Application Note

PENDOTECH
Leading Process Analytics

Augmenting Protein Harvest

With Single-Use Sensors for Chromatography

Capture chromatography, the initial step in protein purification, aims to selectively and swiftly capture the target protein using a specialized resin. The process of isolating and concentrating the protein makes it easier to obtain the starting material required for the subsequent purification steps, which results in a higher yield of purified protein. To ensure a high-quality yield, it's vital to optimize the chromatography step. PendoTECH Mettler Toledo offers a suite of single-use sensors to monitor and control the entire process, facilitating cost-effective optimization for maximum yield.

METTLER TOLEDO

Background

Chromatography separates molecules by impeding their movement through the solvent, using physical characteristics for fractionation. Column chromatography is the most popular method, utilizing a stationary phase-filled tube and a mobile phase. Chromatography offers high resolution, scalability, and reproducibility, making it the primary purification tool in the biopharmaceutical industry [1]. As chromatography requires specialized resins, it can be a costly process. However, the resins are highly specific, owing to stringent quality control measures used during manufacturing. Such resins are manufactured using specialized materials that must meet strict standards, requiring significant research and development efforts [2]. Despite the cost, chromatography resins are essential in scientific research, diagnostics, and biopharmaceutical production, and scientists are continuously finding ways to optimize their performance while managing costs.

Challenges:
In chromatography, several critical parameters must be monitored and controlled at the inlet and outlet of the chromatographic column to ensure optimal performance and separation [3]. These parameters include air detection, flow rate, UV absorption, pressure, conductivity, and pH. Regular monitoring and adjustment of these parameters can help identify and resolve any issues that may arise during chromatography in biopharmaceutical production.

Air detector: Air bubbles in the chromatography column can alter retention times, reduce resolution, and interfere with the separation and detection of target molecules. Minimizing bubble formation and removing any bubbles that form during chromatography is crucial to maintain optimal separation conditions.

Flow rate: The flow rate of the sample into the column is a critical parameter that can affect the separation efficiency and resolution. A high flow rate can lead to loss of resolution, while a low flow rate can result in poor recovery. In addition, accurate flow rate measurement is critical for controlling in-line buffer dilution ahead of the column.

UV Absorption: UV280 absorbance measurement is a crucial aspect of chromatography applications, especially in protein processing. During protein elution from the column, UV sensors direct the flow of liquid into the collection vessel. Once the complete protein has eluted, the UV sensor senses this and signals for the liquid to be discarded. By measuring UV280 absorbance, it is possible to determine protein concentration, monitor column elution, optimize protein capture, assess purity, and monitor column performance. Overall, the use of UV sensors in bioprocessing chromatography is critical in achieving high-quality protein products, making it an essential component of bioprocessing operations.

Pressure: Pressure is a critical parameter used to monitor and control the chromatography process. It helps ensure column integrity, detect clogging or packing issues, and prevent equipment damage. Pressure is also monitored during sample loading and elution to prevent overloading, which can cause pressure spikes and compromise the process integrity, resulting in product loss.

Conductivity: Conductivity is used to detect eluted fractions, assess column packing and equilibration, monitor buffer gradients, and evaluate column cleaning and regeneration effectiveness. Conductivity profiles of different biomolecules and contaminants aid in the collection of specific components, while stable conductivity values indicate proper column packing and equilibration. Monitoring buffer gradients ensures optimal separation conditions. Changes in conductivity during cleaning and regeneration may indicate the presence of residual contaminants or cleaning agents, requiring further validation or optimization.

pH: pH helps ensure proper column packing and equilibration and maintains protein stability and activity. pH adjustments optimize separation conditions, improving selectivity and resolution. Measuring pH also helps monitor buffer gradients during gradient elution, ensuring precise and reproducible separation conditions. pH is crucial as it affects the interactions between biomolecules and the stationary phase, their ionization state, and overall structure and function.

Monitoring and controlling critical parameters are crucial to achieve optimal separation, purity, and recovery of the target molecule during chromatography. Regular monitoring and adjustment of these parameters can help identify and resolve any issues that may arise during chromatography in biopharmaceutical production.

