European Journal of Pharmaceutics and Biopharmaceutics 104 (2016) 30–41

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Nanoparticle tracking analysis of particle size and concentration detection in suspensions of polymer and protein samples: Influence of experimental and data evaluation parameters

Julia Gross [a], Sabrina Sayle [b], Anne R. Karow [b], Udo Bakowsky [a], Patrick Garidel [b,*]

[a] Philipps – University Marburg, Institute of Pharmaceutical Technology and Biopharmacy, D-35032 Marburg, Germany
[b] Boehringer Ingelheim Pharma GmbH & Co. KG, Biopharmaceuticals, Protein Science, D-88397 Biberach an der Riss, Germany

ARTICLE INFO

Article history:
Received 7 November 2015
Revised 16 April 2016
Accepted in revised form 18 April 2016
Available online 20 April 2016

Keywords:
Nanoparticle tracking
Protein
Aggregation
NTA
DLS
Scattering

ABSTRACT

Nanoparticle Tracking Analysis (NTA) is an emerging technique for detecting simultaneously sub-micron particle size distributions and particle concentrations of a sample. This study deals with the performance evaluation for the detection and characterisation of various particles by NTA. Our investigation focusses on the NTA measurement parameter set-ups, as will be shown in this study, are very crucial parameters to correctly analyse and interpret the data. In order to achieve this, we used (i) polystyrene standard particles as well as (ii) protein particles.

We show the highly precise and reproducible detection of particle size and concentration in monodisperse polystyrene particle systems, under specified and constant parameter settings. On the other hand, our results exemplify potential risks and errors while setting inadequate parameters with regards to the results and thus interpretation thereof. In particular changes of the parameters, camera level (CL) and detection threshold (DT), led to significant changes in the determined particle concentration. We propose defined and specified "optimal" camera levels for monodisperse particle suspension characterisations in the size range of 20–1000 nm. We illustrate that the results of polydisperse polystyrene standard particle solution measurements, highly depend on the used parameter settings, which are rarely published with the data. Changes in these settings led to the "appearance" or "disappearance" of particle populations ("peaks") for polydisperse systems. Thus, a correct evaluation of the particle size populations in the sample becomes very challenging.

For the use of NTA in biopharmaceutical analysis, proteinaceous samples were investigated. We analysed protein particle suspensions and compared unstressed and stressed (formation of aggregates) protein samples similar to polystyrene particle analysis. We also studied these samples in two different measuring modes (general capture mode and live monitoring mode) that the commercially available analysis software is offering. Our results stated the live monitoring mode as more suitable for protein samples, as the results were more reproducible and less operator-depending.

In conclusion, NTA is a potential technique and unique in quantitative evaluation of particle suspensions in the subvisible size range, especially for monodisperse suspensions. We strongly urge on not underestimating the influence of the measuring parameters on the obtained results, which should be presented with the data in order to better judge and interpret the NTA results.

1. Introduction

The detection, analysis and characterization of subvisible particles qualitatively and quantitatively remain a main challenge for biopharmaceutical developers [1–3]. The size range of particles covers the sizes from a few nanometres to larger than 100 μm in diameter. There are still analytical gaps existing, such as the lack of techniques able to adequately analyse particle sizes around

Abbreviations: NTA, nanoparticle tracking analysis; CL, camera level; CT, capture time; DT, detection threshold; DLS, dynamic light scattering; PS, polystyrene; SD, standard derivation.

* Corresponding author at: Biopharmaceuticals, Process Science, Protein Science, Boehringer Ingelheim Pharma GmbH & Co. KG, D-88397 Biberach an der Riss, Germany.

E-mail address: patrick.garidel@boehringer-ingelheim.com (P. Garidel).

http://dx.doi.org/10.1016/j.ejpb.2016.04.013
0939-6411/

1 μm and/or covers the whole size range. Consequently the search and development for new and orthogonal techniques to cover these gaps are of high interest [4,5,8].

For pharmaceutical purposes, particles as well as protein aggregates need to be detected, characterised and counted to fulfil authority regulations and to dispel safety concerns [5–8,14]. As one of these concerns the potential immunogenicity risk of protein particles is often mentioned [9,10]. The fact that already small amounts of protein particles may have an intensive immunogenic potential, underlines the need for highly sensitive detection and characterisation methods [10–12]. For example, Freitag et al. [13] highlighted the need for better characterisation of protein aggregates as key to evaluate the immunological effect of aggregates.

Another important point is that authorities, e.g. the USP Expert Committee cycle (2010–2015), consistently update regulations especially on topics related to protein particles in parenterals. An example is the addition of new chapters and revision of existing general chapters that provide guidance on particulate matter content of injectable drug products by the USP [6,7]. In particular the informational chapter <USP1787> describes new (e.g. Flow Microscopy Imaging) and established methods (e.g. Light obscuration). At the same time the FDA points out the need for new and orthogonal methods to control the presence of protein aggregates through the whole process [8]. Besides others, NTA is mentioned as a new and high potential analytical method for particles in therapeutic protein formulations [1,4,8].

