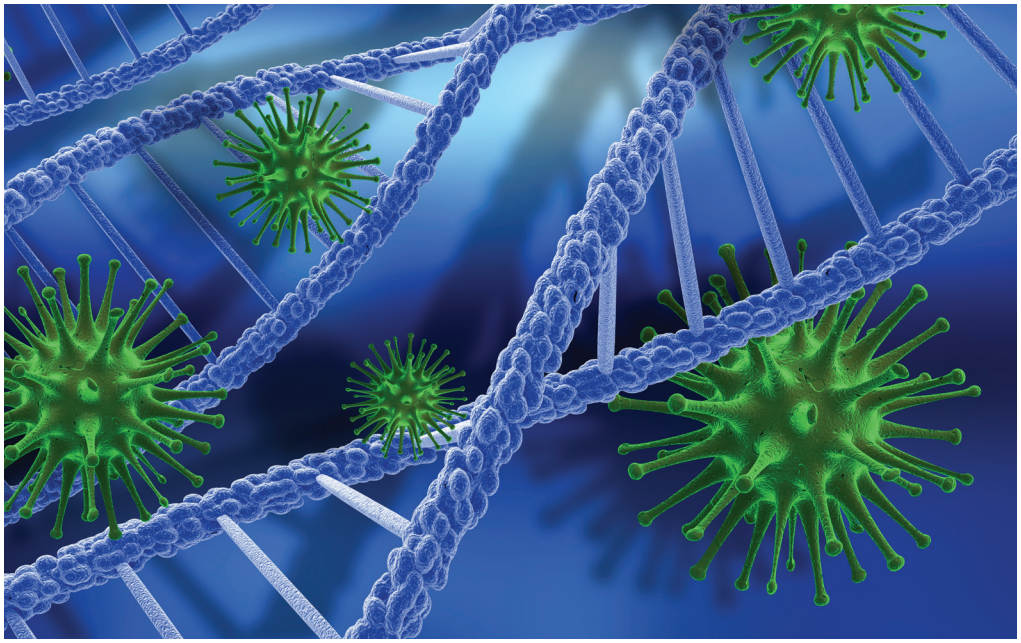
Application Note

PENDOTECH
Leading Process Analytics

Optimize Viral Vector Production

With Real-time UV Monitoring

Viral vector-mediated gene therapy is a technique that uses modified viruses to deliver genetic material into cells to treat or prevent disease.. While viral vectors have high transduction efficiency and stable expression, the process of production and separating empty and full capsids pose challenges to pharmaceutical industries. The METTLER TOLEDO Pendotech PM2 photometer utilizes UV LEDs, providing efficient and reliable measurements of bioprocess fluid streams during processing. In-line UV absorbance sensors integrated with PM2 transmitters can help optimize viral vector production. These solutions represent a significant advancement in viral vector production, promising more efficient and effective gene therapies in the future.



METTLER TOLEDO

Background

Gene therapy is a therapeutic approach that modifies the genes of an individual to treat a specific disease. Its success relies on an effective delivery system. One of the primary benefits of using viral vectors is that they can be modified to remove their pathogenicity. These modified vectors are widely used as a delivery system due to their natural high transduction efficiency and stable expression. Gene therapies based on viral vectors have shown significant progress over the years, with promising results in clinical trials. These therapies can either be in vivo or ex vivo. The three primary viral vector strategies are adeno-associated viruses (AAV), adenoviruses, and lentiviruses [1]. The process involves the delivery of therapeutic genes into target cells and the expression of desired proteins. These proteins can correct a defect or provide missing proteins. This method is being utilized to treat or silence an unwanted gene associated with a harmful factor.

Process

Recombinant viruses are grown under stringent sterile conditions. Their growth is monitored controlled using in-line single-use sensors. The usage of single-use closed bioprocessing systems is on the rise for the production of viral vectors. It provides the advantage of reduced risk of cross-contamination and meets the need of maintaining a sterile environment. Also, integration in automated systems helps in real-time monitoring and control of culture conditions that ensure optimum growth [2]. Recombinant AAV (rAAV) or viral vector manufacturing has made significant progress in recent years; however, there are still substantial obstacles to overcome. Separating full and empty viral capsids remains a challenge, but it is crucial for achieving high yields of functional vectors [3].

UV photometers have been used for decades to detect proteins in post-column elution streams, allowing for automated product pooling and maximized yields. The first generation of UV photometers utilized mercury vapor lamps with broad emission spectra and narrow-bandpass optical filters to select specific wavelengths. UV absorbance at a wavelength of 280 nm detects viral capsids as they primarily consist of protein shells. However, conventional UV absorbance measurements at this wavelength are not precise enough to distinguish between empty and full capsids. On the other hand, nucleic acids absorb at 260 nm, making A260 measurement a useful tool to identify only full capsids. A280 measurement represents the total number of capsids, including both empty and full ones, but not the number of full capsids specifically. Hence, A260/A280 ratios provide a precise approach to determine whether elution peaks primarily indicate empty or full capsids.

