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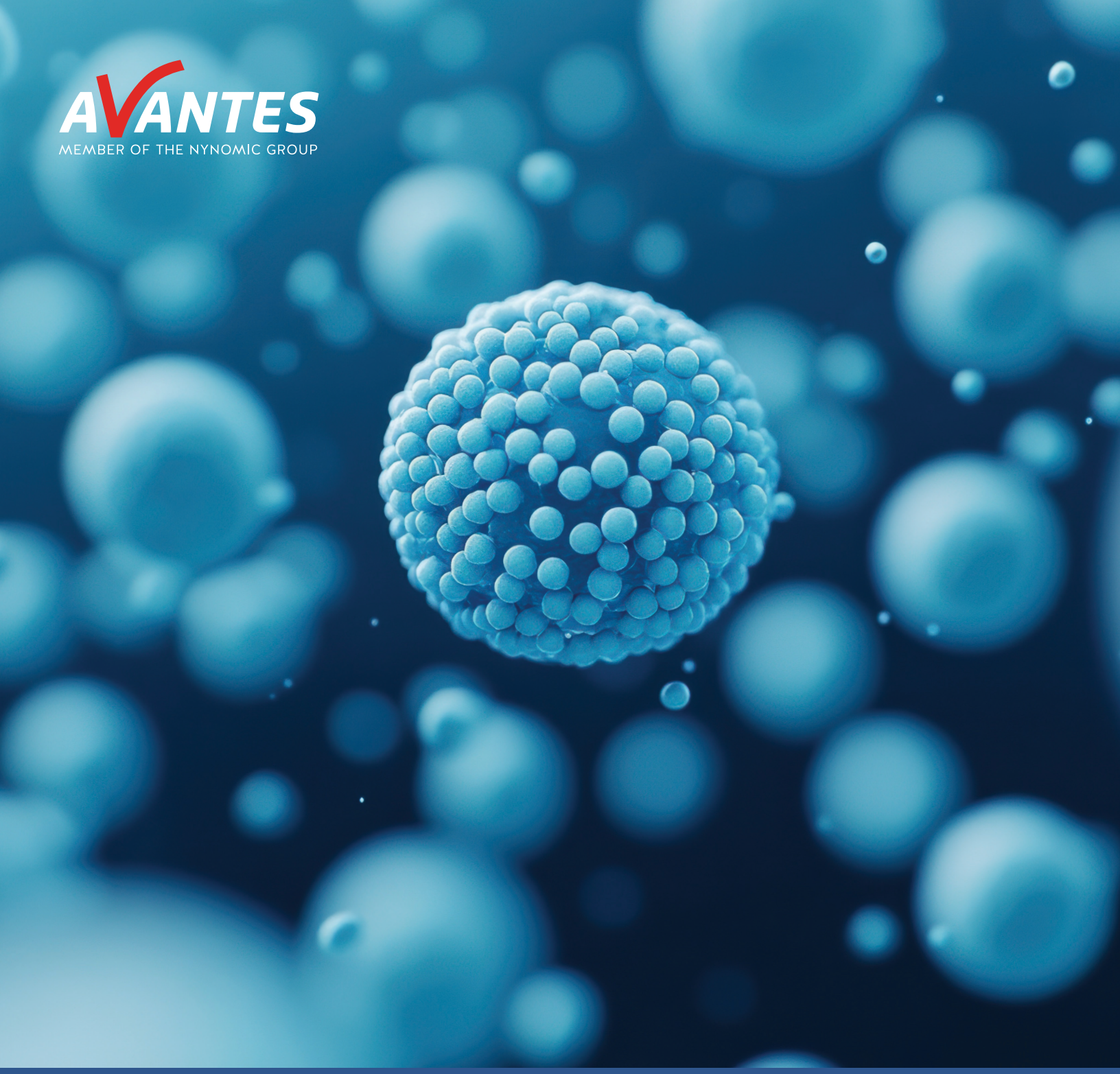

APPLICATION NOTE

REAL-TIME MONITORING IN CONTINUOUS MANUFACTURING OF LIPOSOMAL NANOPARTICLES

INTRO

REAL-TIME MONITORING IN CONTINUOUS MANUFACTURING OF LIPOSOMAL NANOPARTICLES

Liposomal nanoparticles are lipid-based drug delivery systems approved globally for clinical use in cancer therapies, vaccines (such as mRNA COVID-19 vaccines), and immunotherapies. Despite their success, recent findings—like those from the EMA COVID-19 data leak—highlight the need for improved control of critical quality attributes, including RNA stability and particle size, to ensure product safety and efficacy.

