WHITE PAPER

WP2608: Lipid Nanoparticle and Liposome Characterization with FFF-MALS-DLS

Christoph Johann, Ph.D., Wyatt Technology

Introduction

Lipid nanoparticles (LNPs) and liposomes are variations of lipid-based drug carriers differing in their internal structure. In liposomes, a phospholipid membrane encloses an aqueous inner cavity—a synthetic analog to natural cell walls. LNPs include nano-emulsions, micelles and solid lipid nanoparticles (SLN). Liposomes and LNPs serve as versatile drug delivery systems, offering control over composition, structure, and morphology, where these properties can be tailored to each specific pharmaceutical application.

Comprehensive characterization of the particles is essential for rapid and effective pharmaceutical product development as well as for regulatory approval. The basic properties to be evaluated are the size distribution and surface charge (zeta potential). In addition, knowledge of internal structure, mode of drug incorporation, drug loading capacity and drug release kinetics is of vital importance for the development of a successful drug delivery system.

Characterization methods

Various methods are available for characterization of nanoparticle size, including dynamic light scattering (DLS), electron microscopy (EM) and nanoparticle tracking analysis (NTA).

- Batch DLS size distributions are fast and easy to measure and comprise representative ensembles, but only semi-quantitative. Batch DLS is subject to low resolution and bias towards larger sizes.
- EM size and structure analysis offers very high resolution and true quantitative distributions but requires significant sample preparation and is potentially biased due to small, unrepresentative ensembles consisting of just hundreds to thousands of particles.
- NTA offers quantitative distributions over small, possibly unrepresentative ensembles with moderate resolution, limited size range, and bias towards larger particles.

FFF-MALS-DLS is a highly versatile techniques that determines detailed, accurate and quantitative size distributions, while avoiding the biases of small ensembles (EM, NTA) or favoring larger particles (batch DLS, NTA). FFF-MALS-DLS combines sized-based separation, based on field-flow fractionation (FFF), with absolute size and structure determination, using online multi-angle and dynamic light scattering (MALS, DLS). Additional online detectors such as UV/Vis, fluorescence and refractive index provide yet more functionality and produce a powerful analytical system for characterizing drug delivery nanoparticles.

Learn about FFF-MALS-DLS

An FFF-MALS-DLS system comprises two main subsystems:

1. **Separation**: an Eclipse™ FFF device plus standard HPLC components such as pump, degasser and autosampler from leading HPLC vendors.
2. **Characterization:** a DAWN® online MALS instrument incorporating a WyattQELS™ online DLS module and additional HPLC UV or fluorescence modules. MALS determines the rms radius R_g (radius of gyration) while DLS determines the hydrodynamic radius R_h. In addition, R_h can be estimated from the FFF retention time using any of the detectors to detect the peak.

An introduction to FFF and Wyatt's Eclipse can be found in References 1 and 2, as well as www.wyatt.com/Theory/FFF. An introduction to multi-angle light scattering and dynamic light scattering may be found in www.wyatt.com/Theory.

Case study #1: Solid lipid nanoparticles and nanostructured lipid carriers

Lipid nanoparticles with a solid matrix are composed of fully biocompatible, FDA-approved lipids and stabilizers. They can protect the incorporated drug under harsh conditions (e.g. for oral delivery) and the lipid composition can be tailored to optimize drug incorporation and release.

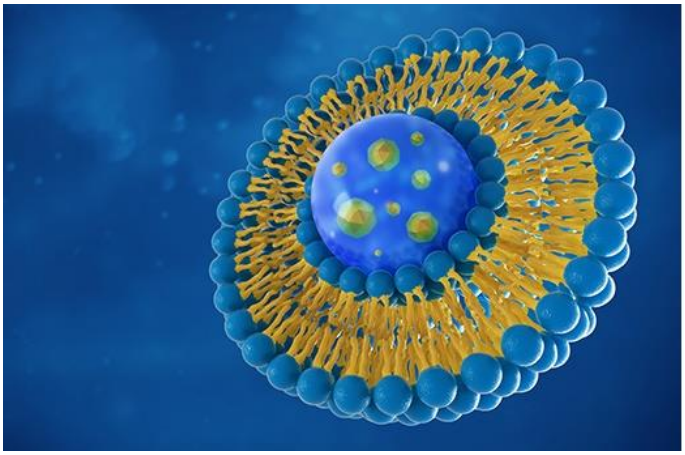

Figure 1. Liposome and lipid nanoparticle formulations enable targeted, effective delivery. Shown is a liposome rendering with a drug payload encapsulated in the core, but it can be as well incorporated in the membrane layer or attached to the surface of the particle.

