HORIBA Scientific

Application Note

Particle Size Analysis of Liposomes

AN174

Liposomes are bilayer vesicles made of phospholipids derived from natural or man-made materials. They are mainly used in the pharmaceutical field for treatment of cancers as carriers of chemotherapeutic drugs to the tumor area. The amount of drug loaded into the liposomes and the size of the liposomes play pivotal roles in the pharmacokinetic and pharmacodynamic parameters of the drug. Hence accurate and rapid measurement of the size of liposomes is essential for novel and effective drug delivery systems.

Introduction

Liposomes are sub-micron particles that are finding important applications in fields such as biotechnology (in applications like siRNA delivery, antibody delivery), cosmetology (emulsions and creams etc.) and the pharmaceutical industry (chemotherapeutic delivery, altering the PK/PD of drug). Liposomes are composed of phospholipids that have a polar end attached to a non-polar chain. When these phospholipids are introduced into an aqueous medium, they self assemble into bilayer vesicles with the polar ends facing the aqueous medium and non-polar ends forming a bilayer as shown in Figure 1.

In pharmaceutical applications the active molecule (drug) is usually incorporated into the liposome either into the hydrophilic pocket or sandwiched between the bilayers depending on the hydrophilicity/ lipophilicity of the drug.

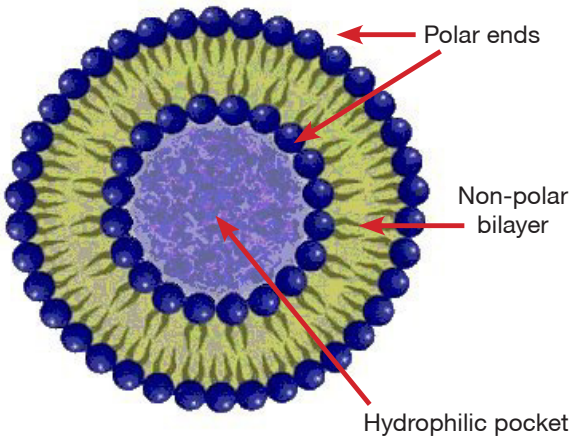

Figure 1: Basic structure of a liposome

Chemotherapeutics such as Doxorubicin and Paciltaxel have been used to treat cancers of various kinds for over two decades. The main disadvantage with these or any other chemotherapeutic drug is their inability to differentiate between a normal cell and a cancer cell. This leads to unwanted side effects such as loss of hair, stomach ulcers and loss in body weight. Drug delivery systems or targeting systems that would channel these and other therapeutics to the area of interest (tumors etc.) have garnered tremendous interest.

DOXIL, a reformulated version of Doxorubicin, was one of the first drugs to be approved that is delivered using a liposome. The Doxorubicin drug lies within the hydrophilic pocket of a liposome coated with PEG (polyethylene glycol) to evade detection and destruction by the immune system. The PEG coating improves the stability and lengthens the half life in circulation. A PEG coated liposome is often referred to as a sterically enhanced, or stealth liposome.

For solid tumors, liposomes are of particular interest because of their easy manipulation and optimum size. It has been known for some time that the tumor areas have leaky vasculature and poor lymphatic drainage. This can be used as a targeting tool with what has been described as Enhanced Permeation and Retention effect (EPR). This refers to the permeation and retention of molecules of sizes 50 to 150 nm in to the tumor area. Hence size measurement is at the center of liposomal drug delivery systems.

Liposome Size Analysis via Dynamic Light Scattering (DLS)

It is clear that rapid and accurate measurement of liposome size is important for the development of drug delivery systems in the pharmaceutical industry. The materials used for making the liposome are very expensive and only a small quantity is available for analysis. DLS instruments like the HORIBA SZ-100V2 are perfectly suited for liposome size measurement. Not only is very little liposome quantity needed, (about 700 uL sample at 0.01 mg/mL) but also the sample can be easily recovered. Measurement is very rapid with times ranging from 30 seconds to 10 minutes depending on sample nature.

