Liposomes

Nicomp® DLS system and AccuSizer® SPOS system

Liposomes are spherical engineered particles made of phospholipids used in the pharmaceutical and cosmetic industries. The size and surface charge of liposomes are important characteristics which require measurement and monitoring. Dynamic light scattering (DLS) is the most common analytical technique used for measuring the size of submicron liposomes. Single particle optical sizing (SPOS) is used to measure the size of liposomes greater than one micron. The Entegris Nicomp® DLS system and AccuSizer® SPOS system are used in laboratories around the world for the accurate measurement of the size and charge (zeta potential) of liposomes.

INTRODUCTION

Liposomes are bilayer vesicles routinely used in the pharmaceutical industry as a drug delivery system for transport of chemotherapeutic drugs to the tumor area. They are composed of phospholipids which have a polar end attached to a nonpolar chain that self assembles into bilayer vesicles with the polar ends facing the aqueous medium and nonpolar ends forming a bilayer. In pharmaceutical applications the active pharmaceutical ingredient (API) is usually incorporated into the liposome, either into the hydrophilic pocket or sandwiched between the bilayers depending on the hydrophilicity of the API, see Figure 1.

One of the first drugs approved which is delivered using a liposome is Doxil, a reformulated version of doxorubicin. The doxorubicin drug lies within the hydrophilic pocket of a polyethylene glycol (PEG) coated liposome. The PEG coating helps to evade detection and destruction by the immune system, improves the stability and lengthens the half-life in circulation. Other applications for liposomes include uses in fields such as biotechnology (siRNA delivery, antibody delivery) and cosmetology (emulsions and creams etc.).

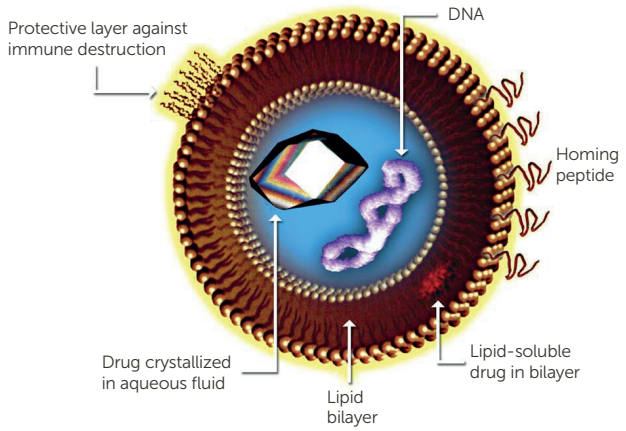

Figure 1. Liposome for drug delivery.

Figure 2. The Nicomp N3000 DLS system.

Liposomes can be classified according to their lamellarity (uni- or multi-lamellar vesicles), size (small, large, or giant) and preparation method. The major types of liposomes are the small unilamellar vesicle (SUV), small multilamellar vesicle (SMV), multilamellar vesicle (MLV), large unilamellar vesicle (LUV), and the giant multilamellar vesicle (GMV).

The size of the liposomes, and the amount of drug loaded into 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.

Most liposomes are submicron (~20 – 250 nm) and the preferred particle size analysis technique is dynamic light scattering (DLS) such as the Entegris Nicomp system, Figure 2. Some novel larger GMV liposomes are too large for DLS analysis (>5 µm) and can be measured using the Entegris AccuSizer single particle optical sizing (SPOS) system, Figure 3.

Figure 3. The AccuSizer SPOS system with SIS sampler.

SIZE MEASUREMENTS DURING PROCESSING

Both the Nicomp and AccuSizer are used to accurately measure the size of liposomes during the manufacturing processes such as extrusion through membranes.[1] The first set of results, shown in Figures 4 through 6, show the size results from the Nicomp DLS system as the liposomes are extruded through membrane filters of decreasing size.

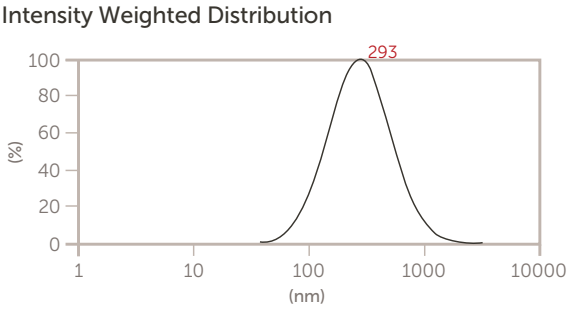

Figure 4. Liposome size before extrusion.

