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The application of STEP-technology® for particle and protein dispersion detection studies in biopharmaceutical research

J. Gross-Rother[a], N. Herrmann[a], M. Blech[a], S.R. Pinnapireddy[b], P. Garidel[a], U. Bakowsky[b,*]

[a] Boehringer Ingelheim Pharma GmbH & Co. KG, BioPharmaceuticals, D-88397 Biberach an der Riss, Germany
[b] Department of Pharmaceutics and Biopharmaceutics, University of Marburg, Robert Koch Strasse 4, 35037 Marburg, Germany

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ABSTRACT

Particle detection and analysis techniques are essential in biopharmaceutical industries to evaluate the quality of various parenteral formulations regarding product safety, product quality and to meet the regulations set by the authority agencies. Several particle analysis systems are available on the market, but for the operator, it is quite challenging to identify the suitable method to analyze the sample. At the same time these techniques are the basis to gain a better understanding in biophysical processes, e.g. protein interaction and aggregation processes. The STEP-Technology® (Space and Time resolved Extinction Profiles), as used in the analytical photocentrifuge LUMiSizer®, has been shown to be an effective and promising technique to investigate particle suspensions and emulsions in various fields.

In this study, we evaluated the potentials and limitations of this technique for biopharmaceutical model samples. For a first experimental approach, we measured silica and polystyrene (PS) particle standard suspensions with given particle density and refractive index (RI). The concluding evaluation was performed using a variety of relevant data sets to demonstrate the significant influences of the particle density for the final particle size distribution (PSD). The most challenging property required for successful detection, turbidity, was stated and limits have been set based on the depicted absorbance value at 320 nm (A320 values). Furthermore, we produced chemically cross-linked protein particle suspensions to model physically "stable" protein aggregates. These results of LUMiSizer® analysis have been compared to the orthogonal methods of nanoparticle tracking analysis (NTA), dynamic light scattering (DLS) and micro-flow imaging (MFI). Sedimentation velocity distributions showed similar tendencies, but the PSDs and absolute size values could not be obtained.

In conclusion, we could demonstrate some applications as well as limitations of this technique for biopharmaceutical samples. In comparison to orthogonal methods this technique is a great complementary approach if particle data e.g. density or refractive index can be determined.

1. Introduction

The interest in biopharmaceutical research and the market of biopharmaceutical products, e.g. therapeutic proteins or nucleic acid-based products has been growing over the last four decades (Walsh, 2014, 2000). In particular, monoclonal antibodies (mABs) appear as highly potential therapeutics for the treatment of autoimmune diseases or cancer (Walsh, 2014). However, one of the major challenges and potential risks of such biomolecules are their colloidal and conformational instabilities (Wang et al., 1999; Chi, 2003).

Proteins in solution can be described as a suspension with colloidal properties (Nicoud, 2015). Proteins naturally occur in a specific structure, the so called native state, allowing optimal biological activity (Anfinsen, 1972). This three-dimensional structure and conformational stability is highly sensitive to environmental influences and changes thereof, might lead to instabilities, e.g. chemical, physical, conformational or colloidal instability (Wang et al., 2007; Dill, 1990; Wang et al., 2014). If a protein monomer is changed in such a manner, this represents a potential nucleation point for aggregation processes. As a result, aggregates are formed with lower effectivity and/or immunogenic potential.

As a further consequence, such protein particles have to be detected due to their size and size distribution for quality and safety reasons in pharmaceutical industry (Rosenberg, 2006; Roberts, 2014). Since protein particles appear highly heterogenic, investigation should be performed by orthogonal techniques based on various particle properties. The most used properties are optical properties like light-scattering techniques. Another typical principle is the determination of

* Corresponding author.
E-mail address: ubakowsky@aol.com (U. Bakowsky).

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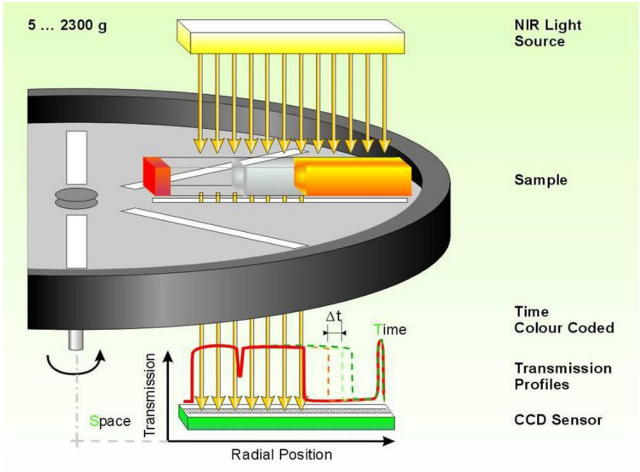

Fig. 1. Schematic configuration and measurement principle of the analytical photocentrifuge system LUMiSizer®.

