WYATT TECHNOLOGY

Whitepaper

Robust and Repeatable Nanoparticle Drug Delivery Characterization with FFF-MALS-DLS

Daniel Some, Ph.D., Principal Scientist, Wyatt Technology Corp. †

Introduction

Nanoparticles hold enormous potential for taRgeted, well-controlled drug delivery. Extensive characterization of nanoscale drug-delivery vehicles is essential to ensure their efficacy and reproducibility. While various methods are available for characterization of nanoparticle size, including dynamic light scattering, electron microscopy and nanoparticle tracking analysis, FFF-MALS is one of the most versatile techniques for determining size, structure and other properties. This article highlights a study demonstrating the benefits of FFF-MALS-DLS for the characterization of nanolipid particles.

The NanoDDS Characterization Challenge

Nano-drug-delivery systems (nanoDDS), increasingly recognized as the basis for the next generation of drug delivery, have already proven significant benefits over traditional formulations. However, nanoDDS are inherently more complex in structure and composition than common drug forms. In order to realize their promise, extensive characterization is required throughout research, development and production.

Techniques for characterization of nanoparticle size are plentiful, varying from high-resolution, poorly samples electron microscopy to low resolution, well-samples batch light scattering. Recent studies have shown that FFF-MALS [1, 2] is a highly beneficial technique for nanoDDS characterization which provides well-resolved and accurate size distributions as well as structure and composition, overcoming many of the limitations of other technologies. This article presents case studies illustrating the characterization of liposomes and nanolipid complexes by FFF-MALS, proving the technique to be an easily adaptable yet powerful characterization tool.

Nanoparticles for drug delivery

The limitations of many conventional pharmaceutical preparations, including solutions, suspensions and emulsions, often include low availability, intolerance, instability, and a lack of sustained effect. One of the most active research directions to overcome these limitations is nanomedicine, which applies nanotechnology to highly specific medical interventions for prevention, diagnosis and treatment of diseases. The surge in nanomedical research during the past few decades is now translating into considerable commercialization efforts around the globe.

Nanoparticles are attractive because nanoparticle fabrications can be precisely controlled, allowing their size, shape, surface charge, stability and other physical characteristics to be modified to influence particle behavior in vivo. These properties are exploited to enhance uptake and bioavailability, efficacy of transfection through the cellular membrane, and other aspects of pharmacokinetics. Nanoparticles also exhibit a large surface to volume ratio, allowing the surface coating to be functionalized with ligands for highly specific applications such as targeting of biomarkers. These features result in a concomitant reduction in the quantity of the drug required and dosage toxicity, which ultimately enables the safe delivery of toxic therapeutic drugs and protection of non-target tissues and cells from severe side effects.

†dsome@wyatt.com

Characterization of nanoparticle drug delivery vehicles

The biological response of nanomedicines depends on specific physico-chemical characteristics. Even small variations in physico-chemical system properties can have a significant impact on nanomedicine performance. Many different nanoDDS have been described, including liposomes, hydrogels, silver-coated nanolipids, dendrimers and colloidal drug carriers, each entailing a different mix of physical and chemical properties. Therefore, in order to maximize the potential of nanoparticles for drug delivery, accurate and detailed characterization is critical in the course of development and production. Successful development of a nanoparticle drug delivery mechanism requires thoughtful selection of the most appropriate orthogonal and complementary characterization techniques.

For the characterization of nanoparticle size and aggregation, several methods are available and routinely used, including dynamic light scattering (DLS), electron microscopy (EM) and nanoparticle tracking analysis (NTA). DLS is one of the most common techniques for particle size measurements, providing low-resolution and semi-quantitative size distributions, albeit with good statistical sampling. DLS is widely considered to be user-friendly and fast, yielding relatively consistent results relating to average size and aggregates. DLS is particularly beneficial for formulation screening and production QC, as a consequence of its implementation in a high-throughput microwell plate reader, the DynaPro® Plate Reader II. However, DLS on its own does not provide particularly good resolution, quantitative distributions, structural or compositional information.

On the other hand, EM is well-suited for detailed studies of nanoparticle size and shape. It provides more detailed insight into the structure and morphology of individual particles via electron micrograph images of very high resolution. Unfortunately, EM is not suitable for routine, statistically valid characterization and requires lengthy and operator-intensive analysis.

