Application Note

PENDOTECH
Leading Process Analytics

Optimizing Ultrafiltration and Diafiltration Process

Using Real-time In-line Single-Use Sensors

Ultrafiltration (UF) is a pressure or concentration gradient-dependent filtration process. It is combined with diafiltration (DF) to provide an efficient and robust process for buffer exchange. UF/DF is a crucial step in the high production of various biopharmaceuticals in its pure form. Most bioprocesses utilize multiple UF/DF cycles throughout the purification and fill/finish steps. The process requires control and monitoring of several parameters to ensure continuous and efficient operation, including pressure, conductivity and pH, and UV280 absorbance for protein concentration measurement. PendoTECH METTLER TOLEDO offers a complete line of single-use sensors for pressure, conductivity, pH, and UV absorbance measurements. These sensors are ideal for real-time monitoring and optimization of the UF/DF process.

This application note illustrates how PendoTECH METTLER TOLEDO single-use sensors can be used to achieve optimal performance in your UF/DF process from process development through clinical and cGMP manufacturing.

Background

Nowadays, most pharmaceuticals are inclined toward producing drug molecules, vaccines, and proteins using mammalian or microbial cells, as it is a cheaper and faster method for producing consistent high-quality drugs. These biologically produced molecules are compatible with the human body and comprise of post-translational modifications[1]. Many biopharmaceutical products including mAbs are recombinantly expressed at large scales for commercial purposes. Cells expressing these mAbs are being cultured in large-capacity bioreactors. Processing these cells to obtain the final desired product includes several steps, which all must be closely monitored and controlled to achieve an efficient process and collect quality product. Cross-contamination caused by failed sterilization is also a major threat that costs the manufacturers both in terms of time and money.

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Process

To achieve maximum expression of molecules of interest, high-volume cultures of different cell lines are used and subjected to various purification methods. The first step involves centrifugation to remove cell debris, followed by depth filtration to obtain a clear supernatant. The next purification step involves chromatography, and the resulting eluent is collected in a recirculation tank for subsequent ultrafiltration/diafiltration (UF/DF) processes. Chromatography is the core purification method utilized in modern bioprocessing. Nearly all bioprocesses includes two or three chromatography steps. In the transition from one chromatography step to next, it is typically necessary to eliminate the buffer from previous step (UF) and replace it with a buffer more suitable for the next chromatography step (DF). In this process, molecules are separated based on their molecular weight and size, and the product is concentrated. Diafiltration is used for desalting or buffer exchange during the ultrafiltration process [2]. The UF/DF process is a critical step that requires careful attention to defined parameters, as any variation can significantly affect the yield and quality of the final product. The Flow sensors are typically used to monitor flow rates in the feed, permeate and retentate lines of the tangential flow filtration (TFF) system. It is essential to maintain the proper flow rate and trans membrane pressure (TMP) to prevent fouling of the membrane, which could result in poor buffer exchange or product loss.

Fluid pressure 1 needs to be monitored and controlled in the feed, permeate and retentate lines. Delayed high pressure at influx causes an increase in trans membrane pressure (TMP) and leads to fouling of the membrane. Pressure excursion outside of the specified operating range can result in membrane damage and/or loss of filter efficiency. Process pressure in the feed, retentate and permeate lines continuously assessed to maintain optimal conditions for efficient filtration [3].

Conductivity 2 assures efficient and complete buffer exchange. It determines the end-point of diafiltration step by measuring ionic strength. Effective buffer exchange relies on monitoring conductivity to determine when unwanted ions are sufficiently removed. A decrease in conductivity indicates the successful removal of unwanted ions, while an increase suggests incomplete buffer exchange. As different buffers have widely varying ionic strengths, hence process conductivity provide an excellent setpoint for the endpoint of the buffer exchange.

Because most proteins are highly pH sensitive, maintaining specific pH 3 throughout the process as well as during storage is crucial for product stability and activity [4]. If the pH varies out of the acceptable range, then protein degradation can occur, and the product may be compromised. In addition pH, like conductivity, can often be used to verify adequate buffer exchange.

UV absorbance sensors 4 typically measuring at 280 nm can monitor protein concentration. In the retentate line, UV sensors can indicate when the target protein concentration had been achieved. An elevated UV 280 absorbance in permeate may indicate the presence of small amounts of protein, providing an early indication of potential product loss. [4]. Therefore, monitoring UV absorbance in the permeate line is an additional safety precaution that can be taken to ensure a more efficient process.