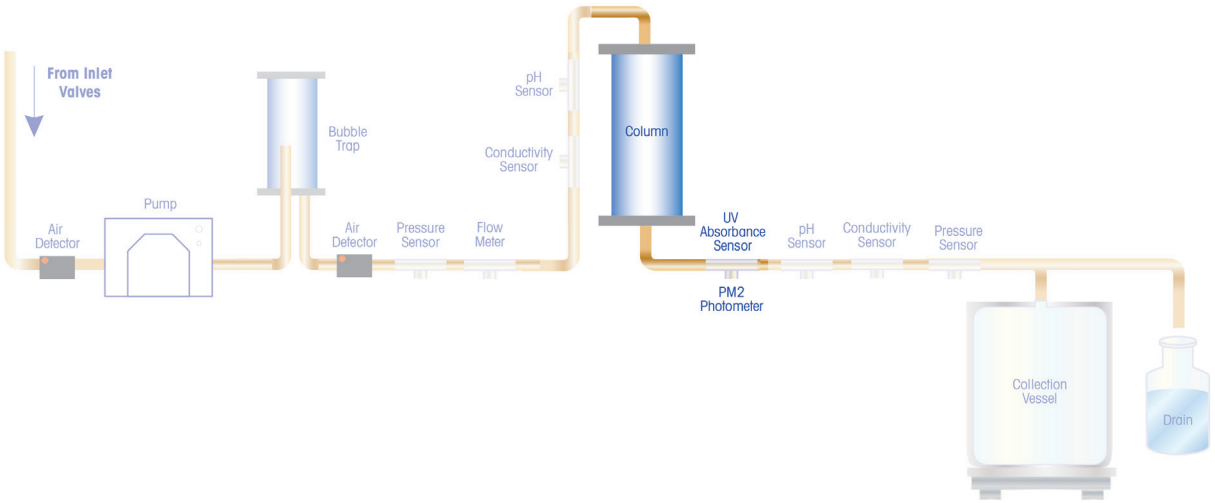


Fig. 1 Process image for chromatographic separation of capsids

Problem

Legacy UV sensors with mercury vapor lamps are problematic because they are large, have a long warm-up period, and can damage optical components over time. These mercury lamps have a strong output peak at 254 nm, a wavelength known to break down chemical bonds in nucleic acids and proteins. While this mechanism is useful for UV sterilization, it can also damage biological products passing through a flow cell, particularly if flow rate is low.

New, alternative light sources have been introduced to overcome issues related to mercury lamps. Ultraviolet light-emitting diodes (UV LEDs) have become popular in the last 30 years due to their compact size, low power consumption, long lifespan, and instant on-off capability. LEDs with narrow bandwidth centered at or near a wavelength of interest, effectively address the limitations of traditional UV-filter photometers. By selecting suitable UV LEDs, it is possible to limit process exposure to harmful UV radiation while maintaining strong output at the desired wavelengths, resulting in excellent signal strength.

UV LEDs can be directly integrated into a transmitter due to their small size and low power consumption, eliminating the need for the lamp and detectors to be mounted on the flow cell. OUnlike traditional mercury-vapor lamps, UV LEDs do not require a warm-up time and can complete a measurement cycle in as little as 100 milliseconds. Two different wavelength UV LEDs can be used with alternating measurement cycles for dual-wavelength measurement. Additionally, UV LEDs are highly stable, providing a long service life with minimal drift, and do not damage products or degrade system components over time. These features make UV LEDs a simple yet powerful tool for viral vector manufacturing, especially in addressing limitations related to the effective separation of empty and full rAAV capsids.

Mettler Toledo Pendotech Solution

UV absorbance measurements with PM2 photometer

METTLER TOLEDO Pendotech Single-Use UV Flow Cells, along with the compact PM2 photometer provide an efficient way to collect data from bioprocess fluid streams during processing with minimal disruption compared to offline measurements. The flow cell is connected to the PM2 system via fiber optic cables, which can be integrated directly with the flow cell holder or optical couplers. The PM2 photometer is a versatile instrument suitable for lab and process applications and is available in benchtop or panel mount versions. Additionally, it can be pre-configured at the factory with seven different wavelength combinations, including 260 nm, 280 nm, 300 nm, 880 nm, 260-280 nm, 280-300 nm, and 280-880 nm. This technique provides the benefit of measuring spectra at two distinct wavelengths in a binary mixture, making it particularly useful for separating capsids. It is capable of accurately identifying both proteins and nucleic acids in a given sample by taking absorbance measurements at 280 nm and 260 nm, respectively. The resulting ratio can be used to determine the purity of the solution.

Fig. 2 METTLER TOLEDO Pendotech's PM2 photometer with transmitter and single-use flow cell

Conclusion

Gene therapy is a promising therapeutic approach that holds great potential for treating specific diseases. With the development of efficient carrier delivery systems, such as viral vectors, and decades of research, it has shown promising clinical outcomes. Mettler Toledo Pendotech's cutting-edge technology offers accuracy, reliability, and ease of use in monitoring and controlling the production of gene therapy products. Our UV absorbance PM2 photometer is designed to be user-friendly and compatible with a wide range of applications. It is specifically designed to provide accurate and reliable measurement of UV absorbance, enabling efficient separation of capsids, andresulting in high yields.

References

[1] X. Li, Y. Le, Z. Zhang, X. Nian, B. Liu, and X. Yang, "Viral Vector-Based Gene Therapy," International Journal of Molecular Sciences 2023, Vol. 24, Page 7736, vol. 24, no. 9, p. 7736, Apr. 2023, doi: 10.3390 IJMS24097736.

[2] "Closed and Aseptic Processes for Cell Therapy Manufacturing." Accessed: May 23, 2024. [Online]. Available: https://cellculturedish.com closed-aseptic-processes-cell-therapy-manufacturing/

[3] Modern Sensor Technologies for Viral-Vector Manufacturing- Improving Ultraviolet Photometric Detection of Full and Empty Adenoassociated Virus Capsids, Joe Hewitt, October 2023, 21(10)si BioProcess Sponsored International

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