NTA makes it possible to analyze nanoparticle population in terms of both size and number. NTA provides a more quantitative size distribution than DLS; however, despite its advantages, the technique has comparable size resolution and inferior sampling statistics.

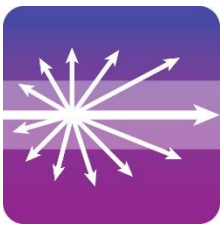
Field-flow fractionation (FFF) for the separation and characterization of nanoparticles has seen increased acceptance in the recent years. FFF provides high-resolution separation of particles as a function of their hydrodynamic size from one nanometer up to several micrometers, the perfect range for use in determining accurate and well-resolved nanoparticle size distributions. Moreover, when the technique is coupled with online spectroscopic, multi-angle light scattering (MALS) and dynamic light scattering detectors, a powerful system is created for versatile, robust characterization of molar mass and size distributions as well as structure and composition. MALS determines molar mass, size (R_g, rms radius) and particle number density. DLS determines hydrodynamic size (R_h, hydrodynamic radius). As shown below, the shape parameter $\rho = R_g / R_h$ indicates conformation or shape. Additionally, spectroscopic techniques such as UV/Vis, refractive index or fluorescence are helpful in determining concentration and composition. FFF-MALS (including DLS) is amenable to the analysis of simple to complex solutions that may include small molecules, proteins, polymers, colloids and/or nanoparticles.

FFF-MALS can provide high-resolution analysis of size, medium-resolution analysis of conformation, and insight into drug or ligand loading into the nanoparticle's interior or onto its surface, with good statistical sampling [3,4]. It can separate and characterize nanoDDS components such as free drug (payload), ligands (targeting mechanism) or unassembled/partially-assembled polymer or protein (nanoparticle building blocks). It is suitable for robust, repeatable, and routine analyses. In addition to sequential MALS, DLS and spectroscopic analyses, FFF even offers online analysis of electrophoretic mobility for determination of well-resolved size/zeta potential distributions. This article presents but a small sample of the potential applications of FFF-MALS-DLS to nanoDDS characterization.

Liposome characterization by FFF-MALS-DLS

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. During development and formulation, the size, structure and drug loading of the liposomes must be assessed. While low-resolution DLS size measurements are often expedient, high resolution analysis of size distributions and structure by FFF-MALS is essential for well-characterized products. In the following case study [5], the analytical results of two liposome samples, one empty and one filled with a therapeutic are reported, using FFF in combination with MALS and online DLS.

Standard (unfractionated) DLS measurements of filled and empty liposomes, acquired with a DynaPro® NanoStar®, are shown in Figure 1. Since filling the liposome core did not, in this system, change its external dimensions, and R_h is basically a measure of the envelope of the particles, both filled and empty liposomes exhibit nearly identical hydrodynamic radii. Therefore standard DLS is not suitable for identifying drug encapsulation in these samples. We will note that there have been observations of other liposomal delivery systems that did exhibit a shift to lower hydrodynamic radius upon drug loading.



For fractionated FFF-MALS-DLS measurements, the Eclipse™ FFF system was followed by a DAWN® HELEOS® MALS detector incorporating a WyattQELS embedded online DLS module, all from Wyatt Technology. The size range accessible to accurate DLS analysis generally depends on flow rate and detection angle; here the DLS scattering angle was approximately 143° in order to extend the R_h measurement from 0.5 nm up to 300 nm. The FFF method was designed with the aid of Wyatt's FFF simulation and optimization software.