In UF/DF process, all parameters must be monitored and controlled in real-time to ensure optimal process performance and product quality. Maximum product recovery and purity continues to be a major challenge for the industry.

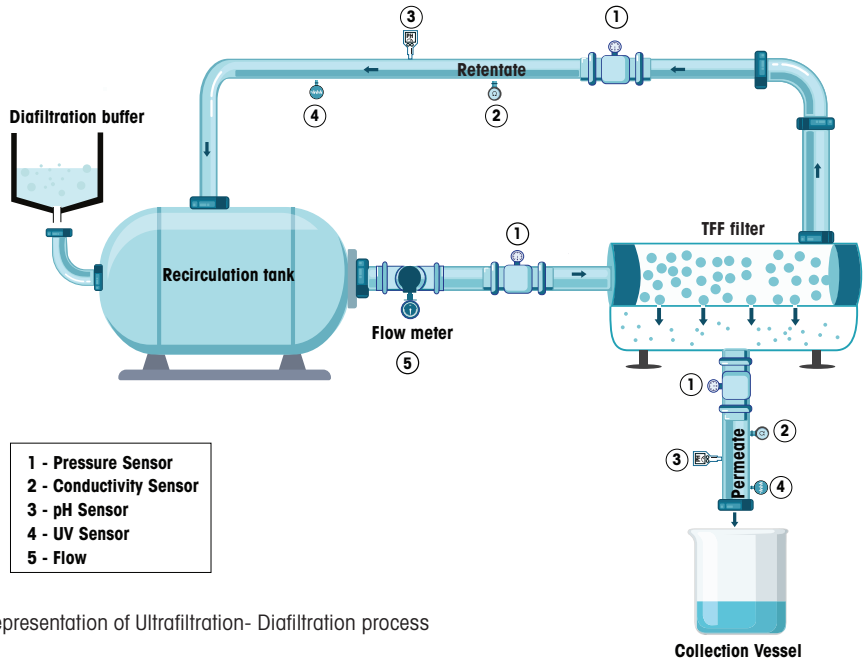


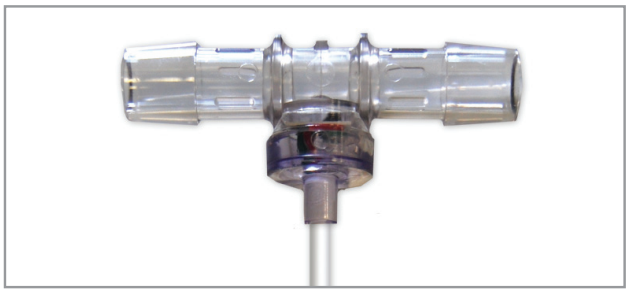
Fig. 1 - Schematic representation of Ultrafiltration- Diafiltration process

PendoTECH METTLER TOLEDO Solutions

PendoTECH METTLER TOLEDO offers a comprehensive range of Single-Use Sensors for downstream bioprocessing operations. Our sensors are designed to provide accurate and reliable measurements, helping our customers to improve their process efficiency and quality.

1 Revolutionize Your Process with Our Single-Use Pressure Sensors

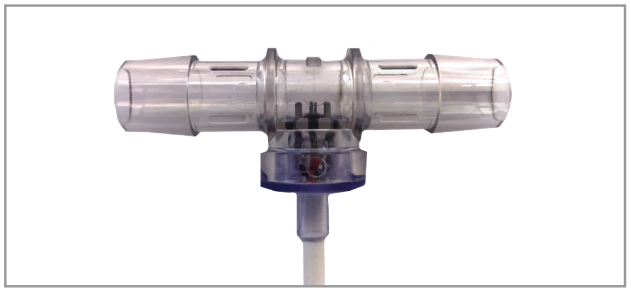
PendoTECH METTLER TOLEDO single-use pressure sensors integrated with High Accuracy Pressure (MEMS-HAP™) chips can accurately measure static and dynamic pressure of gases and liquids in situ. The sensors connect to monitors via an integral connector. Compatible monitors include the PressureMAT® and PressureMAT PLUS monitor/transmitters as well as the new, DIN rail mount PTR Pressure Transmitter. All sensors are qualified for use from -11.5 to 75 psi (-0.79 to 5.2 bar) with accuracy claimed -10 to 60 psi (-0.69 to 4.14 bar).



