Technical note NT-*In situ*_Drug_Control_ EN

D. Jacob[1], H. Anop[1], S. Boj[1], B. Maxit[1], S. Race[2]
1: Cordouan Technologies SAS
2: Xigo nanotools Inc

Key Words: *Dynamic light scattering (DLS), In situ contactless measurements, active pharmaceutical ingredients (API), size distribution, stability, aggregation.*

Pharmaceutics is the science of preparing and dispersing of active pharmaceutical ingredients (also abbreviated as API) their delivery to the target and a formulation of final medicament. Mainly APIs are the small molecules, protein-based therapeutics, based on the DNA and RNA vaccines etc. In order to deliver APIs to their target in living organism, scientists use biocompatible polymers, proteins, drug encapsulation into polymeric shell or into liposome, nanoparticles and many other systems (see fig.1). All steps of the drug development require very strict control of the initial materials, final products as well as their stability in time and size. While the stability of medicines is related to their activity, the size is affecting the permittivity of the APIs through the cell membranes. Both of these parameters have influence on the efficiency of developed medicament and have to be controlled.

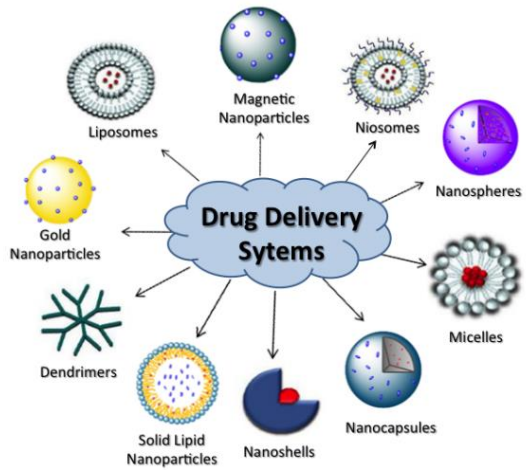

Figure 1. Schematic illustration of the different drug delivery systems [1]

To qualify and detect small molecules, high cost mass spectrometry or nuclear atomic resonance are widely used. In contrast, majority molecules with hydrodynamic size higher than 0.5 nm can be detected with optical methods like dynamic light scattering (DLS, d≥0.5nm[*]), light diffraction (d≥10nm[*]) or optical microscopy (d≥200nm[*]), which have many advantages, including their lower price.

Aggregation of API in liquid biopharmaceutical products remains a major concern, impacting the stability and usability of a product. For example, protein, nanoparticle or dendrimer aggregation can occur during all stages of the lifetime of a therapeutic, including production, purification, sterilization, storage, and delivery processes [2, 3]. The mechanism of API aggregation depends on their origin, and for certain APIs (such as proteins) is still not well understood. In particular, it has been shown that the storage conditions (temperature or light) affect the stability of liquid medicament, such as different type of injection, oral solutions and suspensions etc. In a context of increasingly stringent international health regulations of biopharmaceutical product control, the **in-situ monitoring of the denaturation and degradation process of APIs** during production and storage can be a key competitive advantage for manufacturers and researchers.

Many analytical techniques are routinely used today to study aggregates: dynamic imaging particle analysis (DIPA), micro-flow imaging (MFI), multi-angle static light scattering (MALS) etc. The coupling with

[*] system and instrument dependent

separative techniques (such as size-exclusion chromatography or analytical ultracentrifugation) helps to analyze all drug components individually [4]. Alternatively, batch DLS is another useful optical technique to characterize aggregates at various conditions, including different temperatures. All these recognized techniques are quite complementary and efficient in their range of use, but they require some specific manipulations (preparation, conveying, and handling) with the initial formulation before or during measurement, which can modify the aggregation state of APIs. That's why measuring directly into the factory packing, like hermetically sealed vial, bottle, or syringe without their opening would be preferred in many cases. But up to 2017 such kind of instrument didn't exist. To fix it, **Cordouan Technologies** has developed **contactless in-situ** remote probe to measure the size of nano-object using **DLS** in any transparent container (see fig.2).