To address these challenges, the FDA and University of Connecticut have developed a GMP-compliant continuous manufacturing platform that incorporates real-time process analytical technologies (PAT). This system enables precise, in-process control of key parameters such as particle size, lipid concentration, and drug encapsulation, using tools like UV-Vis spectrometers paired with predictive algorithms.

A core component of this system is an Avantes UV-Vis spectrometer integrated with a multivariate predictive algorithm, enabling accurate, continuous monitoring of drug encapsulation levels. Following this stage, the material undergoes bioburden reduction and purification before final quality testing and readiness for fill-finish operations. This end-to-end approach supports scalable, efficient, and high-quality production of complex nanoparticle formulations.

Using this continuous system, liposomal nanoparticles were not only successfully formed, but also underwent buffer exchange, concentration, and remote drug loading—streamlined into a single, integrated unit operation.

BACKGROUND

Lipid-based nanoparticles, such as liposomes and lipid nanoparticles (LNPs), (figure 1) are widely used drug delivery systems designed to improve therapeutic outcomes. Liposomes consist of a lipid bilayer surrounding an aqueous core, and most approved formulations include phosphatidylcholine, cholesterol, and either a pegylated or charged lipid[3].

Globally, liposomal nanoparticles are approved for a range of clinical uses, particularly in anti-cancer therapies. These typically encapsulate small-molecule drugs like doxorubicin, daunorubicin, cytarabine, and irinotecan, offering advantages such as reduced side effects and longer circulation times. By delivering high drug payloads directly to target sites, such as tumors, liposomes can enhance both efficacy and safety compared to conventional treatments. However, scalable manufacturing of these systems remains a significant challenge[2].

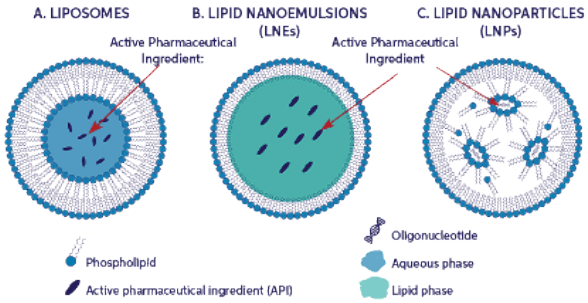

FIGURE 1 Overview of the different classes of lipid-based nanoparticles

For example, doxorubicin-loaded liposomes are used to treat ovarian cancer, AIDS-related Kaposi's sarcoma, and multiple myeloma. These formulations, which typically consist of a single lipid bilayer encapsulating a doxorubicin nanocrystal, help extend circulation time and reduce cardiotoxicity. Developing advanced manufacturing systems to produce these nanoparticles may enable new liposomal drug products carrying one or more small-molecule payloads[2].

Lipid nanoparticles also played a critical role in addressing the COVID-19 pandemic through the development and global rollout of mRNA vaccines. These vaccines use mRNA encapsulated in lipid nanoparticles (mRNA-LNPs) and have shown high efficacy and strong protection, positioning mRNA-LNPs as a promising platform for future vaccines and therapeutics.

mRNA-LNPs are composed of four main components: (1) an ionizable cationic lipid, (2) one or more neutral/structural lipids, (3) the mRNA payload, and (4) buffering systems and water. Together, these elements form a tunable delivery vehicle designed to transport mRNA to specific cells, where it can trigger protein expression for therapeutic effect[3].

Manufacturing liposomal formulations like doxorubicin-loaded nanoparticles requires exceptional process control due to their complexity and sensitivity to formulation variables. Achieving consistent particle size and drug encapsulation is essential for therapeutic performance and patient safety, requirements that are difficult to meet with traditional batch processes. As noted by former FDA Commissioner Dr. Scott Gottlieb and CDER Director Dr. Janet Woodcock, "One of today's most important tools for modernizing the pharmaceutical industry is a process known as continuous manufacturing." This approach helps bridge the long-standing gap between quality assurance and manufacturing efficiency.

