Monitoring protein aggregation in injectable vaccines syringes: a new direct & contact-less technique

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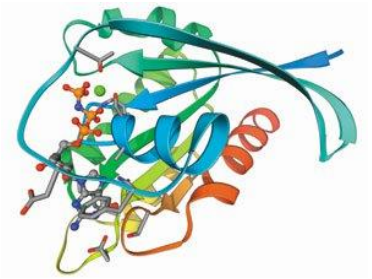
Introduction

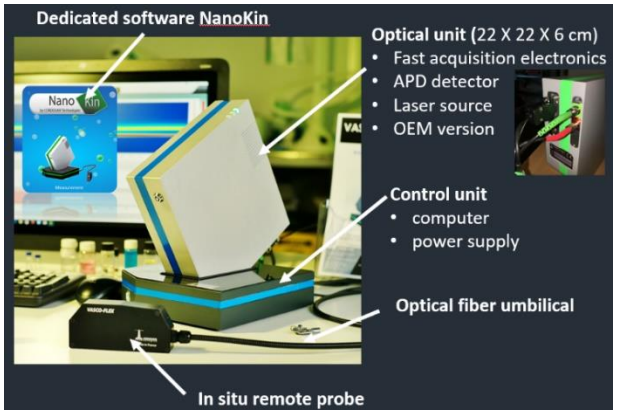
Aggregation of proteins and **active principle ingredients** (API) in **injectable biopharmaceutical products** remains a major concern impacting the stability and usability of a product. Indeed, **Protein aggregation** can occur during all stages of the lifetime of a protein therapeutic, including expression, refolding, purification, sterilization, shipping, storage, and delivery processes [1, 2]. The mechanism of protein aggregation is still not well understood; but it is known that certain manufacturing stages like formulation composition, presence of microbial or vial contaminants during cell culture, and storage influence the risk of chemical degradation, which increases the risk of physical degradation and the formation of aggregates. In particular, it has been shown that the storage container environment plays a role like in prefilled syringes for example where leaking silicone oil from the rubber stopper, and glass delamination can induce aggregates. In a context of more and more stringent international health regulations about the control of biopharmaceutical products, the in-situ monitoring of the denaturation and degradation process of therapeutic proteins during production and storage can be a key competitive advantage for manufacturers and researchers.



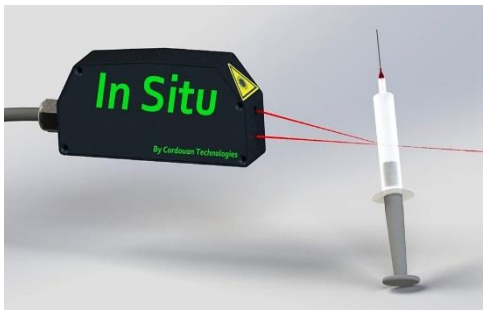
Current measurement techniques and limitations

Many different analytical techniques are used today to study protein aggregates in the one to few hundreds µm range like light obscuration (LO), dynamic imaging particle analysis (DIPA) techniques, micro-flow imaging (MFI), and Coulter counter (CC). For early stage detection and quantification of submicron aggregates, other techniques such as multi-angle static light scattering (MALS) coupled to separative techniques like size-exclusion chromatography (SEC), analytical ultracentrifugation (AUC) and asymmetrical-flow field-flow fractionation (AF4) are routinely used [3]. Alternatively, batch Dynamic Light Scattering (DLS) is another useful optical technique to characterize aggregates when the dilution or shear encountered in SEC or FFF causes the aggregates to disassociate or when aggregation is to be measured under many conditions and/or temperatures. All these recognized techniques are quite complementary and efficient in their range of use but they require some specific preparation, conveying and handling process of the sample before or during measurement which can modify the sample aggregation state. That's why measuring directly into the storage medium like hermetically sealed vial or syringe without any need of sample manipulation and handling would be preferred in many cases. But none of the previously mentioned techniques are fitted to make measurements directly in injectable. A change of paradigm is then needed: **if you cannot bring your sample to the measurement, you have to bring the measurement to your sample**!

In situ contactless measurement: The VASCO Kin© concept



In order to address such kind of applications, Cordouan has developed a new system named VASCO Kin© (see picture on the left) which is based **on real time in-situ contactless remote DLS measurements**. DLS, one of the prevalent technique of choice in colloidal sciences and proteins characterization study is a mature and very powerful technique based on the analysis of scattered light fluctuations caused by the Brownian motion of particles [4]. It allows accurate particle size **measurements from one nanometer up to a few microns** in a minute. Though different measurement configurations are available on commercial DLS systems today, *they all require the user to withdraw the sample with a pipette or an automatic pump and to place/inject the sample into the instrument prior to the measurement, potentially impacting the sample protein aggregation state*.