Figure 2. The typical commercial containers used for liquid medicament storing (left) and the schematic illustration of the contactless in-situ measurements within one of them (right)

Developed in Cordouan Technologies, **Optical Fiber Remote Probe (OFRP)** with its optimized and highly robust opto-mechanical assemblies of OFRP has been designed to perform direct and contactless measurements without any need for sample batching process. This new OFRF currently is available for **VASCO Kin** [5] and **AMERIGO** [6] devices. 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 the 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 (kinetical) particle size analysis with powerful mathematical algorithms.

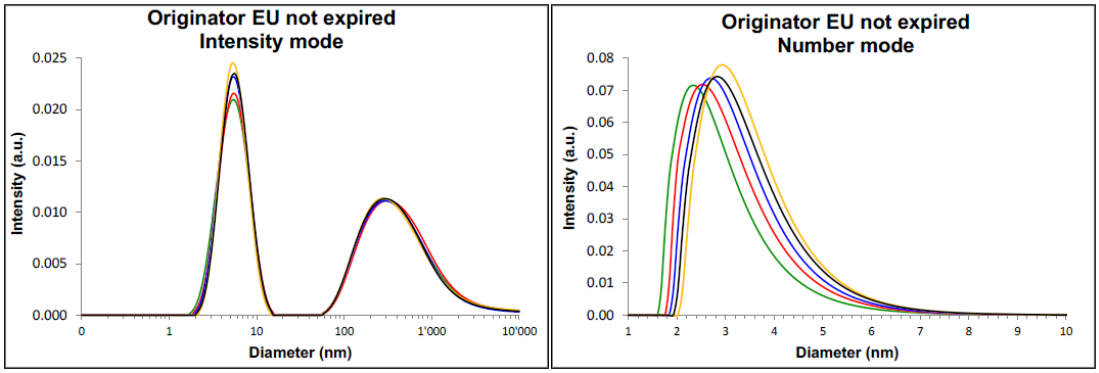

Figure 3. DLS measurements in unopened syringes: comparison of an originator and biosimilar product made by Darpin G., Poirier E. et Arvinte T. from Terapeomic INC in 2018 (unpublished data). Result comparison for 5 different injectable from the same lot.

In order to demonstrate the capabilities of the OFRP, Tudor Arvinte & al (Therapeomic Inc) have made **the contactless in-situ series of particle size measurement into syringes** for o*riginator and biosimilar injectable API products* using VASCO Kin. These suspensions are a complex medium made of a mixture of different ingredients needed for medicament formulation. To check the reproducibility of the DLS contactless

measurements, 5 syringes with *injectable API products* from the same lot were tested with OFRP of Cordouan technologies (fig.3).

For comparison purpose and to evidence possible difference between o*riginator and biosimilar injectable API products*, they have done the parallel measurement at the same conditions into different syringes with the same injectable product produces in the USA and Europe. The particle size distribution results are presented by figure 4.

	Intensity mode		**Number mode**
	⌀ (nm) Peak 1	⌀(nm) Peak 2	⌀(nm) Peak 1
O*riginator US not expired*	5.4±0.1	299±27	2.7±0.2
O*riginator EU not expired*	5.4±0.1	293±11	2.7±0.2
O*riginator US expired*	5.5±0.2	262±37	3.0±0.1
Biosimilar not expired	5.7±0.1	340±27	3.2±0.2
Biosimilar expired	5.8±0.2	306±38	3.1±0.4

