

Characterization of Liposomes and Virus Particles with the Eclipse F coupled to the DAWN EOS

Liposomes and viral particles are of growing interest in pharmaceutical and cosmetic applications. Liposomes are used for drug delivery formulations and as carrier systems for DNA. Viral particles are used for vaccination and immune stimulants. In all applications characterization of the colloidal suspensions is vital and has to cover several aspects. Not only size distribution, but also internal structure is of interest. This refers to shape (spherical vs. elongated), density and properties of the shell. Has a liposome been successfully loaded with a drug? A virus particle preparation loses biological activity during the

concentration and cleaning procedure, can this be correlated with a change in size distribution? Difficult analytical tasks like these cannot be solved with conventional sizing techniques applied on the unseparated sample.

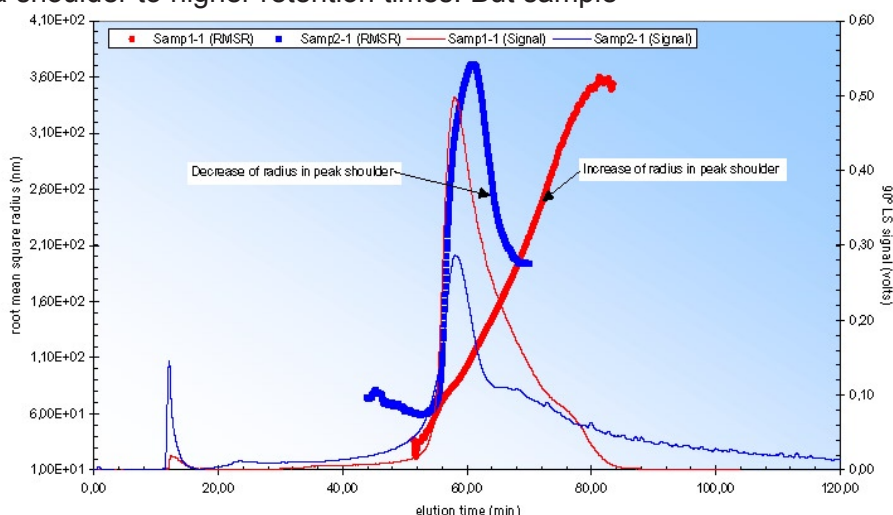
The Eclipse works on the principle of Flow Field-Flow Fractionation, which physically separates the particulates with very high resolution. Each fraction eluting from the channel is characterized according to radius and molar mass. The application note illustrates the wealth of information available from this sophisticated system.

Virus Particle

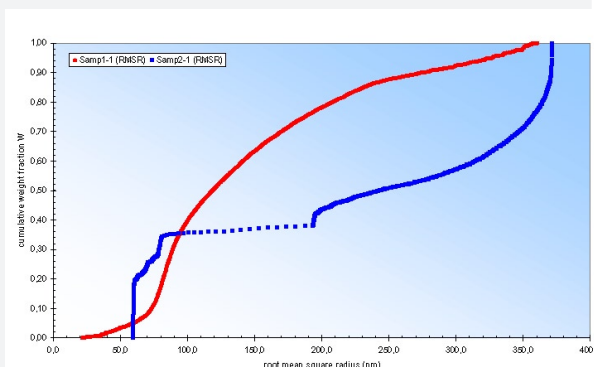
The example shows separation of two virus particle suspensions (red and blue). The 90° LS-signal is plotted along with radius calculated from light scattering. Both samples have a peak with a shoulder to higher retention times. But sample

means, RMS radius is a property of the complete structure, not the external diameter. A hollow sphere has a bigger RMS radius with respect to a filled sphere, because part of the particle mass is closer to the center of gravity.

The shoulder of sample 2 could consist of virus particles with higher internal density. The cumulative distribution plot below shows how different the size distribution of those samples is. The results can be used to differentiate virus particle batches, help to understand and monitor changes during the purification and formulation process.



