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Note

Laser-induced breakdown detection of temperature-ramp generated aggregates of therapeutic monoclonal antibody

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ABSTRACT

The detection and characterization of protein aggregation is essential during development and quality control of therapeutic proteins, as aggregates are typically inactive and may trigger anti-drug-antibody formation in patients. Especially large multi-domain molecules, such as the important class of therapeutic monoclonal antibodies (mAbs), can form various aggregates that differ in size and morphology. Although particle analysis advanced over the recent years, new techniques and orthogonal methods are highly valued. To our knowledge, the physical principle of laser-induced breakdown detection (LIBD) was not yet applied to sense aggregates in therapeutic protein formulations. We established a LIBD setup to monitor the temperature-induced aggregation of a mAb. The obtained temperature of aggregation was in good agreement with the results from previously published temperature-ramped turbidity and dynamic light scattering measurements. This study demonstrates the promising applicability of LIBD to investigate aggregates from therapeutic proteins. The technique is also adaptive to online detection and size determination, and offers interesting opportunities for morphologic characterization of protein particles and impurities, which will be part of future studies.

1. Introduction

Protein aggregation is a major hurdle for therapeutic proteins as the aggregated species typically lack activity and are potentially more immunogenic than protein monomers in patients [1]. The complex pathways of protein aggregation, especially in case of large multi-domain proteins such as monoclonal antibodies (mAbs), lead to aggregates of various size and morphology [2,3]. To cover the full size range from several nanometers to micrometers, an arsenal of analytical tools is needed and employed to investigate aggregates of protein drugs during development and in quality control [3–5]. Not only the size, but also morphology and structure of protein aggregates as well as non-proteinaceous impurities are in the focus of the pharmaceutical scientist and drive the development of new analytical techniques [6,7]. Progress was achieved, especially in the challenging range of micron (1–100 μm) and submicron (100 nm–1 μm) particles, e.g. by flow imaging techniques and resonant mass measurement [8]. As the obtained size information is always linked to the measurement principle and technique, orthogonal methods are highly valued and also appreciated by the regulatory agencies [9].

Laser-induced breakdown detection (LIBD) of particles in solution relies on the effect that the energy threshold to induce a plasma breakdown in the focus of an intense pulsed laser is dependent on the dielectric properties (atom number density and refractive index) and is lower for solids than liquids (and gases) [10]. The detection and counting of breakdown events induced by micron- and submicron particles by means of acoustic and optical detectors has been exploited mainly in the area of environmental analysis. A minimum detectable diameter of about 19 nm was demonstrated with polystyrene standard particles, while theoretical calculations even suggest a minimum size range of about 1 nm [11]. The density of pure mAb, i.e., the inverse of the partial specific volume is typically reported about 1.35–1.37 g/ml [12], but the apparent density of protein aggregates in solution can be substantially lowered because of the entrapped water and irregular shape (e.g., 1.07 g/ml of heat-stressed rituximab [13]). Furthermore, the lower refractive index of proteins of about 1.41 in comparison with polystyrene particles of 1.59 [14], will most likely result in a reduced sensitivity of LIBD toward protein particles. The probability to induce a breakdown by the laser pulse is increasing with both higher particle concentration and larger particle size [15], thus complicating size distribution analysis of real-world samples where both parameters are unknown.

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The application of LIBD for the analysis of protein aggregates is highly intriguing. We therefore built a LIBD setup and tried to study the formation of mAb aggregates upon thermal unfolding. Using a temperature-ramped setup to induce aggregation, the breakdown incidence, i.e. the ratio of observed breakdowns to the number of laser pulses within 30 s, was monitored. A significant increase in breakdown incidences was observed after protein aggregation demonstrating that LIBD in principle can be used for characterization of protein formulations, and the high potential of the method has to be further exploited in future studies.

2. Materials and methods

2.1. Sample preparation

Stock solutions of an IgG1 monoclonal antibody (mAb) in 10 mM phosphate buffer at pH 7.2 or 5.0 with or without 140 mM sodium chloride (NaCl) and 280 mM mannitol were used [12]. Samples of 0.2 mg/ml mAb were prepared by dilution with the corresponding buffer and filtered through a 0.2 μm PVDF syringe membrane filter (Pall Life Sciences, Port Washington, New York, USA) immediately before the experiments.

