NovaSheet
Technology: AF4 - SLS/DLS
Application: Liposomes

Analysis Conditions / System Set-up

Sample:	Liposome Characterization
Solvent/Eluent:	20 mM Tris-HCl pH 7,4; 150 mM NaCl; 0,1 mM EDTA
Injection Volume:	50 µL
Sample Concentration:	5 mg/mL
Separation System:	Postnova AF4-10.000 Series Asymmetric Flow FFF
Detector 1:	Postnova PN3000DLS On-line Dynamic Light Scattering Detector
Detector 2:	Postnova PN3240 4-Channel UV/Vis Detector (Length 210 nm)

Liposomes, or lipid vesicles, are of major interest as model systems for the study of biological membranes and for an increasing number of different pharmaceutical applications, e.g. as drug delivery systems. Membrane imbedded substances like the trans-membrane located parts (TMS) of trans-membrane proteins influence the size of each liposome and potentially the liposome-liposome fusion.

The native size of liposomes, with and without TMS, depends on the production procedure. Different procedures are known in the literature and two were used for the following four liposome samples:

Sample 1: Liposomes sonicated, no TMS
Sample 2: Liposomes sonicated, with TMS
Sample 3: Liposomes extruded through membrane with 50 nm pore size, no TMS
Sample 4: Liposomes extruded through membrane with 50 nm pore size with TMS

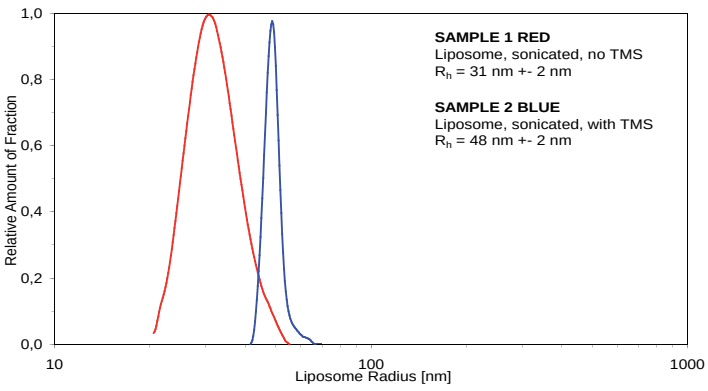

Figure 1: Particle Size Distribution obtained from FFF-DLS.

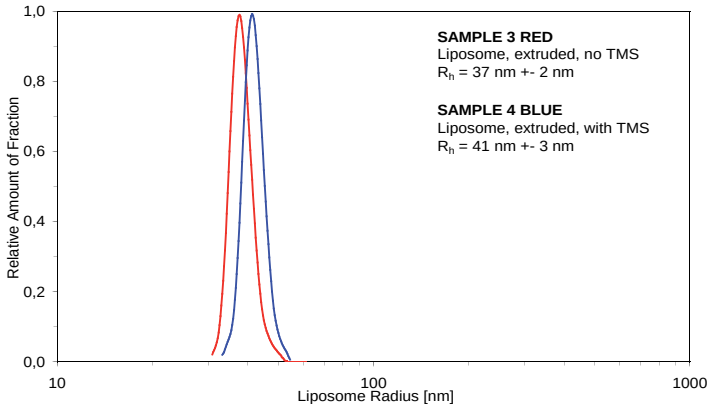

Figure 2: Particle Size Distribution obtained from FFF-DLS.

Liposomes generated by sonication with no TMS (sample 1) showed the smallest size with an Rh of 31 nm. However, this sample also showed the broadest distribution. As soon as TMS was added (sample 2), a higher state of order was reached -- the size distribution narrowed dramatically and the average size increased to 48 nm, the largest size of all samples. On the other hand, the liposomes produced by extrusion showed fewer differences. The extruded liposomes without TMS (sample 3) showed a bigger size (37 nm) than the corresponding sonicated sample. Interestingly, the extruded liposome containing TMS also showed a bigger size (41 nm) than the corresponding sample without TMS. However, the differences between the extruded samples were not significant compared to the sonicated counterparts. The extruded sample without TMS shows a quite narrow size distribution, other than the distribution of the sonicated liposome without TMS.

These Results clearly show that AF4 combined with on-line DLS is a powerful tool to determine very small differences in the average size and size distribution of liposomes. While DLS alone can provide average size measurements, the combination of the AF4 size-based separation with on-line DLS is highly sensitive to small differences. Moreover, the size distributions are determined directly with no assumptions as to the form of the distribution, Gaussian, etc. For example, the results of both sonicated samples show the possibility of aggregates due to the tail on these peaks. The results also show, that the structure of a liposome is dependent on the chemical nature of the liposome, as well as the generation technique.

Why use AF4-DLS for Liposome Characterization?

- High resolution separation of nanoparticles, even a few nanometers can be distinguished.
- Very fast, gentle and nearly interaction free sepa-ration without stationary phase.
- Separation of complex matrices without sample preparation or filtering.
- Easy direct coupling of AF4 with many powerful detection systems as on-line and off-line DLS, UV, RI, SLS, etc.