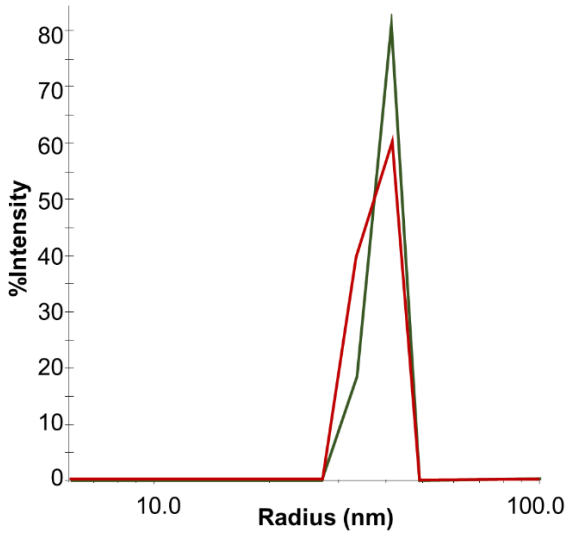
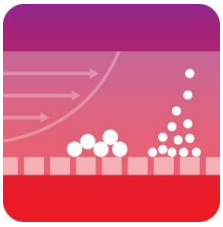


Figure 1. DLS measurements of empty (red) and filled (green) liposomes using a DynaPro NanoStar batch DLS detector. The sizes are indistinguishable within the relatively low resolution of this technique, providing no positive indication of their loading.



Both R_g and R_h are plotted against FFF elution time in Figure 2. The results from duplicate runs demonstrate excellent reproducibility of the FFF-MALS method. Figure 2 shows that the R_h values for empty and filled liposomes are nearly identical, bearing out the initial batch DLS data (Figure 1) albeit with much higher resolution. However, R_g values for these two liposomes do not overlay, which indicates that both liposomes have different degrees of encapsulation.

Root-mean square radii R_g were then plotted against hydrodynamic radii R_h for these two liposomes (Figure 3a). The slope of R_g vs. R_h plot yields the shape factor ρ indicative of the structure of the liposomes. For the empty liposome sample $\rho = 1.0$, consistent with a spherical shell structure. For the filled liposome sample, however, $\rho = 0.77$, in excellent agreement with that of a solid sphere structure of uniform density. The shape factor may also be used to determine the aspect ratio of solid rods or ellipsoids. Wyatt's ASTRA software for MALS analysis also provides absolute number densities of nanoparticles that have a known, real refractive index, shown in Figure 3b.