1 (red) has increasing radius for the whole peak, whereas for sample 2 (blue) the radius decreases after the peak maximum. The DAWN EOS reveals a significant difference between the two samples. Flow-FFF separates according to hydrodynamic radius. How can radius calculated by light scattering decrease to higher elution times? This is possible if the internal structure of the particle changes. The RMS-radius (abbreviation for root mean square radius) is the averaged distance of the particle mass from the center of gravity. This



Liposomes

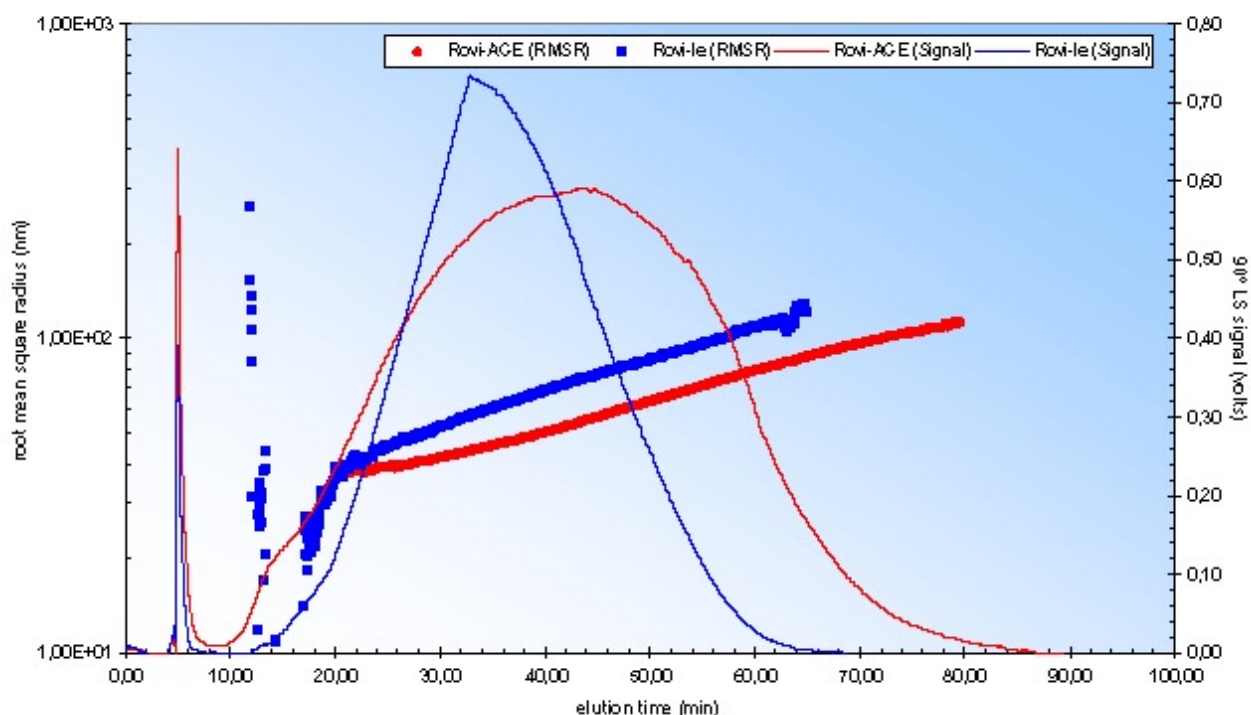
Liposomes are cell models synthesized from phospholipids, mostly lecithin. A double membrane separates the interior of the spherical shape and creates a closed cavity. Liposomes prepared from lecithin are non-toxic and can be used to transport drugs for pharmaceutical and cosmetic applications into the body. Size distribution is important because small liposomes cannot carry enough drug quantities to be effective and over sized liposomes do not penetrate the skin in cosmetic applications and can cause severe complications in IV injections. High resolution analysis of the size distribution is imperative both for development and for quality control.

Liposomes manufactured by ROVI GmbH in Germany have been analysed using the Eclipse F system.