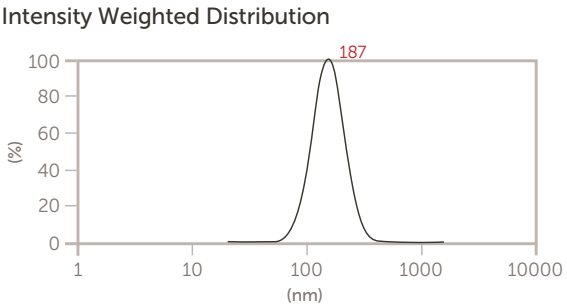

Figure 5. Liposome size after extrusion through 0.4 µm membrane three times.

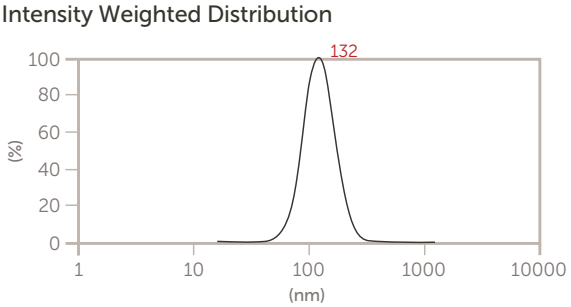

Figure 6. Liposome size after extrusion through 0.1 µm membrane three times.

Giant multilamellar vesicle (GMV) liposomes were manufactured using a sugar-doped lipid film hydration process. The size was then reduced using centrifugation and extrusion through membrane filters. The change in liposome size was monitored using the AccuSizer SPOS system as seen in Figures 7 through 10.

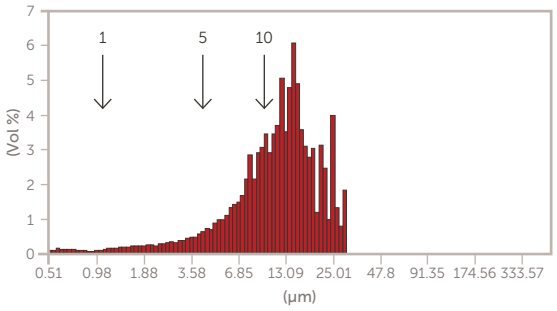

Figure 7. GMV liposome before centrifugation.

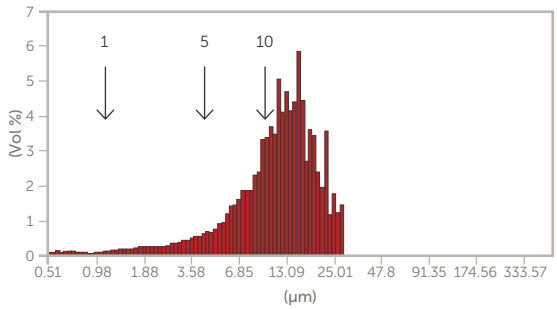

Figure 8. GMV liposome after centrifugation.

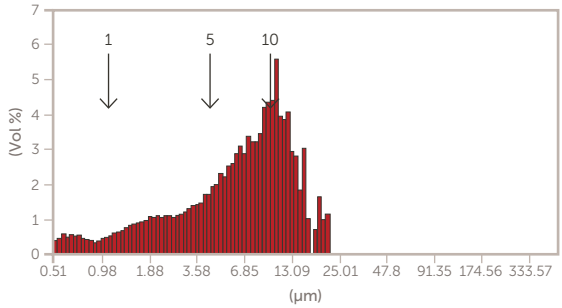

Figure 9. GMV liposome after extrusion through 5 µm filter.

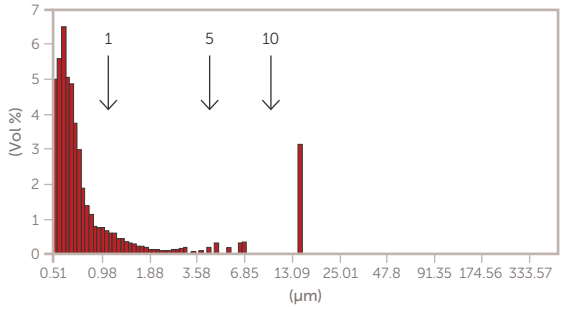

Figure 10. GMV liposome after extrusion through 10 µm filter.

CATIONIC COATED LIPOSOMES

Liposomes composed of the cationic lipid DOTAP (N-[1- (2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl-sulfate), have been shown to be an effective carrier for anionic RNA and DNA nucleotides. Cationic liposomes provide advantages that include high encapsulation efficiency of nucleotides and high cellular uptake due to the overall cationic electrostatic charge on the lipid bilayers, To prevent the aggregation induced by serum, cationic liposomes have been PEGylated to increase circulation lifetime, and allow the accumulation in tumor tissue.