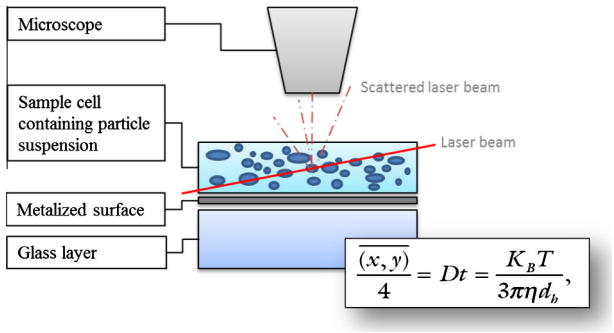

Fig. 1. Principle of NTA measurements and Stokes–Einstein equation (adapted from NanoSight). The sample in the sample cell is illuminated by a laser beam. The particle movement is recorded via light scattering by a CCD camera and the software tracks each particle and determines the diffusion coefficient of the Brownian motion. Based on Stokes–Einstein equation (see above) the particle size is then calculated as the mean-squared particle path in two dimensions (x, y), k_B as the Boltzmann's constant, T as the absolute temperature, t as the measurement time, η as the viscosity and d_h as the hydrodynamic diameter [25,33].

The technique of Nanoparticle Tracking Analysis (NTA) was first mentioned in 2006 as commercial set-up for size and concentration measurements of colloidal suspensions [15–17]. Until today, NTA was used for various applications in different research and industry fields as well as for various sample compositions, e.g. cellular vesicles, virus particle, microvesicle and exosome, gold nanoparticle conjugation, fullerenes or proteins [18–25].

The technique offers the simultaneous, multiparameter analysis of nanoparticles in liquid suspensions, concerning size distribution, concentration and direct and real-time visualisation [26]. The measurement principle and the instrument configuration have been reviewed extensively (Fig. 1) [26–28]. In short, the sample (liquid) is pumped into a sample cell that is illuminated by a laser. The particle movement is subsequently recorded by tracking the light scattering centres of the particles by a charge-coupled device (CCD) camera. Hereby the Brownian motion of particles is analysed in real-time by a CCD camera and each particle is simultaneously but individually visualised and tracked by a specific image tracking, analysis and processing software. The diffusion coefficient of each tracked particle is calculated and based on the Stokes–Einstein equation (Fig. 1, equation) and the particle size (hydrodynamic diameter) for each particle can be calculated, assuming a sphere. Because each particle (scattering centre) is tracked separately, the resulting estimate of particle size distribution is not suffering from the limitation of being an intensity weighted, z-average distribution, which is normal in conventional ensemble methods of particle sizing [11,12,17]. The particle concentration is derived by counting these tracks. The results can be presented as a histogram showing the size distribution and the corresponding particle concentrations [26–28].

The commercial NTA system developed by Nanosight Ltd. offers various options as well as challenges for the operator, such as different capture modes or variable measurement and evaluation parameters that need to be set for each measurement. The key parameters are summarised in Table 1 [17,27,28] and the operator needs to consider each parameter for each sample. Video recording of the tracks is the first step of the measurement process. The parameters capturing time (CT) and camera level (CL) – combination of camera shutter (=time while camera shutter is open) and camera gain (=sensitivity of the camera) – define recording time and quality of the video and have to be optimised for each single measurement by the operator. In the common capture mode, the CT value can be set based on a matrix (suggested by the software) considering the expected particle concentration with a maximum CT of 215 s. As in most cases for real-life samples the concentration is unknown, and the maximal CT should be chosen. The second step, the data analysis and evaluation process, involves another set of parameters. For some of the parameters mathematical

Table 1
Overview measurement and evaluation parameters of the NTA System (Nanosight Ltd).

Measurement step	Parameter	Description
Video recording	**Camera level CL** (Camera gain and shutter)	Defines the length of time the camera shutter is open and the sensitivity of the camera
	Capture time/duration	Total number of frames of the video recording
Tracking analysis and evaluation	Screen gain (Gain and Brightness)	Combination of the intensity value for each pixel and the brightness of the video images (no influence on the evaluation)
	Detection threshold DT	Minimum intensity required for an area of light to be assigned to a particle
	Blur	Intensity profile of particles
	Minimum track length	Minimum number of frames a particle must be tracked to be considered as completed
	Minimum expected particle size	Imposed minimum particle size by operator, related to maximum distance between two successive positions of a particle. Software applies an exclusion zone around particles Large minimum size implies a large exclusion zone around the particle as the larger particles move slower than small particles