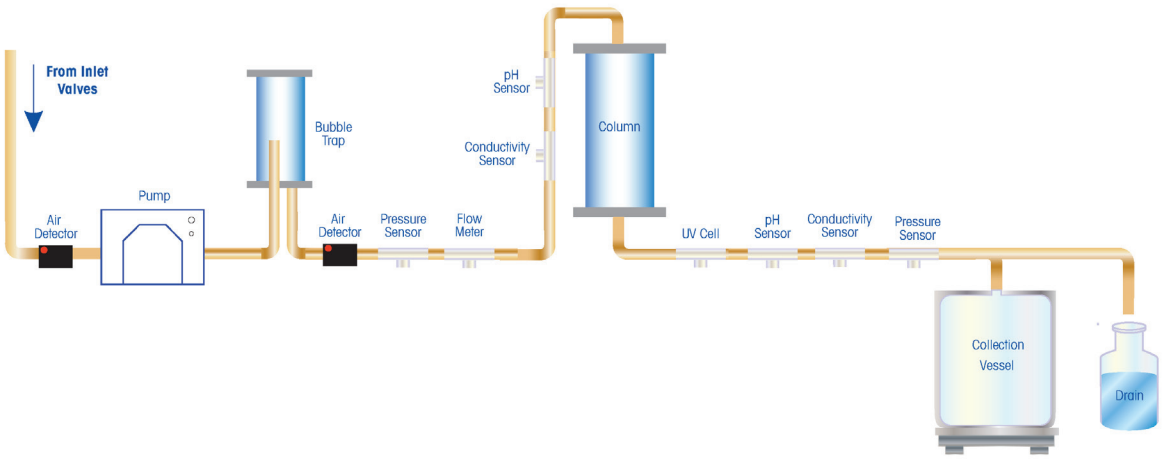

Fig. 1 Schematic representation of control measure parameters for the chromatographic separation process

PendoTECH Mettler Toledo Solution:

Air detector:

The air detector sensor is a powerful tool designed to detect the presence of air or liquid in various applications. It utilizes a 24V DC supply and offers a digital 5V output for air and 0V output for liquid. With the ability to detect bulk air defined as over ½ the cross-section empty, this sensor offers reliable and accurate monitoring of your system. The sensor also features an integral red LED that lights up when liquid is present, providing easy identification and monitoring of liquid levels. The sensor comes with an integral 8-foot cable with an M12-style connector, which ensures easy connectivity and installation. Additionally, for organizations looking for a panel mount installation option, an optional mating connector is available.

Fig. 2 PendoTECH Air in Tube Detector

Flow meter:

PendoTECH® offers Single Use Rotary Flow Meters that provide a cost-effective and precise method to measure flow in your process. They are an excellent alternative solution to the existing reusable rotory flow meters available in the market when used with tubing. These flow meters are suitable for filtration processes, chromatography, and other applications. Despite their name, they can also be used repeatedly or for long-term applications. There are two sizes available for scalability purposes. The tubing can be easily fitted over the hose barb on the rotor and secured with a cable tie or other means. The reusable flow sensor electronics can be connected to a flow monitor via a 3-foot (1-meter) electrical cable, and extensions are available. Compatible monitors include the PendoTECH Flow Monitor, a PendoTECH PressureMAT-PLUSTM, or a PendoTECH TFF Process Control System (DAQ), PendoTECH Data Acquisition System, or other qualified third-party monitors.

Fig. 3 Rotary Flow meter

UV-Vis absorbance sensor:

The photometer is a highly adaptable instrument designed for use in both laboratory and process settings and is available in both benchtop and panel mount versions. It boasts factory configuration with seven different wavelength combinations, making it easy to integrate into a monitor with data acquisition capabilities. With two 4-20mA output signals and a local display, it allows for easy reading and viewing. The photometer is compatible with a variety of data acquisition devices and control systems and supports digital communication protocols. Its non-invasive, real-time measurement of UV absorbance enables reliable identification of the presence or absence of a target molecule, while its ability to monitor concentration changes and detect absorbance peaks enhances its utility. The Photometer also offers a low-cost solution for single-use applications, with flow cells that can be repeatedly cleaned and reused. Furthermore, it supports a range of flow cell sizes and path lengths to accommodate different applications and process scales.