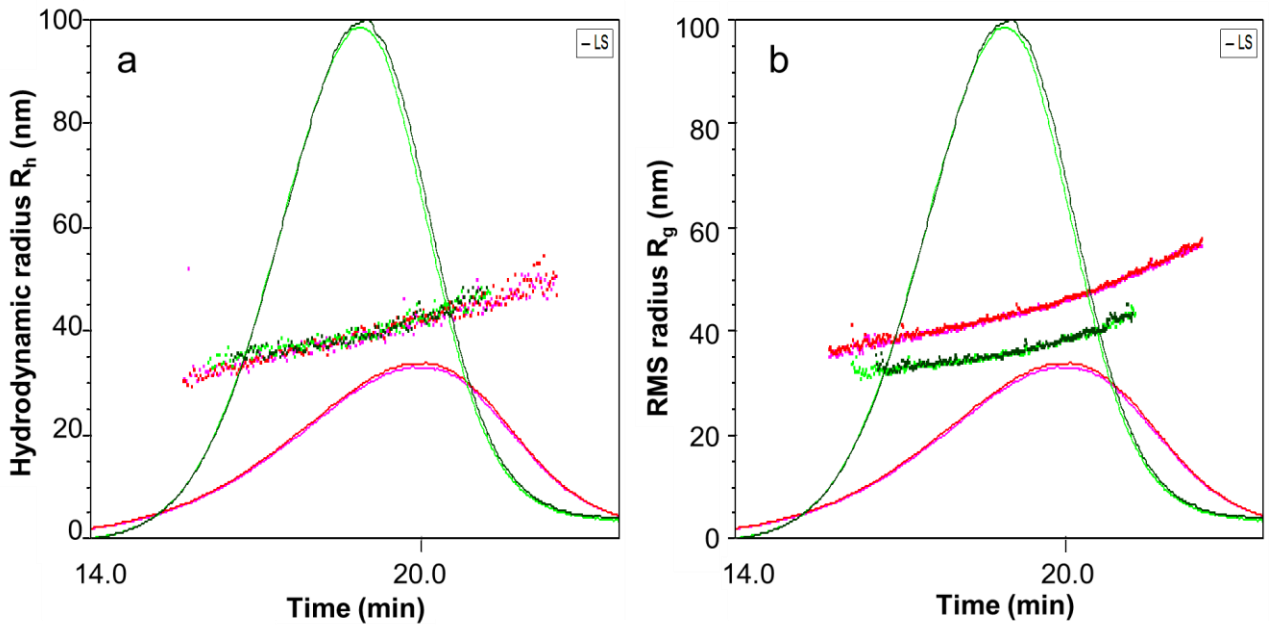


Figure 2. Hydrodynamic radius (a) and root-mean square radius (b) plotted against elution time overlaid with 90° LS signals for empty liposome sample (red) and filled liposome sample (green). The R_h and R_g values are determined by the respective online DLS and MALS detectors. The results from duplicate runs of each sample are shown here to demonstrate the excellent reproducibility of the FFF-MALS analysis.

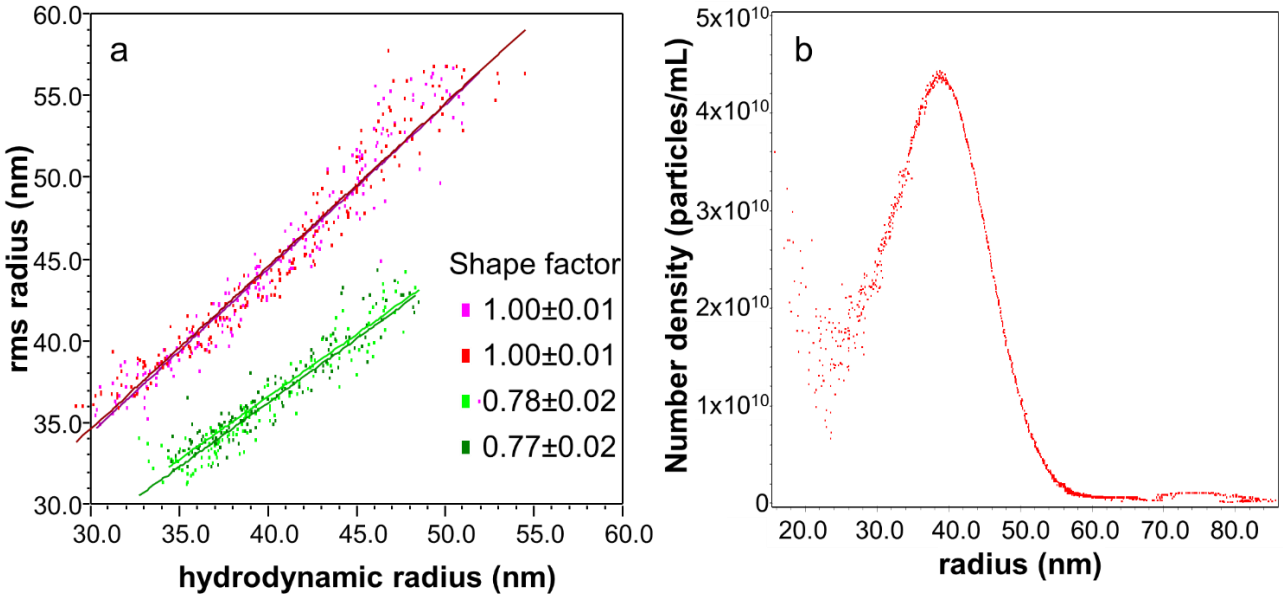


Figure 3. a) Root-mean square radius, R_g, plotted against hydrodynamic radius, R_h, for empty liposome sample (red) and filled liposome sample (green). The slopes for empty and filled liposomes are 1.0 and 0.77, respectively. b) Number density calculated for each particle size.

Silver-nanolipid complex characterization by FFF-MALS

In another case study, FFF-MALS was used to analyze nanolipid complexes (NLC) as carriers functionalized with anti-microbial microsilver particles [6]. Functionalized nanoparticles were prepared by incubating two different NLC dispersions (5.0% and 10.0% w/w) in two different microsilver dispersions (0.1% and 0.15% w/w) for a total of 4 different preparations, in order to assess silver loading onto the NLC. The resulting dispersions could not be differentiated by DLS or zeta potential. However, FFF-MALS showed distinct and highly repeatable size differences, depicted in Figure 4. Saturation of the NLCs clearly occurs when exposed to 0.15% w/w microsilver dispersions, but not 0.1%, leading to size changes on the order of 10-15 nm in radius. With repeatability better than 1% in size and accurate, quantitative, high-resolution distributions thanks to true hydrodynamic size separation prior to online light scattering measurements, FFF-MALS readily illuminates the effect of the four different preparative conditions on the complex formation and helps determine the optimal production conditions.