Cationic liposomes created and studied at UC Davis[2] contain the cationic lipid DOTAP for the encapsulation of micro RNAs. The size of the liposomes is critical because eventually these are injected intravenously into mice. Therefore, the final size should not be much greater than around 100 nm. The size result for these cationic coated liposomes is shown in Figure 11.

Gaussian Summary

Mean diameter	132.5 nm
Standard deviation	44.0 nm (33.2%)
Normalized standard deviation (Coeff. of Var'n)	0.332
Variance (P.I.)	0.110
Chi squared	0.577
Baseline Adj.	0.000%
Z-Avg. Diff. Coeff	3.51E-008 cm²/s

Intens-WT Gaussian Distribution

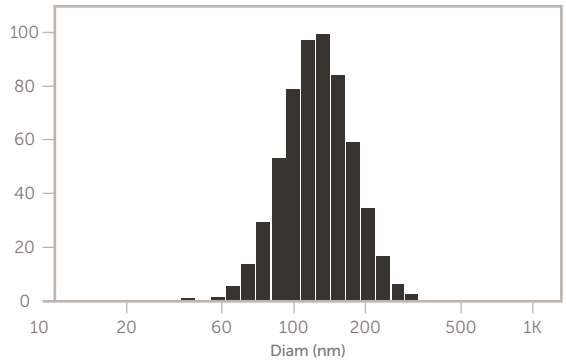

Figure 11. Cationic coated liposome size result.

LONG CIRCULATION LIPOSOMES

Another liposome studied in the Ferrara Lab at UC Davis[2] is labeled with ^{64}Cu to act as a nanotracer to improve the visualization of head and neck tumors via Positron Emission Tomography (PET).[3] This liposome is a particular formulation, a standardized combination of HSPC/cholesterol/DSPE-PEG2K, which creates a highly stable, long-circulating liposome (LCL) that is good for a number of different applications. In this formulation, a molar ratio of 55.5:39:5.0 mol/mol/mol of HSPC/cholesterol/DSPE-PEG2K was used, and then functionalized with 6-BAT-PEG-lipid for 64Cu radiolabeling. ^{64}Cu liposomes accumulate in various cancers and provide both a sensitive tracer and an indication of the biodistribution of nanotherapeutics.

The size of the ^{64}Cu LCL liposome is shown in Figure 12.

Gaussian Summary

Mean diameter	122.8 nm
Standard deviation	16.2 nm (13.2%)
Normalized standard deviation (Coeff. of Var'n)	0.132
Variance (P.I.)	0.017
Chi squared	0.173
Baseline Adj.	0.078%
Z-Avg. Diff. Coeff	3.78E-008 cm²/s

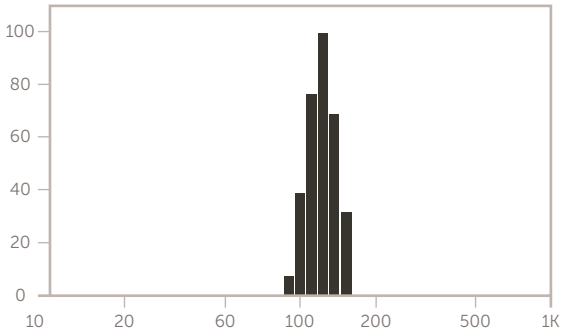

Figure 12. ^{64}Cu labeled LCL liposome size result.

TEMPERATURE SENSITIVE LIPOSOMES

Additionally, temperature-sensitive liposomes have been formulated to enhance the thermal induced release of these particles' contents at a particular target site. In one study, a pH-sensitive complex between doxorubicin (Dox) and copper (CuDox) in the core of lysolipid containing temperature-sensitive liposomes (LTSLs) was formed.[4] These liposomes were composed of DPPC:DSPE-PEG2k:MPPC (86:4:10, molar ratio) where DPPC is 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine, DSPE-PEG2k is 1,2 distearoyl-sn-glycero-3-phosphoethanolamine-N-Methoxy polyethyleneglycol-2000, and MPPC is 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine. Copper TEA liposomes (Copper (II) gluconate, triethanola-mine (TEA) were separated from nonencapsulated copper TEA to induce a salt gradient across the liposomal membrane. The size of the MPPC-Copper TEA liposomes is shown in Figure 13.