Rovisome® Liposomes are manufactured from lecithin that is highly purified and consists to 80% of pure phosphatidylcholin. Carefully dried lipids are dispersed in water and the multilaminar vesicles are treated by proprietary procedures using ultrasonic or extrusion (French Press) to create unilaminar liposomes in the optimal size range of 30 nm to 150 nm diameter. The process can be used for empty liposomes or to generate loaded liposomes, e.g. with vitamins. Several different types of loaded liposomes have been characterized and differences in the size distribution as a function of the internalized drug have been detected.

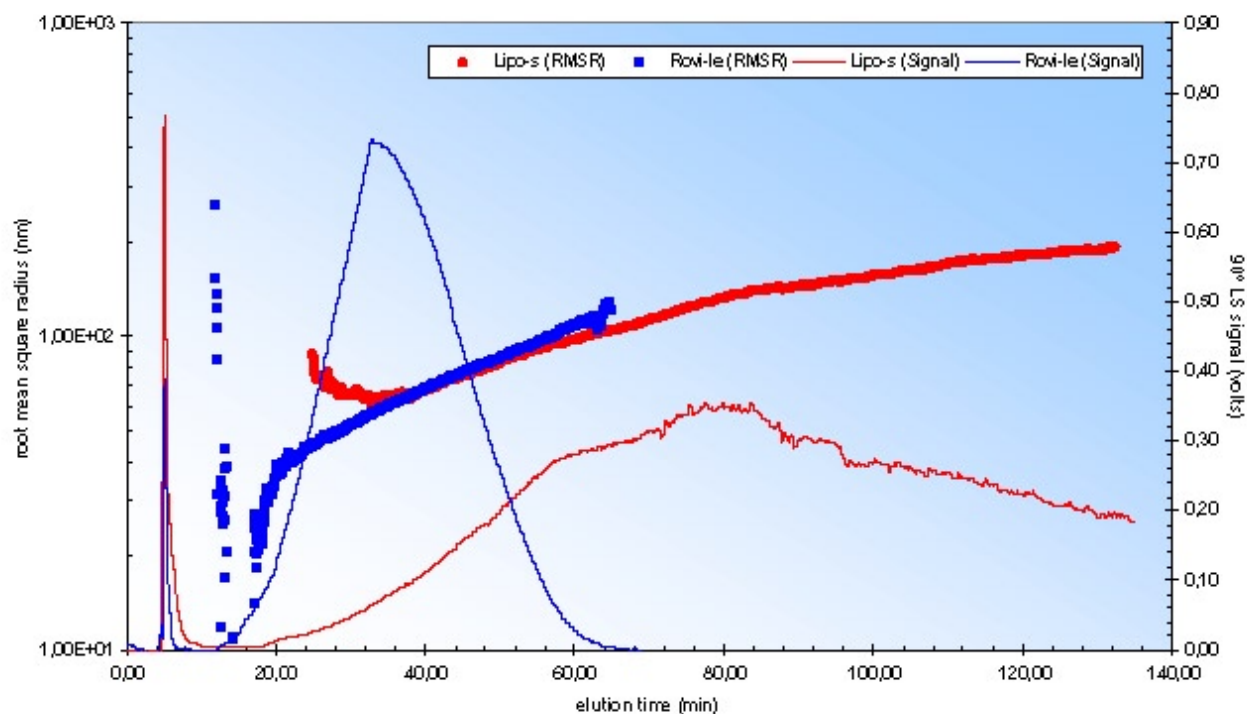
Size distribution of empty liposomes (blue) and liposomes filled with vitamins A,C and E (red) have been determined using the Eclipse F system coupled to a DAWN EOS. Sizes are between 30 and 100 nm which is the optimal range. Significant is the difference in elution behaviour of the empty and filled sample: filled liposomes elute in a broader peak and retention increases for part of the sample, but the RMS-radius is not increasing. At a given elution time, the radius for a filled liposome is smaller compared to the empty one.

The result is consistent with the expected increase of density with drug loading. Higher density inside the liposome will decrease RMS-radius with respect to hydrodynamic radius. Because Flow-FFF separates according to hydrodynamic radius, the RMS-radius should decrease at a given elution time. The result verifies that the drug has been encapsulated. Because the analysis is easy to do and fully automated, it can be used in QC applications.



