A trick of the light

Novel technologies for sizing liposomal drug-delivery particles

Since their inception in the mid-1960s, liposomes have become an important, if not the critical, nanoparticle in the pharmaceutical industry, with applications ranging from magnetic resonance imaging to biosensors to drug delivery. In the area of drug delivery there are presently over 30 liposome formulations involved in clinical trials at the U.S. Food and Drug Administration, targeting breast, prostate and ovarian cancer, among others.

Because of their relatively high carrying capacity, lower toxicity and protection against dilution, liposomes are excellent carriers of therapeutic biological molecules such as antibodies, peptides and proteins. Liposomes also offer a protective environment for drug compounds, encasing water-soluble drugs in an aqueous pouch. One of the most important physical characteristics of these drug-delivery nanoparticles is their size and size distribution, physical characteristics affecting the stability and efficacy of the encapsulated drug. The determination of the size and uniformity of the liposome has classically been performed by a host of techniques, including electron microscopy, fluorescent microscopy and HPLC. Recently, novel light-scattering technologies have become available and their application to liposome characterization is discussed in detail in the present article.

Two types of light-scattering technologies, SLS (static light scattering; also known as classical MALS (multi-angle light scattering)) and DLS (dynamic light scattering; also known as quasi-elastic light scattering), have been applied extensively to biomolecular characterization. These two light-scattering techniques often have complementary roles.

SLS or MALS measures the time-averaged intensity of scattered light from the molecules in solution at one or a plurality of angles. The intensity is proportional to the concentration and molecular mass of the molecule in the solution (Figure 1). MALS detectors, in particular, are widely used in measuring the absolute molecular mass of proteins and other polymers independent of the shape of the molecules. The angular dependence of the scattered light yields the size of the molecule, known as the RMS (root mean square) radius. MALS measurements can be carried out either in a stand-alone ‘batch’ mode or, more often, in conjunction with a separation technique (SEC (size-exclusion chromatography) or FFF (field flow fractionation)).

In contrast with MALS, DLS analyses the rapid time-intensity fluctuations in the scattered light, due to the Brownian motion of the molecules in solution, to determine a molecule’s size. The rate of the time-intensity changes is directly related to the translational diffusion of the particle, from which the hydrodynamic size and size distribution of the molecules is determined (Figure 2). DLS size distributions provide information about the homogeneity of the molecules quickly and without perturbing the sample. Typically DLS is performed manually in batch experiments using high-quality quartz cells, although recently DLS plate readers, using disposable or quartz microplates, have become available for high-throughput DLS experiments. DLS plate reader systems increase productivity by a factor of ten or more over conventional batch DLS systems.

Liposome characterization by DLS

Liposomes, containing 48% brain PC (phosphatidylcholine), 20% brain PE (phosphatidylethanolamine), 12% brain PS (phosphatidylserine) and 20% cholesterol, were prepared by the extrusion method. Briefly, the lipid mixture in chloroform was evaporated using a nitrogen stream

Key words: biomolecular characterization, drug delivery, light scattering, liposome, nanoparticle.
and then thoroughly dried with a vacuum pump for 2 hours to remove residual organic solvent. The dried lipid film was then hydrated with 25 mM Hepes buffer (pH 7.5) to form LMVs (large multilamellar vesicles). The LMV suspension was disrupted by five freeze–thaw cycles. The lipid suspension was then forced through a polycarbonate filter (80 nm pore size) 21 times. DLS was performed with a DynaPro DLS instrument (Wyatt Technology) using a 10 second acquisition time at 37°C. The liposomes, containing 50 mM lipid, were spun at 13,000 rev./min for 10 minutes with a micro benchtop centrifuge before being subjected to DLS measurement. The laser power was adjusted to keep the intensity between 500,000 counts and 2,000,000 counts. The results were then processed with the DYNAMICS software program (Wyatt Technology).

The mean size and the uniformity of the size distribution were calculated with the regularization algorithm provided by this software. The results show that the extrusion method typically yields homogeneous liposomes with a mean diameter of 88 nm (Figure 3). The homogeneity of the liposomes is indicated by the percentage polydispersity. Higher values of percentage polydispersity indicate increasing heterogeneity, or the presence of particles of sizes different from the mean value but that cannot be resolved by DLS. Percentage polydispersity of 15% or less typically indicates a homogeneous size distribution.

Electron microscopy studies yielded similarly consistent results, although the size dispersion appears to be larger than indicated by the DLS measurements. Batch DLS experiments provide size and size distributions in a few minutes, with as little as 12 µl of sample. Complete control over the solution conditions for the measurement (e.g. no dilution or change in ionic strength) is often critical to understanding the effects of conditions or additives on the size and size distribution. Although DLS cannot resolve particles similar in size by a factor of 2–5 in radius, the technique provides an indication of heterogeneity without separation.

High-resolution liposome characterization by FFF–MALS

Combining a fractionation technique, such as an AFFF (asymmetric cross-flow FFF), and an on-line MALS detector provides high-resolution characterization of liposome sizes. Because of the absence of a stationary phase, FFF is able to separate a sample for subsequent light-scattering analysis without the artefacts associated with size–exclusion columns. MALS systems, such as the DAWN instrument (Wyatt Technology), with 18 scattering angles, provide a measure of the RMS radius directly from the angular variation of the light scattered by the particles. Unlike DLS, in which the size is calculated from the measured rate of motion of the particles, the FFF–MALS system provides a direct size determination with no assumptions. Using this combined instrument system, the size distribution of a pharmaceutical liposome formulation was determined. Figure 4 illustrates the RMS radius compared with volume, superposed over the 90° light-scattering signal. Note that in contrast with SEC separation, the smaller liposomes elute first in
Product Focus

The distribution of liposome sizes is shown in Figure 5. The data show a distinct peak at approximately 95 nm, but the distribution is by no means monodisperse, as it has components as small as 80 nm and up to over 150 nm. These data show the enormous resolving power of the FFF–MALS. Since the sample is separated into its individual size components, much more specific distribution information is produced. Fractionation approaches typically require 20–30 minutes of elution time and result in the dilution of the sample by a factor of 10–100, or more.

Conclusion

Information about the solution behaviour of liposome particles is critical for the successful development of pharmaceutical liposome drug formulations. MALS and DLS are insightful complementary techniques for the determination of the size and homogeneity of liposome preparations in solution. MALS in combination with a fractionation technique, such as FFF, provides a high-resolution analysis of the size distribution, which is particularly valuable for complex liposome particle formulations (e.g. coated stealth liposomes). Capturing the high-resolution size distributions takes more time and often perturbs, or dilutes, the sample compared with DLS. DLS provides fast and useful information on the size and homogeneity of the liposome sizes, without perturbation of the sample. New DLS plate reader systems improve the productivity of liposome formulation development by screening hundreds of buffer conditions or preparations automatically.

The liposome characterization by DLS section is adapted from a Wyatt Technology application note, submitted by Xiaocheng Chen, Jose Rizo-Rey Laboratory, University of Texas Southwestern Medical Center at Dallas, USA. The high-resolution liposome characterization by FFF–MALS section is taken from a Wyatt Technology application note by Christoph Johann, MD, Wyatt Technology Europe.

References


Bob Collins is Product Manager for Wyatt Technology, the leader in MALS technology and related systems, including FFF, DLS and on-line viscometry. He was formerly a co-founder of Protein Solutions, manufacturer of the DynaPro biomolecular sizing instrument and Vice President of Product Management for Proterion Corporation, manufacturer of tools for biophysical characterization, including the Aviv CD and DynaPro instruments.

email: info@wyatt.com