PROJECT OBJECTIVES – NEW APPROACH FOR COMMERCIAL BASED SCALE MANUFACTURING OF LIPID BASED NANOPARTICLE FORMULATION

In a six-year collaborative effort between the FDA and the University of Connecticut, a novel approach to commercial-scale manufacturing of lipid-based nanoparticle formulations was developed[1]. This foundational work led to the development of DIANT Pharma's scalable, GMP-compliant continuous manufacturing system, purpose-built for producing complex nanoparticle therapeutics with precision and efficiency.

At the heart of this system is an ethanol injection method using a customized turbulent jet in co-flow, which enables the formation of liposomal nanoparticles with tight control over critical quality attributes such as particle size, polydispersity, and drug loading[2]. To support this level of precision, the platform integrates advanced Process Analytical Technologies (PAT), (figure 2) including:

- (PAT 1) Online particle size analysis (InProcess LSP)
- (PAT 2) Turbidity-based lipid concentration monitoring (Optek)
- (PAT 3) UV-Vis spectroscopy (Avantes) for real-time drug encapsulation tracking

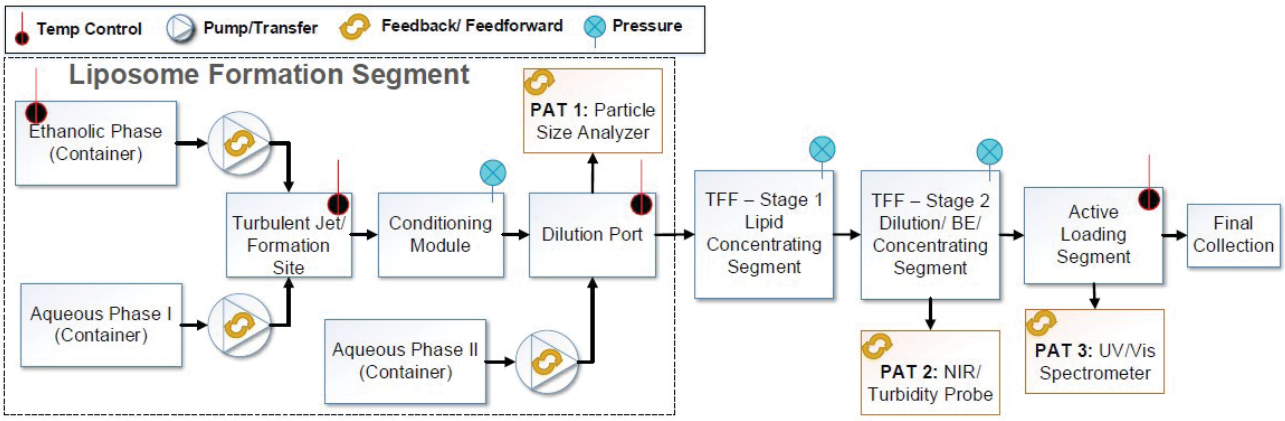

FIGURE 2: System block-diagram outlining key areas in the process from nanoparticle formation to drug loading (final product), ready for fill/ finish[1].

Why This Matters:

Consistency, Efficiency, and Safety: Continuous processing ensures robust and repeatable manufacturing, resulting in safer, more reliable drug formulations.

Scalability on Demand: The system's design allows rapid scale-up to meet rising demand—crucial in public health emergencies like pandemics.

Real-Time Quality Control: Integrated PAT tools minimize waste and reduce batch failures by continuously monitoring product quality throughout production.

ROLE OF AVANTES UV/VIS SPECTROSCOPY

An Avantes UV-Vis spectrometer, integrated with a multivariate predictive algorithm, plays a critical role in determining doxorubicin encapsulation efficiency in real time[1] (Figure 3). Positioned inline as part of the system's process analytical technology (PAT) suite, the spectrometer provides high-sensitivity, repeatable measurements of drug concentration throughout the active loading stage. Its seamless integration with other PAT tools enables a dynamic feedback loop that ensures process parameters remain within specification[2]. By detecting deviations rapidly, the system can maintain tight control over encapsulation, ensuring each nanoparticle batch meets therapeutic targets. This real-time insight eliminates the need for time-consuming offline testing, allowing the material to move directly into bioburden reduction and purification. Once processed, the drug substance is stored and ready for the fill line following final quality checks. Overall, the spectrometer has significantly accelerated development timelines and improved production reliability, making it a key enabler of efficient, high-quality continuous manufacturing.