On the contrary, the VASCO Kin is a fully agile DLS system which circumvents that issue thanks to its unique Optical Fiber Remote Probe (OFRP). The OFRP, an optimized and highly robust optomechanical assemblies is designed **to make direct and contact less measurements without any need for sample batching process**. Connected to an Optical Unit by mean of a special optical fiber umbilical, the OFRP injects a laser beam into the sample and collect the light scattered by the sample in the backward direction at an angle 170°(see picture on right) . A highly sensitive single photon Avalanche Photodiode Detector (APD) connected to a dedicated fast acquisition electronic board monitors in real time the scattered light intensity fluctuations which are converted into time-resolved particle size kinetic analysis with powerful mathematical algorithms. More detailed information about the VASCO Kin can be found in [5]

Measurement in vaccines syringes

In order to demonstrate the capapbilities of the VASCO kin to achieve contactless in-situ measurement into syringe, we have made series of particle size **measurement on a commercial injectable flew vaccine** (see fig 1- left). This vaccine is a complex medium made of a mixture of many different ingredients : desactivated and fragmented Flew Virus from three different stem cells, several excipients like ppi water, Potassium chloride, Sodium chloride, buffered saline solution, and traces of chicken eggs proteins (ovalbumin), Formaldehyde, Neomycin, Octoxinol 9, etc. For the purpose of the measurement the vaccine syringe was removed from it plastic bilster[1] (see figure 1) and placed in a dedicated mount at a distance of 6 cm in front of the prob (see fig 1-right) ;

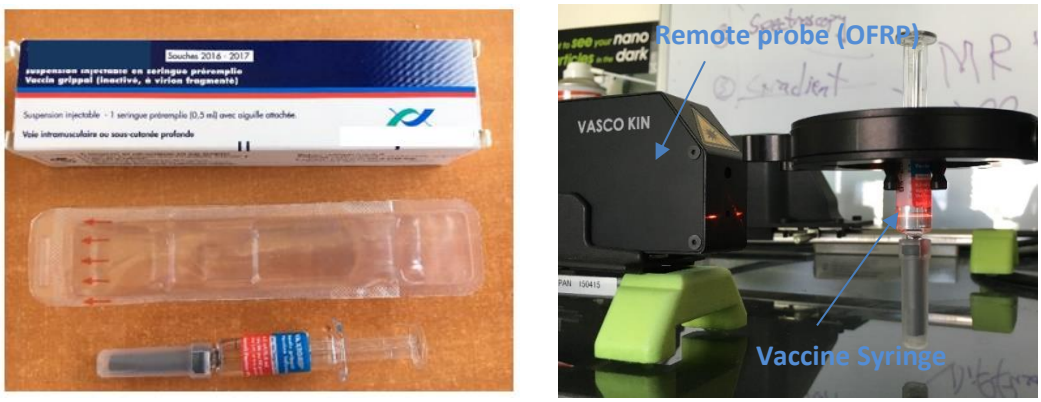


Figure 1: The VAXIGRIP vaccine syringe out of its blister (left); Measurement setup (right) with the VASCO Kin remote head mounted on a dedicated translatable stage and placed in front of holder designed for the purpose of the experiment

For comparison purpose and to evidence possible vaccin aging effects, we have stored one vaccin in a fridge at 7°C while another vaccin was stored at room temperature for 8 months. The two vaccins were then measured in the same conditions the same day. The particle size distribution results are presented below:

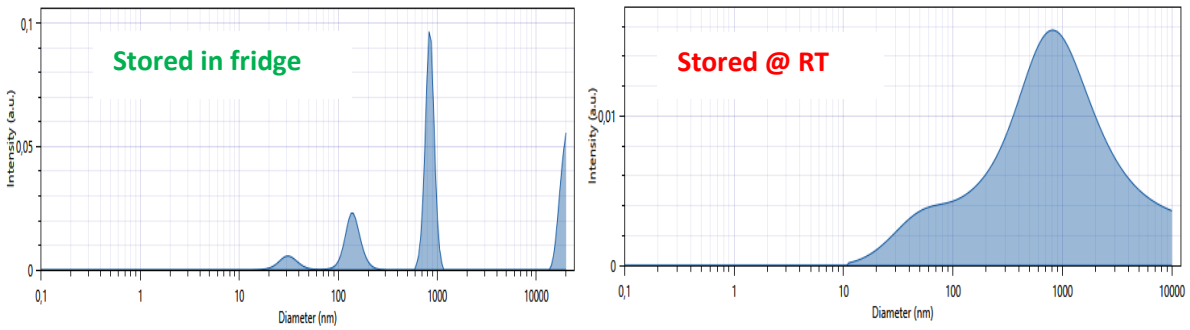


***Figure** 2: Particle size distribution measurement results (X axis: size in nm; Y axis Amplitude in arbitrary unit) of a vaccine stored in a fridge (top) and of a vaccine stored at room temperature for 8 months (bottom)*

[1] *For verification purpose, some tests have also been made without removing the plastic blister giving very consistent results with the measurements outside the blister.*

For the vaccine stored in a fridge (fig 2 -top) one can notice the relative complexity of the sample particle size distribution exhibiting three distinct populations ranging from 30 nm up to 800 nm. These peaks correspond to virus fragments and proteins. One can also notice the presence of few aggregates beyond 10 µm. By comparison (fig 2- bottom), the vaccine stored at room temperature clearly shows some noticeable changes in the particle size distribution which now appears like a broad continuum from 10 nm to 10 µm and beyond. This variation would certainly deserve further investigation to understand the reason for this change. But, to the best of our knowledge these preliminary results are the first demonstration of an in situ DLS measurement into injectable syringe.

Conclusion

We have presented the **first practical demonstration of a contactless in situ particle size measurement of a commercial injectable vaccine directly into a syringe.** By eliminating sample batching steps, the VASCO kin system with its innovative optical fiber remote head is opening up new fields of application to particle size measurement systems, in particular for the **in-situ monitoring of protein aggregation in biopharmaceutical injectable products**. The VASCO kin can also be used to monitor in real-time Nano particle synthesis Kinetic in various type of reactor configuration (double jacket glass reactor, high pressure &high temperature Super Critical CO2 autoclaves, microwave reactors, micro fluidic chips, etc) or for instrumental coupling [6].

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Further reading:

http://www.pharmtech.com/analyzing-protein-aggregation-biopharmaceuticals

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