The drawback of SLNs lies in their limited drug loading capacity, a result of the crystalline structure of the lipid core. To overcome this limitation, nanostructured lipid carriers (NLCs) were developed, suitable for higher drug loading.

In the early stages of NLC development, the morphologies of the solid SLN and nanostructured NLC were not known. It was unclear how the liquid and solid phases were arranged in the particles, and if the particles had spherical or other shapes. These properties are important to maximizing drug loading and controlling drug release.

In a study to clarify these questions[3], three different LNP types were compared: a nano-emulsion, which was known to have spherical droplets, solid SLN, and an NLC preparation which contained a mixture of liquid and solid lipids. The samples were analyzed with FFF-MALS, EM and batch DLS.

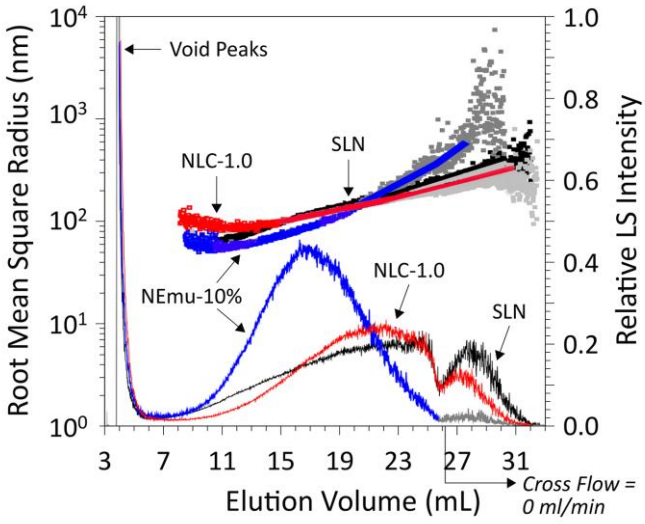

Figure 2. AF4-MALS fractogram of a nano-emulsion (NEmu-10%, blue), SLN (black) and NLC (red) samples. The 90° LS signals are overlaid with calculated RMS radius from MALS. The radius of the nano-emulsion is smaller between 11 mL and 20 mL elution volume compared to the other two samples, indicating an elongated structure for SLN and NLC (Figure adapted from Ref. 3)

Standard off-line (batch) DLS is a low-resolution technique which could not reveal a high level of detail with respect to size or structure. However, it did indicate higher polydispersity for SLN and NLC compared to the micro-emulsion, suggestive of inhomogeneity, which was born out in the FFF-MALS and EM results.

FFF-MALS provides a much higher level of detail than off-line DLS. It showed that, over the main fractogram populations, both SLN and NLC had higher R_g values at a given elution time compared to the nano-emulsion (Figure 2). As the retention time in FFF is directly related to the hydrodynamic radius, it was concluded that both types of lipid particles were not spherical but were elongated. These findings confirmed the interpretation of EM images which indicated a mixture of needles and ellipsoidal structures decorated with droplets. Hence the three techniques complemented each other to better understand the morphology of these particles.

Case study #2: Preclinical characterization of lipid-based nanoparticles

According to a recently published study,[4] particle size distribution and stability are key attributes for the evaluation of the safety and efficacy profile of medical nanoparticles. The authors state in the abstract: "Measuring particle average size and particle size distribution is a challenging task which requires the combination of orthogonal high-resolution sizing techniques, especially in complex biological media. Unfortunately, despite its limitations… batch mode dynamic light scattering (DLS) is still very often used as the only approach to measure particle size distribution in the nanomedicine field… AF4-MALS-DLS is a powerful method for the pre-clinical characterization of lipid-based nanoparticles."

The publication supports this conclusion by comparing characterization data from DLS, AF4-MALS-DLS and TEM. Three different Lipidots™ formulations were prepared and their size distribution measured on freshly prepared samples, after three and six months of storage, and after incubation with serum. Figure 3 shows an example comparing the fractograms and calculated RMS radius from MALS for a fresh lipid nanoparticle compared to the sample stored for 6 months at 4°C.