Figure 2: HORIBA SZ-100V2 nanoparticle analyzer

The DLS technique is able to measure much smaller sizes than traditional static light scattering instruments, allowing the measurement of liposomes from below 20 nm up to 1 µm with a high degree of accuracy. The SZ-100V2 has a size range of 0.3 nm to 10 µm.

Liposome Measurement Considerations

Viscosity is one of the most important parameters for DLS. Usually aqueous media are the dispersed phase in liposomes but if the medium is an organic solvent, then it is important to measure the viscosity of the solution and incorporate it as part of the measurement. Viscosity directly affects the Brownian motion of nano-particles and thus the calculated liposome size result.

Another important aspect is the concentration of the sample. Concentrations in the range of 0.05 to 0.01 mg/mL are ideally suited for most liposome size measurements. Sample should be diluted if it is very concentrated (>0.1 mg/mL). If the concentration is below 0.01 mg/mL then an initial measurement should be made to check for the counts/sec. If the counts/sec is below 10 kilo counts/sec the results will not be accurate and the sample should be concentrated and measurement should be repeated. Another factor affecting the results is the presence of impurities or agglomerates. These may skew the results and sometimes show an increased liposome size rather than showing a distinct bimodal distribution of liposome and impurity. Hence it is important to always filter the samples before measuring.

SZ-100V2 Liposome Size Results

A small volume of liposome sample was purchased for analysis. The small volume necessitated using the 50 µL disposable microcell for particle size analysis. The measurement was made with the SZ-100V2 Nanoparticle Size Analyzer at the 90 degree sizing detector for 120 seconds. The reported Z-Average particle size was 138.6 nm.

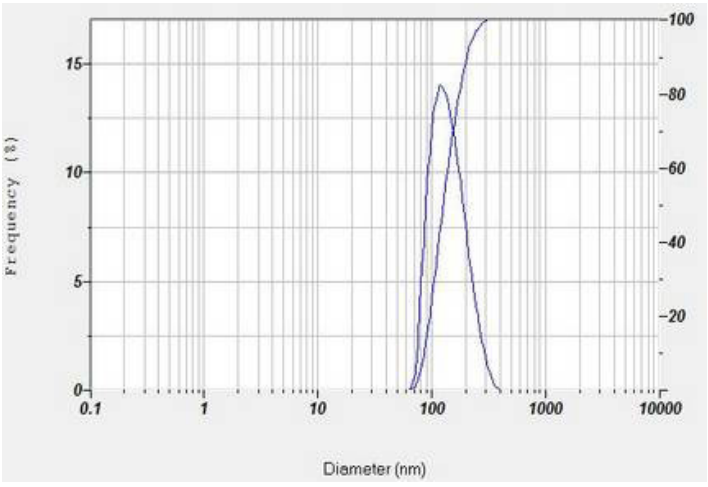

Figure 3: Particle size distribution for a liposomal material as measured by the SZ-100V2.

Scattering Angle	: 90
Temperature of the Holder	: 25.1 °C
Dispersion Medium Viscosity	: 0.893 mPa·s
Transmission Intensity before Meas.	: 32408
Distribution Form	: Standard
Distribution Form(Dispersity)	: Polydisperse
Representation of Result	: Scattering Light Intensity

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	137.6 nm	47.8 nm	112.0 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	137.6 nm	47.8 nm	112.0 nm

Cumulant Operations

Z-Average : 138.6 nm

Figure 4: Particle size results for the liposome.

Conclusions

The research of liposome vesicles for targeted drug delivery requires particle size analysis. The measurement of nano-sized particles such as liposomes may be accomplished with dynamic light scattering instruments like the HORIBA SZ-100V2. The ultimate goal is to provide some control over pharmacokinetic and pharmacodynamic parameters by monitoring the size of liposome vesicles.