Centrifugal Field-Flow Fractionation Hyphenated with Dynamic Light Scattering for Size Characterization of Nanoparticle Systems for Biopharmaceutical Applications

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Application	Medicine, Drug Delivery
Technology	CF3-DLS
Info	Postnova CF2000, Malvern Zetasizer Nano ZSP
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Introduction

Biodegradable nanoparticles (NPs) as drug delivery systems (DDS) for biomedical applications have become extremely attractive due to their stability in the blood stream and controlled release characteristics. In this study, polymer-based, non-cytotoxic NPs, in particular 200 nm PLGA NPs, were characterized according to their size and integrity of the particle formulation. PLGA was chosen due to its biodegradability, biocompatibility, controlled release characteristics and approval for therapeutic devices by the US Food and Drug Administration (FDA). Special focus was laid on the size and stability of these potent DDS over time in cell culture medium. Therefore, Centrifugal Field-Flow Fractionation (CF3) hyphenated with dynamic light scattering detection (DLS) was performed using such complex medium as eluent. With this setup, physiological conditions were closely mimicked and influences of the surrounding media as well as the sample preparation could be significantly minimized thereby offering a realistic insight into the behavior of such DDS under potential real-life application conditions [1].

Figure 1: Instrumental Setup of the CF2000 from Postnova Analytics (top left), the Malvern Zetasizer Nano ZSP (bottom) and the Principle of Centrifugal FFF (top right).

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CF3-DLS Measurements in Cell Culture Medium

Prior to the start of the investigations, the CF3-DLS setup was slightly modified in order to prevent microbiological contamination of the system during the measurements in cell medium (RPMI 1640). In particular, after thorough cleaning of the system, antibiotics were added to the cell medium and the eluent was cooled down to 5 - 7 °C. By these means, system integrity could be ensured for at least 44 h. Afterwards, by adding a defined amount of PLGA NPs to the respective cell medium solutions, incubation studies were performed at 37 °C mimicking physiological conditions. In order to investigate the influence of cell medium components on the PLGA NPs size and integrity over incubation time, thus treated samples were subsequently characterized via CF3-DLS. Analysis at distinct time intervals (directly after start of incubation, at 1 h, 4 h and 20 h) thereby allowed monitoring of PLGA NP size alterations in cell medium over incubation time. A representative overlay of the CF3-DLS fractograms obtained for the respective incubation time intervals in pure RPMI 1640 (blue) and in RPMI 1640 containing 10% fetal calf serum (FCS) as additive (green) is displayed in Figure 2 and Figure 3.

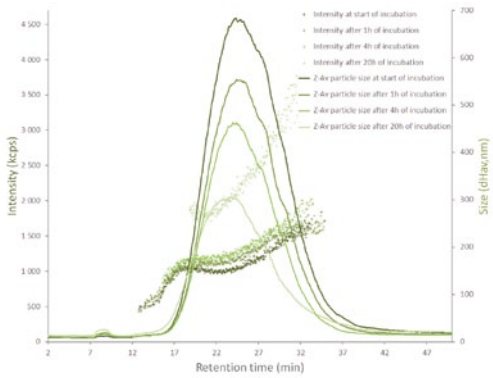

Figure 2: CF3-DLS-fractograms of 200 nm PLGA NPs after different incubation times (0 h, 1 h, 4 h and 20 h) in RPMI 1640 (Hydrodynamic radii highlighted as dots in lighter colors to the corresponding intensity trace).

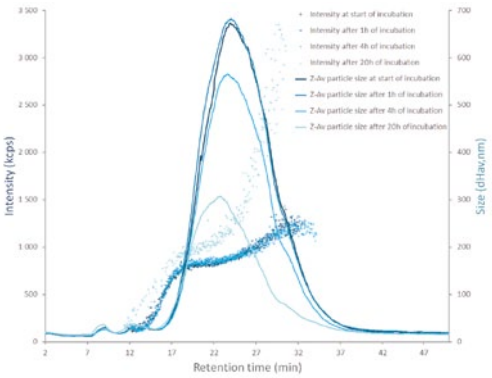

Figure 3: CF3-DLS-fractograms of 200 nm PLGA NPs after different incubation times (0 h, 1 h, 4 h and 20 h) in RPMI 1640 containing 10% FCS (Hydrodynamic radii highlighted as dots in lighter colors to the corresponding intensity trace).

Results and Discussion

The obtained results clearly indicate that while staying practically constant over the first hour of incubation, the size of the PLGA NPs significantly increased with prolonged incubation time. Moreover, the increase in NP size was observed in both RPMI 1640 with and without 10 % FCS, however the absolute size increase in latter medium was notably higher (Table 1). This finding might be related to additional agglomeration of FCS components onto the PLGA NP surface. Likewise, the simultaneous decrease in the overall particle numbers observed by the decreasing DLS signal intensity determined after prolonged incubation is a strong evidence for the increasing agglomeration and subsequent sedimentation of PLGA NPs in both respective cell media.

Diameter [nm]		
Incubation Time [h]	Pure Cell Medium	Cell Medium incl. 10 % FCS
0	181 ± 24	189 ± 49
1	180 ± 24	190 ± 45
4	186 ± 26	190 ± 39
20	207 ± 33	325 ± 256

Table 1: Particle size alterations after incubation in RPMI 1640 with/without 10 % FCS for 200 nm PLGA NPs obtained from CF3-DLS analysis.

Conclusion

The hyphenation of Centrifugal FFF with Dynamic Light Scattering has proven to be an ideal tool for the investigation of particle sizes even in complex matrices such as e.g., cell medium. CF3-DLS thereby for the first time not only enabled the detection, but also the evaluation of the particle integrity as well as the monitoring of particle size alterations of PLGA NPs directly in cell medium over time. Thus, CF3-DLS gives valuable information on the potential of biocompatible PLGA NPs towards their application in drug delivery systems (DDS). Therefore, this technology can provide detailed insights into NP behavior under physiological conditions, where other particle sizing technologies such as batch-DLS usually fail.

References

[1] Kohl Y., Spek S., Halley F., Spallek M. J. et al., Nanosafety Conference 2013, Biopolymer Nanoparticles for Therapeutic Applications: Synthesis, Characterization and Assessment of Biocompatibility.

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