

'SINGLE PARTICLE TRACKING' FOR SIZING CATIONIC LIPOSOMES IN BLOOD

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The therapeutic capabilities of macromolecular drugs are being actively investigated by the pharmaceutical community. Examples are nucleic acids like small interference RNA (siRNA) and genes. To avoid degradation of these macromolecular drugs in the blood circulation or extracellular matrix, they are usually packed into non-viral vectors, such as cationic polymers or liposomes, to form drug complexes of ~100 nm with the negatively charged nucleic acids based on electrostatic interactions. Knowledge of the size distribution of those drug complexes after intravenous injection is important as the size of the complexes determines to a large extent their capacity to leave the blood stream and to penetrate into tissues. While the size distribution of the complexes can be easily measured in a clear suspension by dynamic light scattering, it cannot be measured in more complex biological media such as plasma or whole blood, which contain all kinds of scattering components. To address this issue, we have developed a novel technique, based on single particle tracking (SPT) microscopy, for studying the size distribution (and aggregation) of nanoscopic drug complexes in biological fluids.

We have built a single particle tracking setup with which one can image the diffusional movement of dispersed individual fluorescent nanoscopic particles. The trajectories of individual particles are obtained using custom developed software. By calculating the diffusion coefficient for each trajectory, a distribution of diffusion coefficients can be obtained which can be transformed to a size distribution with the Stokes-Einstein equation. The raw measurement is finally refined by a Maximum Entropy deconvolution process.

Using the new single particle tracking sizing technique, we have, for the first time, been able to follow the aggregation behavior of drug carriers, in this case cationic liposomes (Dotap/Dope) in whole blood (at 37°C). As expected, the non-pegylated liposomes were found to aggregate quite strongly over time. Aggregation of the liposomes was largely inhibited by inclusion of high amounts of Dspe-PEG (5 and 10 mol%), but not by inclusion of small amounts of Dspe-PEG (< 5 mol%).

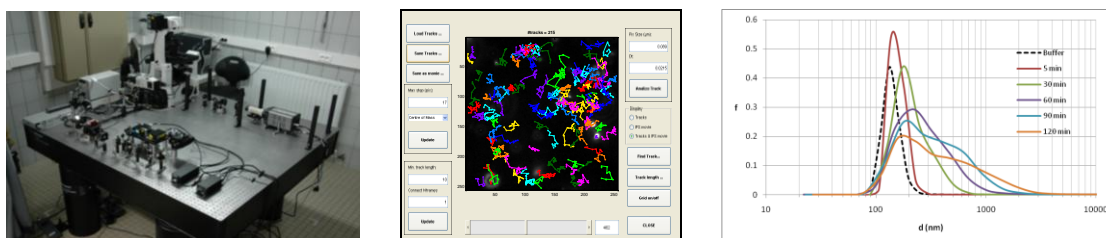


Figure 1: Single particle tracking setup, trajectories of diffusing nanoparticles and size distributions of liposomes that aggregate over time in whole blood.