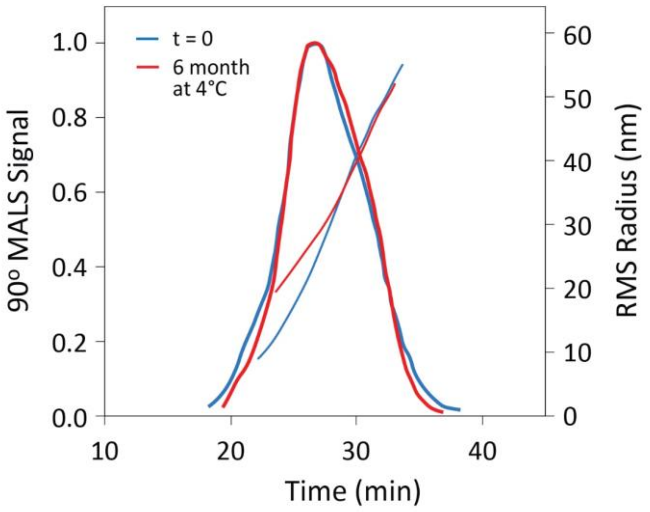

Figure 3. AF4-MALS fractogram of Lipimage815™ which is prepared from a mixture of lipid, lecithin and a dye solution as the active ingredient[3]. The overlay compares the sample at t=0 and after 6 months of storage, indicating an increase of the RMS radius between 23 and 30 minutes of elution time. (Figure adapted from Ref. 4)

Under batch DLS the two samples looked identical. FFF-MALS indicated a clear increase in R_g at early elution times, which points to the formation of more elongated particle shapes. Monitoring these subtle differences with FFF-MALS, undetectable by batch DLS alone, can impact significantly the product development of drug nanocarrier systems.

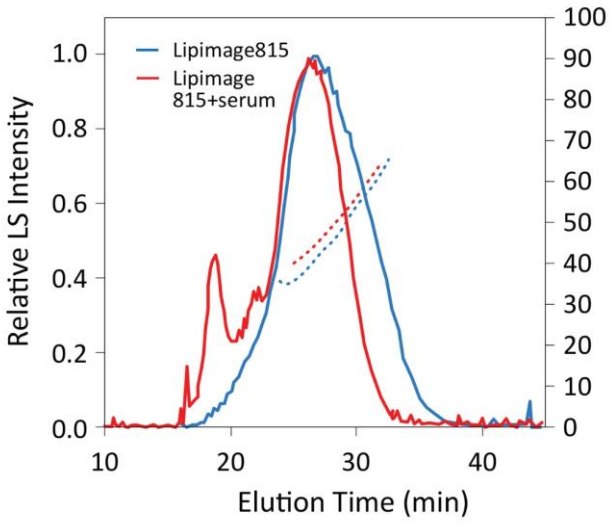

Figure 4. AF4-DLS (QELS) fractogram of lipid nanoparticles incubated with serum. Compared to the original sample, the peak becomes narrower. These slight changes cannot be detected by DLS or NTA. (Figure adapted from Ref. 4)

If the drug is formulated for intravenous injection, an important consideration is the interaction of the lipid nanoparticles with serum. The biological matrix makes the analysis even more challenging; neither batch DLS nor nanoparticle tracking analysis (NTA) can produce meaningful results.[4] However, separation of other components from the lipid nanoparticles by FFF enables effective characterization by online light scattering, as shown in Figure 4.

Case study #3: Drug transfer from liposomes

Liposomes are commonly used as carriers for delivery of drug compounds or RNA-based therapeutics. Drugs may be encapsulated in the liposome's core, incorporated into the lipid bilayer, or attached to the outer surface. The aim of drug formulation with liposomes is the targeted transport and release of the drug on site in the patient's body.

In the case of parenteral delivery, drug and liposome may be subject to different clearance kinetics leading to premature loss of the drug even before the liposome itself is removed from the blood stream. Lipophilic and amphiphilic drugs can redistribute from liposomes to lipoproteins, cell membranes of blood cells and to high-abundance proteins such as serum albumin.

A predictive model

A test for ex vivo prediction of the degree of drug loss consists for example of donor liposomes which carries the drug and acceptor lipid particles such as large multilamellar vesicles. To analyze the degree and kinetics of drug transfer, donor and acceptor particles must be separated and quantified. This model for drug transfer is important in order to develop an efficient liposome-based drug delivery formulation. Traditionally this has been done by bench-top centrifugation and radiolabeling.

Using FFF-MALS a new, elegant predictive test has been developed which is label free, precise and convenient[5]. The donor liposome is prepared with a narrow size distribution which can be baseline separated by FFF from the bigger multilamellar vesicles functioning as acceptors. Dye molecules, detectable by UV absorbance or fluorescence, serve as model drugs that are transferred from donor to acceptor.