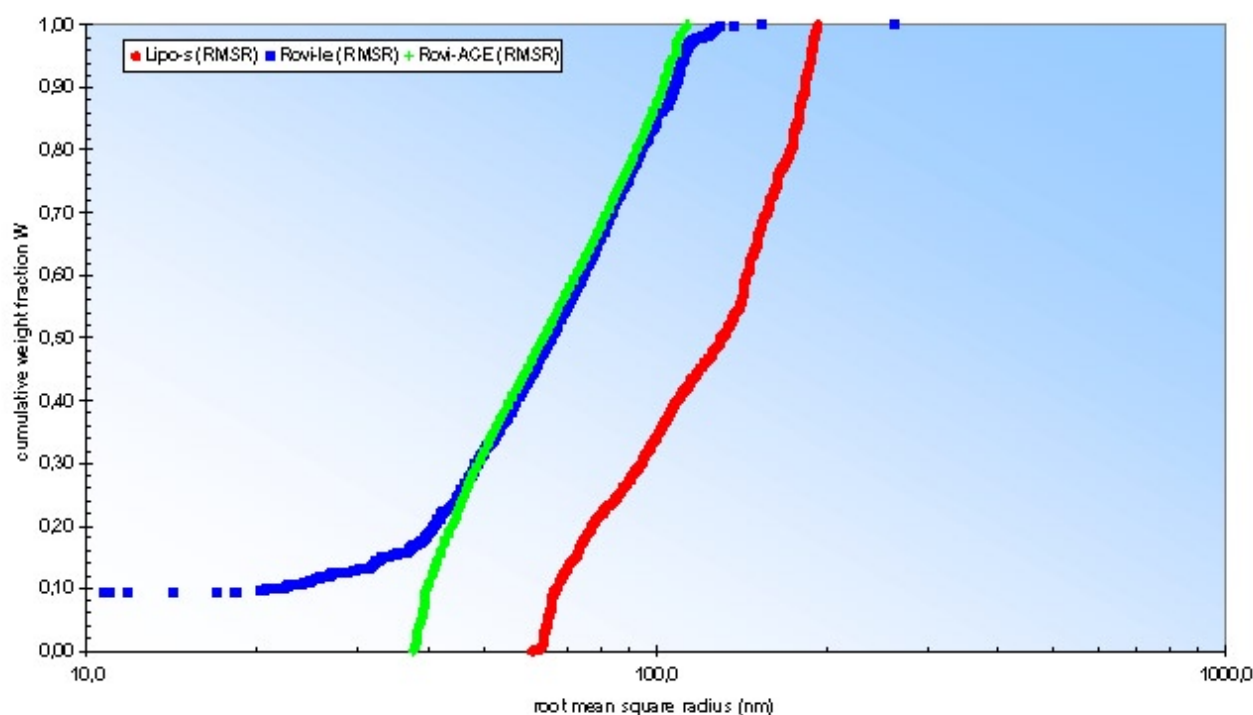
Quality of the lecithin is crucial to obtain liposomes with narrow size distributions. The plot compares two liposomes. Sample Rovi-LE (blue) has been prepared from high purified lecithin, sample Lipo-

s (red) from a low-grade material which contains only 25% phosphatidyl cholin. Size distribution of sample Lipo-s is much broader and extends far above the optimal size range.