2.2. Temperature-ramped LIBD setup

A schematic illustration of the temperature-ramped LIBD setup is illustrated in Fig. 1. A pulsed (10 Hz) Surelite Nd:YAG laser (Continuum, San Jose, California, USA) was frequency-doubled to 532 nm. A lens with a focal length (f) of 20 mm was used to focus the beam into a quartz glass cuvette (Hellma Analytics, Müllheim, Germany). The center of the focus was positioned 1 mm from the cuvette inner wall. The laser energy was recorded using a beam splitter and laser energy sensor (Ophir Optronics, Jerusalem, Israel). The cuvette was placed in a cell holder which was connected to a Thermo Haake C25P temperature controlled water bath (Thermo Electron, Karlsruhe, Germany). Using a lens of f = 40 mm, the emitted light from a LIBD event passed a long-pass filter and was detected by a photodiode at an angle of 60° to the incident laser beam. The electrical signal of the photodiode was amplified and collected using a Tektronix TDS 620A oscilloscope (Tektronix, Wilsonville, Oregon, USA). The oscilloscope also received the electronic trigger signal from the laser source for timing. The LIBD signal was observed after about 300 ns and the electronic signal was transferred via a GPIB-to-USB converter (National Instruments, Austin, Texas, USA) to a personal computer (PC). The data from the photodiode and the laser energy sensor were recorded and processed using LabVIEW 2010 (National Instruments Corporation, Austin, Texas, USA). The software logged the laser energy and signal amplitude of the photodiode for every individual pulse.

With a laser pulse energy of 39 ± 4 μJ, the breakdown incidence of highly purified water (MilliQ Plus 185, Merck Millipore, Billerica, Massachusetts, USA) and the filtrated formulation buffers were 0.1 and 0.7 breakdowns/30 s, respectively, over a period of 5000 laser pulses (ca. 8 min). The cuvette was filled with 2 ml of the sample and sealed by a rubber stopper equipped with a thermocouple. A linear temperature ramp from 20 °C to 95 °C in 75 min (1 °C/min) was adjusted by using the build-in function at the control panel of the water bath. The temperature in the solution was recorded using a HH147U thermo logger (Omega Engineering, Stamford, Connecticut, USA) connected to the PC.

The signal amplitudes were analyzed in order to identify LIBD events using a threshold value above the background signal amplitude with Origin 8 SR6 (Originlab, Northampton, Massachusetts, USA). The breakdown incidence was calculated in time bins of 30 s. Using the recorded temperature data over time, the temperature where the breakdown incidence suddenly increased ($T_{agg,LIBD}$), was determined at the intersection of two tangents fitted to the cumulated breakdown incidence at the baseline and the slope.

2.3. Differential scanning calorimetry (DSC)

DSC thermograms from 20 to 96 °C at 1 °C/min were obtained from the mAb samples at a concentration of 0.8 mg/ml using a VP-DSC MicroCalorimeter (MicroCal, Northampton, Massachusetts; now Malvern Instruments) as described previously [16]. The apparent melting temperatures (T_m values) of the CH2 and the CH3 domains as well as the Fab part were obtained from the peak maxima of the melting transitions.

3. Results and discussion

The breakdown incidence represents the number of LIBD events per time bin of 30 s. At the starting temperature of 20 °C, a breakdown incidence of 0–5 breakdowns/30 s was observed for all 0.2 mg/ml mAb samples in 10 mM phosphate buffer at pH 7.2 (Fig. 2). Subsequently, the temperature of the sample was linearly increased with a heating rate of 1 °C/min. The breakdown incidence was constant at the initial level until a sudden onset of breakdown events occurred. This onset was observed for all three mAb formulations at the neutral pH but the $T_{agg,LIBD}$ was slightly lower for the sample without NaCl or with mannitol (Table 1).

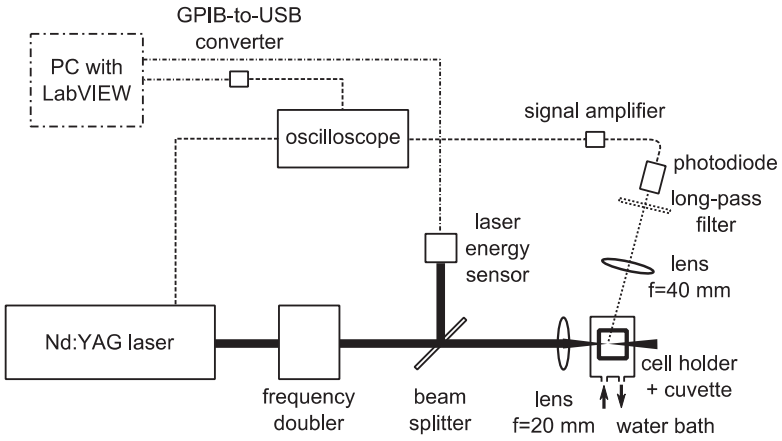

Fig. 1. Schematic illustration of the temperature-ramped LIBD setup including data acquisition using a LabVIEW virtual instrument (VI).