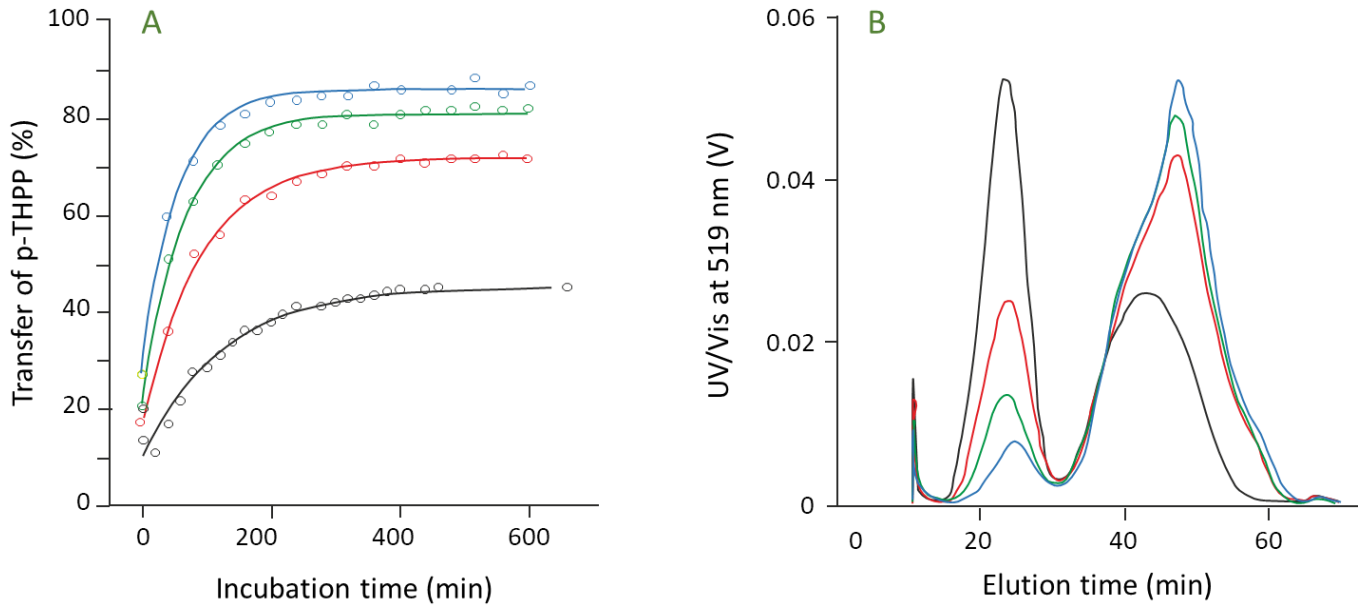

Figure 5. The effect of lipid mass ratio on the kinetics of p-THPP transfer (A): Donor to acceptor mass ratio of 1:0.8 (black), 1:2.6 (red), 1:5.4 (green) and 1:9.1 (blue). Fractogram measured at t=0 (B). The donor is the early eluting peak, followed by the broader peak of the acceptor vesicles. The peak areas vary according to the different mass ratios applied. (Figure adapted from Ref. 5).

The donor and acceptor particles are incubated for defined time periods, then separated by FFF to resolve the two populations. The presence of dye in each population is quantified by UV or fluorescence, at the same time that the size distribution is confirmed with MALS.

Figure 5 shows results from this study. The per cent of transferred drug is shown as a function of incubation time for several acceptor:donor lipid mass ratios. Not surprisingly, the transfer increases with higher acceptor mass.

Even at extreme ratios of acceptor and donor (typical of true serum conditions), quantification by the FFF method is still precise, unlike centrifugation-based methodologies. Another advantage of FFF is that the method can be applied to many different drug models to study the dependence of drug transfer on lipophilicity and water solubility.

For drug models which had a lower UV extinction and high donor-acceptor ratios, a high sample load is required. The semi-preparative (SP) Eclipse FFF channel was successfully used for these experiments; the SP channel maintains baseline resolution even with 1100 µg injected sample mass (30x – 100x more than typical analytical FFF injected mass).

Significance of the FDA liposome guidance for industry

In April 2018 the FDA published a guidance document for liposome products[6], important for new drug applications involving liposomes. With this publication, the FDA raised the bar with respect to the quality of data expected in a new drug application.

The section on physicochemical properties lists critical quality attributes (CQAs) which should be provided. Whereas in the past only the mean size value was expected, now the full distribution profile is specified. Batch DLS was sufficient for mean size, but determination of reliable, quantitative distributions over large ensembles requires a separation method, such as FFF, coupled to MALS and DLS.

Per the new guidance, stability and liposome integrity changes (i.e., drug release, encapsulation efficiency and size) must also be investigated. Some of the required tests are quite difficult to perform without FFF. The case studies discussed above illustrate how the FDA requirements on this topic can be met. Drug loading, encapsulation efficiency and leakage can all be examined with an assay which includes a separation and characterization with FFF-MALS-UV or FFF-MALS-fluorescence. If additional off-line analysis is needed, fractions can be readily collected with a standard HPLC fraction collector.