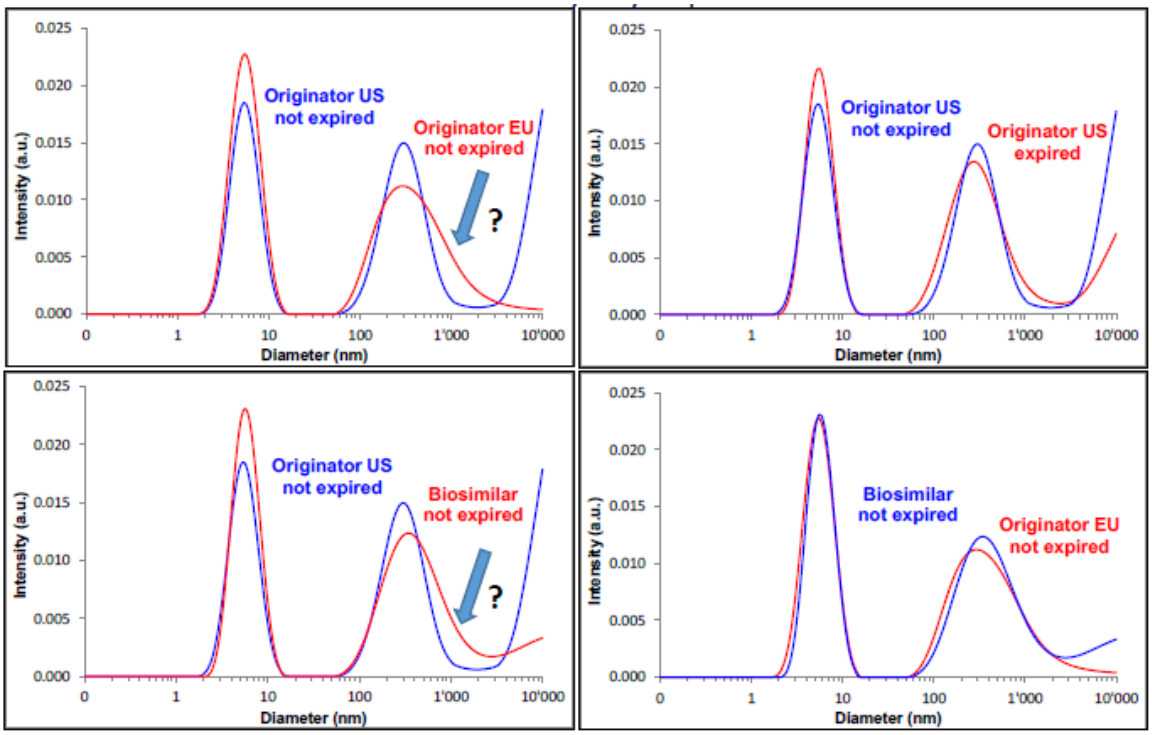

Figure 4. Particle size distribution measurement results of originator and biosimilar injectable made by Darpin G., Poirier E. et Arvinte T. from Terapeomic INC in 2018 (unpublished data).

For the biosimilar injectable (fig. 4), we can notice the shift of the particle size distribution to the higher values and widening of the particle size distribution (higher polydispersity). The same shift has been detected for the injectable after their expiration date (EU, US, and biosimilar expired). These variations could be used for quality control of the injectable without opening the syringes, as well as for the comparison of different product quality. But, to the best of our knowledge, these preliminary results are one of the first demonstrations 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 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 quality control of 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 CO_2 autoclaves, microwave reactors, micro fluidic chips, etc.) or for instrumental coupling [6].

References:

[1] M. S. Gunay et al, Current Neuropharmacology, 2016, 14, 376-391.
[2] E.Y. Chi, "Excipients and their Effects on the Quality of Biologics", AAPS J. , 2015
[3] M. Hasija, L. Li, and N. Rahman et al., *Vaccine: Development and Therapy*
[4] R. Manning et al., BioPharm International 27 (12)
[5] https://www.cordouan-tech.com/products/vasco-kin/
[6] https://www.cordouan-tech.com/products/amerigo/
[7] A. Schwamberger et al, Nuclear Instruments and Methods in Physics Research B 343 (2015) 116–122

Further reading:

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