

Liposome Size, Concentration and Structural Characterization by FFF-MALS-DLS

Liposomes are often used as nanocarriers to encapsulate in their cores hydrophobic or highly toxic drugs, and deliver these drugs safely to target tissue. During drug nanocarrier research and development as well as production quality control, it is of great importance to monitor liposome size distributions accurately while also verifying drug encapsulation. **FFF-MALS-DLS**, consisting of field-flow fractionation (FFF) combined with multi-angle light scattering (MALS) and dynamic light scattering (DLS), is a powerful tool for characterizing the size, concentration and structure of large nanoparticle ensembles.

Method

Encapsulation might cause changes in liposomal dimensions, but that is not always the case and other effects could cause such changes. Therefore, a more sophisticated analysis is warranted than mere size. Here, we report the analyses of two liposome samples, one empty and one filled with drug, by means of an **Eclipse®** FFF system followed by a **DAWN®** MALS detector with embedded **WyattQELS™** DLS module. The WyattQELS detector was placed at approximately 143° in order to extend the r_h measurement to well over 60 nm. The FFF separation method was optimized with the aid of Wyatt's proprietary **FFF simulation software**. **ASTRA** software was used to collect and analyze the light scattering data to determine size and concentration (number density).

Results and Discussion

FFF separates particles according to hydrodynamic radius. Thanks to upstream separation, quantitative size distributions by FFF-MALS-DLS provide far more

resolution and quantification than does batch (unfractionated) DLS. Online DLS directly measures the hydrodynamic radius r_h sequentially for each eluting size fraction, while MALS measures simultaneously the root-mean square radius r_g . The shape factor ρ , which is defined as the ratio $r_g : r_h$, provides important structural information: it can discriminate between empty and filled shells or quantify the axial ratio of a uniform ellipsoid.

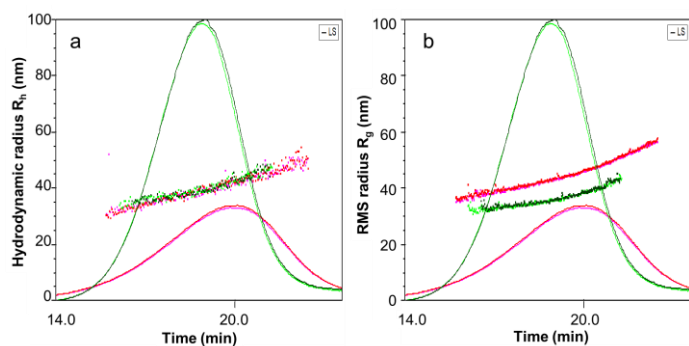


Figure 1. Hydrodynamic radius (a) and root-mean square radius (b) plotted against FFF elution time, overlaid with 90° LS signals for empty (red) and filled (green) liposomes. The r_h and r_g values are determined by the respective online DLS and MALS detectors. The results from duplicate runs of each sample are overlaid to demonstrate the excellent reproducibility of FFF-MALS-DLS analysis.

Both r_g and r_h are plotted against elution time in Figure 1. The results from duplicate runs demonstrate clean separation and excellent reproducibility of the FFF-MALS-DLS method. Figure 1a shows that the r_h values for both empty and filled liposomes are well-overlaid, suggesting that drug loading does not impact hydrodynamic size. However, as shown in Figure 1b, r_g values for these two liposomes do not overlay, which indicates different internal structures.

Figure 2a plots r_g against r_h ; ρ is the slope of the linear fit. The values of ρ for these two populations correlate precisely to empty and filled liposomal structures.

In addition to size and structure, FFF-MALS can determine quantitative size distributions (number density vs. size) of size-fractionated nanoparticles if the refractive index of the constituent material is known. For lipids this is quite straightforward and Figure 2b provides the quantitative nanoparticle concentration analysis.

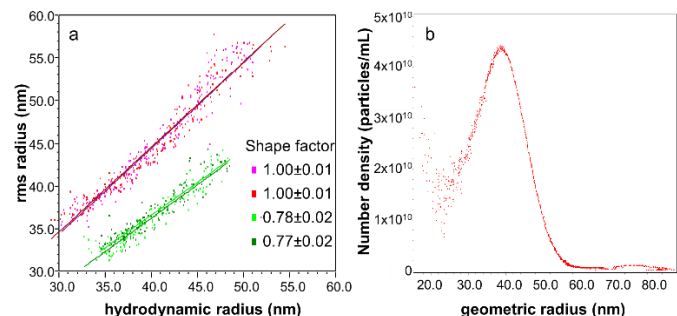


Figure 1. a) Root-mean square radius, r_g , plotted against hydrodynamic radius, r_h , for empty (red) and filled (green) liposomes. The slopes for empty and filled liposomes are 1.0 and 0.77, respectively, corresponding closely to the theoretical shape factor values for shells and uniform particles. b) number density distribution of liposome sizes determined by FFF-MALS.

Conclusion

For liposomes or other nanoparticles, FFF-MALS-QELS provides an easily adaptable yet powerful characterization tool to obtain information on particle size, size distribution, particle count, as well as structure—all without making assumptions about the particles or their composition. FFF-MALS-DLS instrumentation is essential for robust drug nanocarrier development and quality control.