Summary

The case studies presented above highlight the capabilities of FFF for high-resolution separation of lipid particles. Coupling to MALS and DLS produces comprehensive characterization of the size and shape distribution through simultaneous measurement of mean square radius R_g and hydrodynamic radius R_h. The studies demonstrated how drug loading and drug transfer can be examined with FFF-MALS and how the method contributes to stability investigations

Finally, the significance of FFF-MALS in view of the FDA guideline for liposome drugs is evident. The FDA will require information on size distribution instead of just average size and other detailed investigations on drug loading and stability. In view of this evidence, the Wyatt FFF-MALS-DLS system incorporating an Eclipse FFF device followed by a DAWN MALS detector with an embedded WyattQELS online DLS module is an indispensable tool in drug delivery research and development.

References

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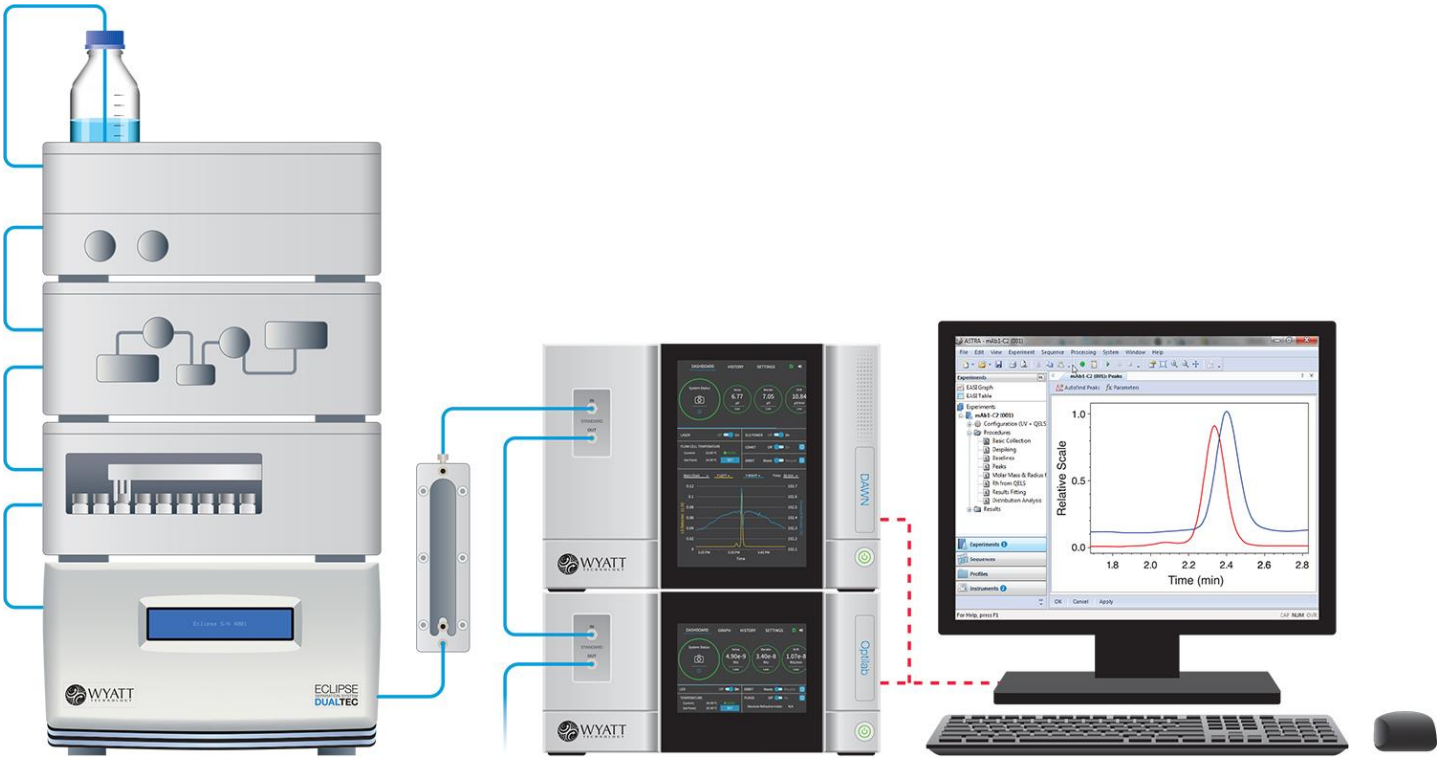

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