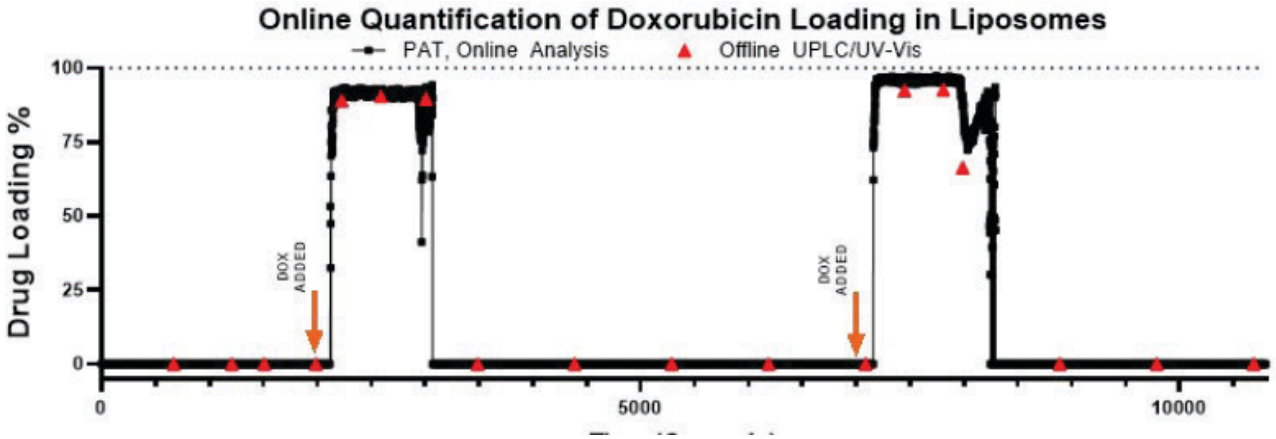

FIGURE 3: Example of Data from drug concentration and drug encapsulation measurement[1].

The instrument used by DIANT Pharma and the University of Connecticut was the AvaSpec_ Mini4096CL which has been replaced by the superior AvaSpec-Nexos 4096 (see image below) which features the same detector, standard replaceable slit, superior electronics, stray light rejection, signal to noise and spectral resolution. For more information please reach out to an Avantes Sales Engineer at info@avantes.com or infousa@avantes.com

IMPACT AND CONCLUSION

The continuous processing system, when integrated with powerful online and inline process analytical technology, enables faster development for future drug products. This novel continuous manufacturing platform features real-time process control strategies, rapid assessment of process parameters and material attributes, and ensures adequate control over product quality and performance. It also offers flexibility to scale production by extending run times, allowing manufacturers to better respond to shifts in demand.

Specifically, continuous processing for downstream applications—such as the production of drug delivery vehicles like liposomes—enhances control over the physical properties of nanoparticles. The high-throughput system developed at UConn exemplifies this approach, with the potential to connect directly to an API processing stream for a fully integrated, end-to-end continuous manufacturing solution. To date, the team has demonstrated a successful 3-hour controlled run, producing tens of liters of doxorubicin-loaded liposomes that meet RLD specifications using a lab-scale system.

Overall, continuous processing presents significant advantages, including cost savings, reduced processing time and footprint, fewer batches, and the potential for higher quality drug products—all contributing to a more efficient and economical approach to pharmaceutical manufacturing.

REFERENCES

1) U.S. Food and Drug Administration. (2021). Continuous manufacturing of liposomes and lipid nanoparticles. FDA Science Forum. https://www.fda.gov/media/149013/download

2) Costa, A. (2020, April 28). Continuous processing of nanoparticles: An emphasis on liposomal formulations. American Pharmaceutical Review. https://www.americanpharmaceuticalreview.com/Featured-Articles/563719-Continuous-Processing-of-Nanoparticles-An-Emphasis-on-Liposomal-Formulations/

3) DIANT Pharma Inc. (2024, April 25). Continuous processing and enhanced control of lipid-based nanoparticles: LNPs and liposomes. https://diantpharma.com/continuous-processing-and-enhanced-control-of-lipid-based-nanoparticles-lnps-and-liposomes/

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