WYATT TECHNOLOGY

APPLICATION NOTE

AN8006: In-line monitoring of liposome size by RT-MALS

Introduction

In liposome and lipid nanoparticle (LNP) drug-delivery applications, particle size is a critical quality attribute (CQA) that impacts bioaccessibility, retention time and biodistribution. Microfluidization is a common method for large-scale production of liposomes within a well-defined size range. The resulting particle size is dependent on a number of process parameters, such as chamber pressure and temperature, which can drift over the course of time.

Current on-line and off-line control methods do not sample the entire lot, and only provide average sizes, potentially hiding pockets of out-of-spec material. Real-time multi-angle light scattering (RT-MALS) ensures batch consistency by in-line monitoring for size of the entire batch; the collection stream can be diverted to waste if the particle size falls out of specification.

Materials and Methods

Crude liposome solution containing heterogeneous liposomes with sizes from 150 nm to 800 nm was passed through a microfluidizer. The internal chamber pressure was initially held at 8,000 psig, then changed to 11,000 psig after 25 minutes to simulate an undesirable drift in the manufacturing process. Upon exiting the chamber, the solution passed through the ultraDAWN™ RT-MALS instrument at a flow rate of 4 mL/min, resulting in a lag time (RTD, residence time delay) of just 22 seconds between the time product exits the chamber and time of measurement. OBSERVER™ software was programmed to acquire ultraDAWN data, measure z-average radius 30 times per minute and trigger a diversion of the exit stream if particle size deviated by 2 nm from the nominal CQA value.

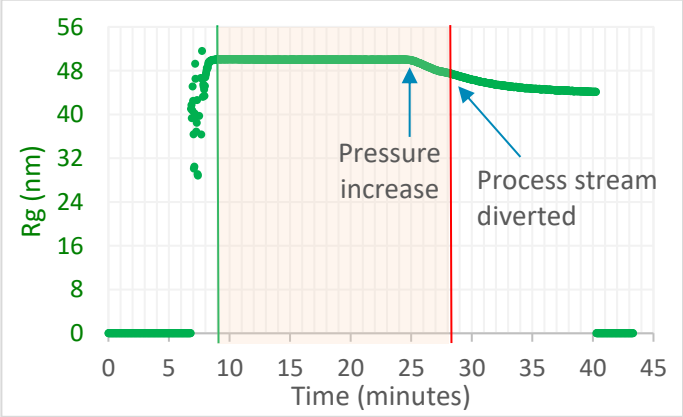

Figure 1. Trace in OBSERVER software of weight-average molar mass measured by ultraDAWN, indicating the achievement of the desired process endpoint.

Results and Discussion

Liposome R_g was observed to be 50 nm, constant to within 0.5%, as long as the chamber pressure was maintained at 8,000 psig. The size decreased when the internal chamber pressure was increased to 11,000 psig. OBSERVER successfully triggered diversion of the process stream when the size decreased below 48 nm. Using the standard procedure of final QC testing alone, the entire batch would have been discarded; with RT-MALS-based

monitoring and control, only about half the batch was discarded and the rest, which was collected, met specification.

In addition, flagging the issue led to re-calibration of the chamber prior to running the next batch, avoiding further losses.

Conclusions

The addition of in-line RT-MALS provided substantial value to this liposome production process, reducing waste of precious product and alerting productions staff to the need for system maintenance.