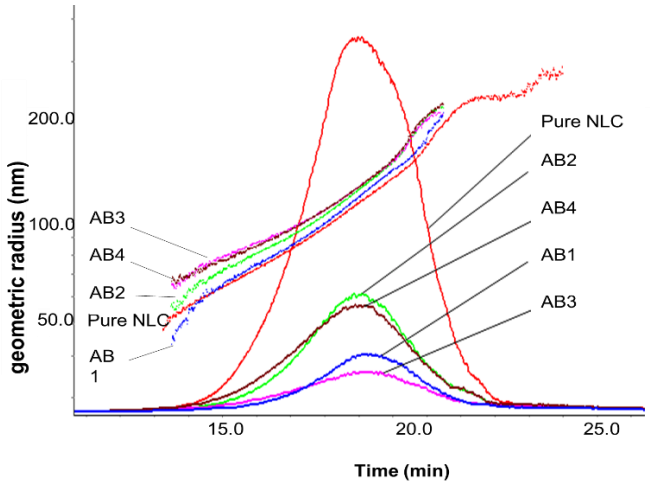


Figure 4. Highly accurate sizing of silver-nanolipid complexes by FFF-MALS. Preparations as follows: AB1 - 5% w/w NLC, 0.1% w/w silver; AB2 - 10% w/w NLC, 0.1% w/w silver; AB3 -5% w/w NLC, 0.15% w/w silver; AB4 - 10% w/w NLC, 0.15% w/w silver.

Summary

The surge of research into nanomedical drug delivery mechanisms has highlighted the importance of accurate and detailed characterization techniques, for the development and clinical use of therapeutic nanoparticles, to ensure efficacy, safety and reproducibility. A number of characterization techniques including DLS, EM and NTA are routinely used, but do not always provide the requisite level of detail and rich information.

FFF-MALS-DLS is a more versatile option than the common sizing techniques, and here we have not even scratched the surface in terms of spectroscopic analyses or online zeta potential measurements [7]. The case studies prove that for liposomes and related carrier vehicles, FFF-MALS-DLS is an easily adaptable yet powerful characterization tool to obtain information on particle size, size distribution, particle count, as well as structure. Already utilized extensively to address environmental and synthetic polymer nanoparticles, exosomes and protein aggregates, further adoption of this technique by the nanoDDS community will enhance R&D efforts to optimize physico-chemical properties of nanoparticles in the service of human health.

References

1. S. Podzimek, *Light Scattering, Size-exclusion Chromatography and Asymmetric Flow Field Flow Fractionation* (John Wiley & Sons; Hoboken, NJ, USA, 2012).

2. P.J. Wyatt, Journal of Colloid and Interface Science 197, 9–20 (1998).

3. A. Hinna, F. Steiniger, S. Hupfeld, M. Brandl, J. Kuntsche. *Asymmetrical flow field-flow fractionation with on-line detection for drug transfer studies: a feasibility study*. Anal. and Bioanal. Chem. 406(30), 7827-7839 (2014)

4. J. Kuntsche, C. Decker, A. Fahr. *Analysis of liposomes using asymmetrical flow field-flow fractionation: Separation conditions and drug/lipid recovery*. J. Separation Sci. 35(15), 1993-2001 (2012).

5. M. Chen and V. Lin, presented at the 2011 International Light Scattering Colloquium, Santa Barbara, CA.

6. C.M. Keck and K. Schwabe, *Silver-Nanolipid complex for application to atopic dermatitis skin Rheological characterization, in vivo efficiency & theory of action*. J. Biomed. Nanotech., 2009.

7. http://www.wyatt.com/files/literature/app-notes/mp-pals/FFF-PALS-and-SEC-PALS-[poster].pdf