Gaussian Summary

Mean diameter	139.3 nm
Standard deviation	30.8 nm (22.1%)
Normalized standard deviation (Coeff. of Var'n)	0.221
Variance (P.I.)	0.049
Chi squared	2.059
Baseline Adj.	0.000%
Z-Avg. Diff. Coeff	3.34E-008 cm²/s

Intens -WT Gaussian Distribution

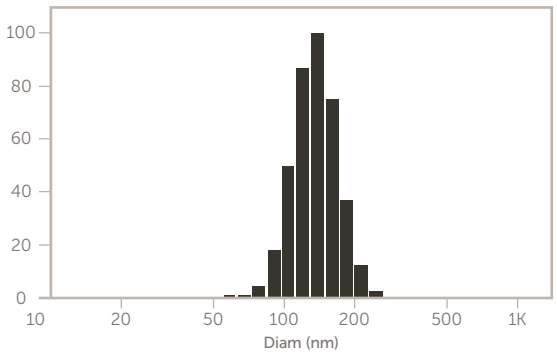

Figure 13. MPPC-Copper TEA liposomes.

Once these liposomes have been prepared and the size is verified, they are loaded with the therapeutic drug doxorubicin using the TEA gradient-doxorubicin goes into the liposome as TEA comes out.

ZETA POTENTIAL OF ZR-89 LABELED LIPOSOMES

In another study[5], Zr-89 labeled liposomes were created to evaluate the pharmacokinetics of long-circulating liposomes over one week. The radioactivity is sequestered in the hydrophilic interior cavity, in the lipid bilayer or on the surface of the liposome. The liposomes in this study were measured on the Nicomp DLS system for size distribution, with values ranging from 114-120 nm. The zeta potential was also measured on the Nicomp using the phase analysis light scattering mode (PALS) and the dip cell. Measurement settings included applying a 12 V/cm electric field across the 0.4 cm gap between electrodes and using the Smoluchowski limit. The results from multiple measurements are shown in Figure 14.

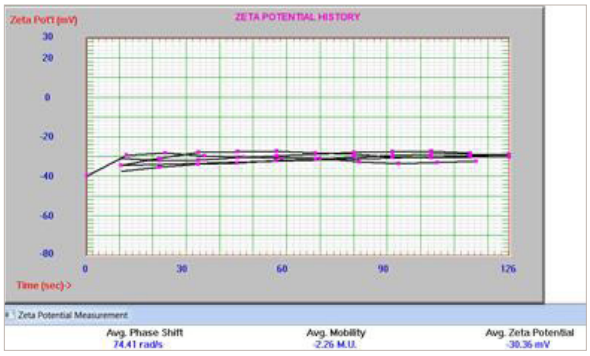

Figure 14. Zeta potential results for NH2-Peg2K liposomes.

Note the extremely consistent results across repeated measurements.

References

[1] Aoki, N. & Hashimoto, M., Hashimoto Electronic Industry CO. and Yoshimura, T. Liposome Engineering Laboratory, *Measurement of Liposome Size Distribution Using Nicomp 380 and AccuSizer 780 AD,* presentation, July 2013

[2] Thanks to Elizabeth Ingham, Azadeh Kheirolomoom and Jai Seo from the Dr. Katherine Ferrara Lab in the Department of Biomedical Engineering at UC Davis for sharing these data and helping to create this document

[3] Mahakian, L. et.al., *Comparison of PET Imaging with 64Cu-Lipsomes and 18F-FDG in the 7,12-Dimethylbenz[a] anthracene (DMBA)-Induced Hamster Buccal Pouch Model of Oral Dysplasia and Squamous Cell Carcinoma,* Mol Imaging Biol (2013)

[4] Kheirolomoom, A. et.al., *Complete regression of local cancer using temperature-sensitive liposomes combined with ultrasound-mediated hyperthermia,* Journal of Controlled Release, 172 (2013)

[5] Seo, J. et.al., *The pharmacokinetics of Zr-89 labeled liposomes over extended periods in a murine tumor model,* Nuclear Medicine and Biology 42 (2015)

FOR MORE INFORMATION

Please call your Regional Customer Service Center today to learn what Entegris can do for you. Visit entegris.com and select the Contact Us link to find the customer service center nearest you.

TERMS AND CONDITIONS OF SALE

All purchases are subject to Entegris' Terms and Conditions of Sale. To view and print this information, visit entegris.com and select the Terms & Conditions link in the footer.

Entegris

Corporate Headquarters
129 Concord Road
Billerica, MA 01821
USA

Customer Service
Tel +1 952 556 4181
Fax +1 952 556 8022
Toll Free 800 394 4083