sedimentation velocities as it characteristically depends on particle size. Centrifugation, as a well-established principle, covers a wide size range compared to other orthogonal principles. The principle is exemplary used in analytical ultracentrifugation (AUC) or density gradient centrifugation (Schaefer et al., 2012). A novel approach is the analytical photocentrifuge LUMiSizer® using the STEP-Technology®. The STEP-Technology® was developed by L.U.M. GmbH (Berlin, Germany) and first commercialized in 1998. In 2004 an updated centrifuge instrument, the LUMiSizer®, was released for the analysis of dispersion stabilities and characterization of nanoparticles. This device combines the fundamentals of sedimentation with a specialized optical detection system for transmission/absorbance measurements. To the best of our knowledge the technique was not used for formulation development of proteins in a nanoparticle scale, e.g. antibodies, until now. For that reason, the following case study aims at investigating the STEP-Technology for protein formulation applications.

The measurement principle of the STEP-Technology® used in the LUMiSizer® is shown in Fig. 1. The particle suspension is presented in a rectangular cuvette “window” along with the whole sample. For measurement, this cuvette is placed horizontally into the centrifuge. The sample is positioned between a light source and a CCD sensor (CCD: charged-coupled device) for the detection of transmitted light. This configuration enables the acquisition of the space and time-resolved extinction/transmission profiles (STEP) at any position and any time during the course of the experiment and thus, provides more information than usual AUCs. In the beginning the particles are homogenously distributed in the whole sample. The intensity of the transmitted light ***I*** is detected by the CCD sensor over the whole sample and the first extinction profile is captured. Sample centrifugation forced, the particles to move from the meniscus to the cuvette bottom depending on their mass, size and shape properties. This process is observable in real-time due to transmission measurements. The light intensity of the transmitted light ***I*** detected at the sensor increases over time from the meniscus as the particles move down and therefore allow a higher part of the incident light I_0 to pass the sample d. The detection method is described by the Beer-Lambert law (Eq. (1)):

$$E = -\lg\frac{I}{I_0} = \varepsilon c d \tag{1}$$

E	extinction
I	transmitted light
I_0	incident light
ε	extinction coefficient
c	particle concentration
d	path length

Thus, a set of extinction profiles, or transmission profile fingerprint, is obtained. The software provided by the manufacturer gives the user various options to evaluate the obtained data. Most commonly the sedimentation velocity is determined based on the captured extinction profiles over time. For further calculations, e.g. PSDs, the Stokes law (Eq. (2)) is applied. For this purpose, the provided software employs a simple correlation (Eq. (3)):

$$u = \frac{d^2(\rho_P - \rho_l)g}{18\mu} \tag{2}$$

$$u = \frac{r_m ln\frac{r_m}{r_0}}{t_m} \tag{3}$$

u	sedimentation velocity
d	particle diameter
ρ_P	particle density
ρ_l	liquid density
g	gravitational acceleration
μ	liquid viscosity
r_m	measurement position
t_m	measurement time
r_0	meniscus position

More detailed information and sources can be found at the homepage of L.U.M. (Berlin 2016).

The determination of further particle properties such as density is another approach which supports measurements and enables the application in a wider field. However, centrifugation as an ensemble method is not a particle-by-particle technique and quantitative evaluations are hard to achieve. In addition, required material data (refractive index and density) are hard to determine for biopharmaceutical samples. Another remaining challenge is the measurement of high concentration samples, because a high particle load with sedimenting particles may counteract the directed centrifugation direction. In addition, a high particle load and high protein concentration is prone for multiple scattering (Walter et al., 2015). These effects can be avoided using improved hindering functions for evaluation and improved analysis approaches as shown by Walter et al. (2015, 2014).

The aim of the present study was the evaluation of the STEP-Technology® used in an analytical centrifuge for applications in biopharmaceutical research. Hence, the measurement and evaluation challenges as well as requirements must be identified first. Furthermore, the applicability of the system for standard particle suspensions (silica and PS) as well as standardized protein samples (mAB1 and mAB2) must be confirmed and the comparison to orthogonal methods (nanoparticle tracking analysis, dynamic light scattering and micro-flow imaging) is necessary.

2. Material and methods

2.1. Materials

Nanosphere™ Size Standards (Polystyrene) of 100 nm size (Cat No 3100A) were purchased from Thermo Scientific (Fremont, USA). As per the manufacturer’s information, the depicted size was determined and verified with 102 ± 3 nm by DLS and Transmission electron microscopy (TEM). The aqueous suspension with a particle concentration of 1% w/w contains traces of detergents. Particle density was stated as 1.05 g/cm^3 and the determined refractive index was 1.59 at 589 nm and 25 °C. The monomodal silica standard particle suspension (SediTest, Prod.-No. 272-15504) was provided by Dr. Lerche KG (Berlin, Germany). According to the product information sheet, the particles