCO KIN for the study and survey of protein and API injectables biopharmaceutical application.



Example of In-situ contactless DLS measurement comparison between originator and biosimilar injectable API products in un-open syringes (Tudor Arvinte & al, Therapeomic Inc, 2018-Not published data).

Conclusion

During any stage of the biopharmaceutical product development cycle, active principal ingredients and proteins are susceptible to modification. Proteins are particularly fragile and can aggregate, ultimately affecting the overall usability of the final product. The fragility of proteins and other active ingredients warrants the use of sophisticated measurement techniques and platforms that can reliably monitor ingredient stability over the product's lifecycle.

The VASCO KIN is one such instrument that is being successfully employed by pharmaceutical companies for the continuous measurement and monitoring of proteins, among other active ingredients, in biopharmaceutical product development. This platform offers real-time contactless measurements, reducing the risk of disturbing therapeutic samples, and offer clear visualizations of protein aggregates, nanoparticle synthesis, the stability of suspensions, and modifications in particle sizes.

References and Further Reading

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7398748/
- https://www.cordouan-tech.com/products/vasco-kin/
- https://www.sciencedirect.com/science/article/abs/pii/S0168583X14009306