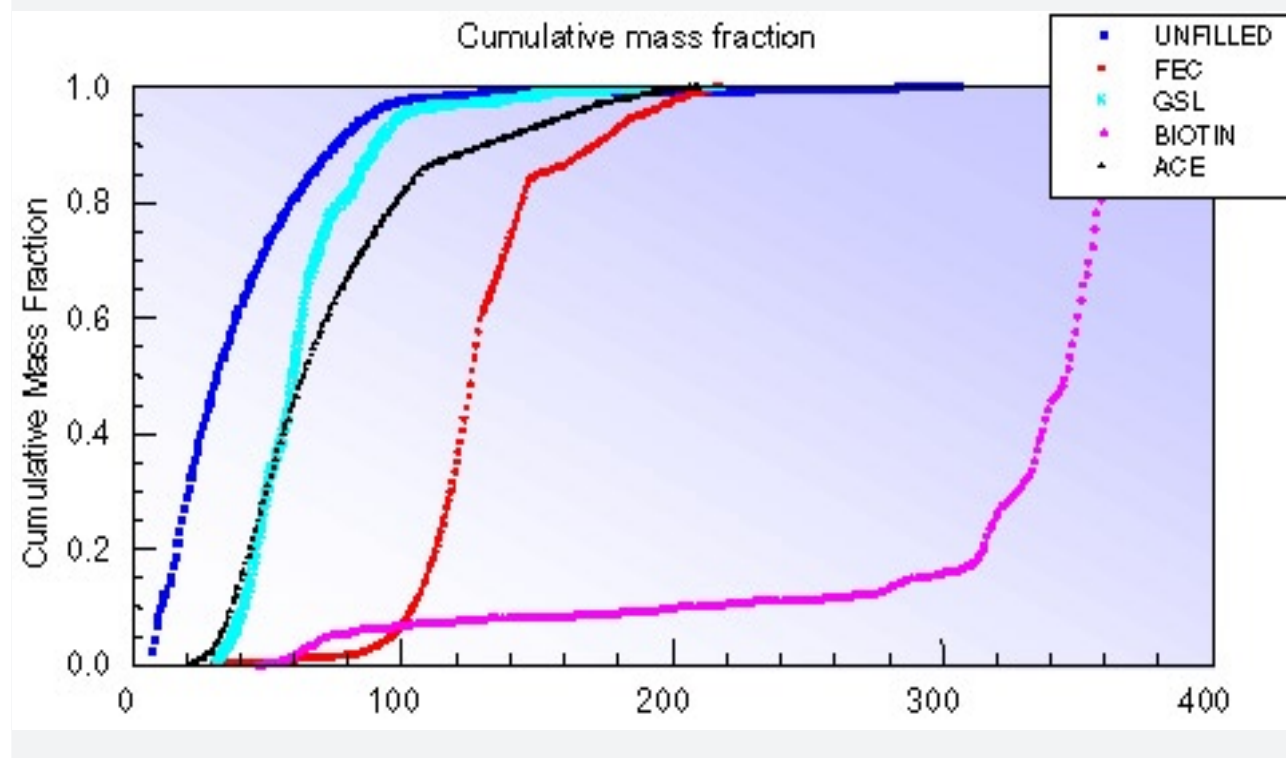
Cumulative mass distribution plot comparing the three different liposome preparations. Filled liposomes (green), empty (blue) and the sample

from low-grade lecithin (red). The cumulative plot shows that the size distribution does not change due to drug loading



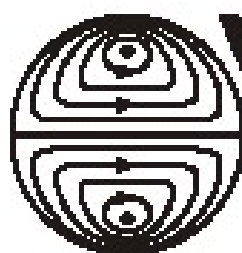
Taking a closer look at the size distribution of several filled liposomes, it turns out there is a shift to bigger sizes as a function of the drug loaded.

The high resolution of FFF allows to monitor which factors are responsible for the shift in size and to optimize the production process.



Literature:

- [1] C. Johann, Asymmetrische Fluss-Feld-Fluss-Fraktionierung zur Charakterisierung von Polymeren und Partikeln, GIT 2/98
- [2] John H. van Zanten, Vesicle Size Distributions measured by Flow Field-Flow Fractionation coupled with Multiangle Light Scattering, Biophysical Journal, 74, 3264-3272 (1998)
- [3] D. W. Shortt, Dierk Roessner, P.J. Wyatt; Absolute measurement of diameter distributions of particles using a multiangle light scattering photometer coupled with field-flow fractionation, American Laboratory, November 1996
- [4] Philip. J. Wyatt, High-Precision Measurement of Submicrometer Particle Size Distributions, Langmuir, 13, 3913-3914



**Wyatt
Technology**
Europe GmbH

In der Steubach 9, D-57614 Woldert
Tel.: +49(0)2684-979161
e-mail: info@wyattde www.wyattde