Fig. 4 PendoTECH PM2 Photometer

Pressure sensor:

Our state-of-the-art single-use pressure sensors from PendoTECH METTLER TOLEDO offer the latest in bioprocessing technology. Integrated with High Accuracy Pressure (MEMS-HAP™) chips, they provide highly accurate measurements of static and dynamic pressure for both gases and liquids. The sensors connect seamlessly to monitors via an integral connector and are compatible with our PressureMAT® and PressureMAT PLUS monitor/transmitters, as well as our new DIN rail mount PTR Pressure Transmitter. These sensors have a wide range of use, from -11.5 to 75 psi (-0.79 to 5.2 bar), with accuracy specifications from -10 to 60 psi (-0.69 to 4.14 bar). They're an ideal choice for any organization looking to optimize their bioprocessing operations with advanced technology.

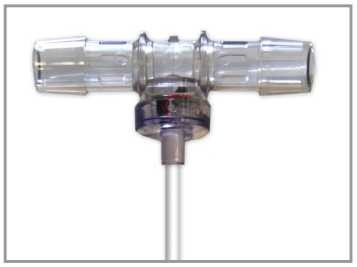

Fig. 5 PendoTECH Single-Use Pressure Sensor

Conductivity sensor:

Our Single-Use Conductivity Sensors™ and Conductivity Monitor from PendoTECH METTLER TOLEDO are an ideal choice for organizations looking for highly accurate conductivity and temperature measurements without requiring sensor calibration. The sensor monitors feature automatic temperature compensation that normalizes conductivity readings to 25°C (77°F). 4-20mA outputs are available for both conductivity and temperature can transmit the readings to a higher-level control system, such as a PLC or DCS. An RS-232 output also enables data collection on a PC. These sensors are designed for conductivity measurement in the range of 0.1 to 100mS/cm and process temperature in the range of 2°C to 50°C (35.6°F to 122°F). With this wide range of measurement capabilities, these sensors can optimize your bioprocess, making it more efficient and productive.

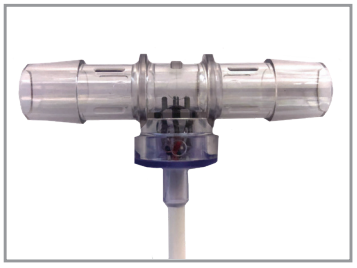

Fig. 6 PendoTECH Single-Use Conductivity Sensor

pH sensor:

Our Single-Use In-line pH Sensors from PendoTECH METTLER TOLEDO are equipped with advanced InSUS 307 pH probe technology, delivering accurate and reliable pH measurements in downstream bioprocessing operations. These sensors are designed to perform with great precision within the pH range of 3 to 10, with an accuracy of ± 0.10 pH when operating within ± 1.50 pH units of the 1-point process calibration point. With a quick response time of under 20 seconds between pH 4 and 7, they can capture rapid pH shifts due to process changes. The InSUS 307 pH sensors are also rated for a temperature range of 5-60°C and a pressure range of 4 bar at 25°C, 2 bar at 40°C, and 1 bar at 60°C, making them a highly versatile and effective choice for bioprocessing operations.

Fig. 7 Single-use pH probe

Chromatography is a crucial step in purifying proteins from complex mixtures in the biopharmaceutical industry. However, several critical parameters must be monitored and controlled for optimal performance and separation, including air detection, flow rate, UV absorption, pressure, conductivity, and pH. PendoTECH Mettler Toledo offers a range of single-use sensors that provide highly accurate measurements and ensure effective monitoring and control of these parameters. These sensors are designed to be easily integrated into bioprocessing equipment and help organizations optimize their bioprocessing operations for maximum yield, purity, and quality of protein products. With advanced technology and reliable performance, PendoTECH Mettler Toledo's sensors provide an essential component of bioprocessing operations and are critical in the scientific research, diagnostics, and biopharmaceutical production industries.

References:

[1] M. Hedhammar, A. E. Karlström, and S. Hober, "Chromatographic methods for protein purification."

[2] A. T. Hanke and M. Ottens, "Purifying biopharmaceuticals: knowledge-based chromatographic process development," Trends Biotechnol, vol. 32, no. 4, pp. 210–220, Apr. 2014, doi: 10.1016/J.TIBTECH.2014.02.001.

[3] J. A. Asenjo and B. A. Andrews, "Protein purification using chromatography: selection of type, modelling and optimization of operating conditions," Journal of Molecular Recognition, vol. 22, no. 2, pp. 65–76, Mar. 2009, doi: 10.1002/JMR.898.