PendoTECH Single-Use Pressure Sensor

2 Enhance Your Single-Use Bioprocessing with Our Advanced Conductivity Sensors

PendoTECH METTLER TOLEDO Single-Use Conductivity Sensors™ and Conductivity Monitor are used for highly accurate conductivity and temperature measurements without the need for sensor calibration. The sensor monitor offers automatic temperature compensation that normalizes conductivity readings to 25°C. Additionally, it has 4–20mA outputs for both conductivity and temperature that can transmit the readings to a higher-level control system such as a PLC or DCS. An additional RS-232 output enables data collection on a PC. The sensors are designed for conductivity measurement in the range of 0.1 to 100mS/cm and temperature in the range of 2°C to 50°C.



PendoTECH Single-Use Conductivity Sensor

3 Optimize Your Bioprocess with Our Single-Use In-Line pH Sensors

PendoTech METTLER TOLEDO Single-Use In-line pH Sensors integrated with InSUS 307 pH probe technology provide accurate and reliable pH measurements in downstream bioprocessing operations. It is designed to operate well 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. It has a quick response time of < 20s between pH 4 and 7 which enables the sensors to capture rapid pH shifts due to process changes. The InSUS 307 pH sensors are rated for a temperature range of 5-60°C and pressure range of 58.01 psi (4 bar) at 25°C, 29 psi (2 bar) at 40°C, 14.5 psi (1 Bar) at 60°C.

PendoTECH Single-Use pH probe

4 Maximize Your Process Efficiency with Our Single-Use UV Flow Cell

Single-Use UV Flow Cells with a compact PM2 photometer and fiber optic cables utilise UV absorbance primarily to monitor protein concentration. The PM2 photometer can be factory configured with seven different wavelength combinations: 260nm, 280nm, 300nm, 880nm, 260-280nm, 280-300nm, and 280-880nm. Dual-wavelength measurements are useful in a number of bioprocessing applications. For example, the 280nm/300nm configuration is ideal for measuring very-low to very high-protein concentrations. The 260nm/280nm dual-wavelength combination is useful for differentiating full and empty capsids in viral vector production. The photometer is designed to be easily integrated into a PLC or DCS system with data acquisition capability.

PendoTECH METTLER TOLEDO offers a wide range of single-use sensors and monitors designed specifically for bioprocessing applications. With transmitters available in both benchtop and panel mount versions, these systems are well suited for process development, clinical and cGMP manufacturing.

PendoTECH Single-Use UV Sensor

All the sensors are:

- Made from robust, industry standard materials (polysulfone or polycarbonate).
- Tested to be biocompatible (USP Class VI and ISO 10993-5 Compliant).
- Gamma & X-ray compatible for sterilization.
- Available in various connection options (hose-barb or sanitary flange).
- Designed for seamless integration with transmitters for data acquisition and control.

We at PendoTECH METTLER TOLEDO fully understand the importance of cost-effectiveness, efficiency, and risk mitigation in downstream bioprocessing operations. Our Single-Use Sensors are designed to address these challenges by providing accurate and reliable measurements while eliminating the need for calibration. Our sensors are well validated and sterilizable by industry standard methods, making them easy to integrate into any single-use assembly.

Moreover, our sensors are plug and play, ready to use right out of the package. They are pre-calibrated, which eliminates the need for calibration before each cycle, saving time and labor. This feature also ensures that the measurements are accurate and consistent, which helps to enhance the process yield and efficiency. By integrating our Single-Use Sensors for real-time process control, biopharmaceutical manufacturers can significantly improve their process efficiency, yield, and cost-effectiveness while mitigating the risk of cross-contamination.

References:

[1] V. Gupta, M. Sengupta, J. Prakash, and B. C. Tripathy, "Production of Recombinant Pharmaceutical Proteins," Basic and Applied Aspects of Biotechnology, p. 77, 2017, doi: 10.1007/978-981-10-0875-7_4.

[2] R. C. Houp, "Ultrafiltration and Diafiltration".

[3] M. C. Vincent-Vela, B. Cuartas-Uribe, S. Álvarez-Blanco, and J. Lora-García, "Analysis of an ultrafiltration model: Influence of operational conditions," Desalination, vol. 284, pp. 14–21, Jan. 2012, doi: 10.1016/J.DESAL.2011.08.030.

[4] R. Ghosh, Y. Wan, Z. Cui, and G. Hale, "Parameter scanning ultrafiltration: Rapid optimisation of proteinseparation," Biotechnol Bioeng, vol. 81, no. 6, pp. 673–682, Mar. 2003, doi: 10.1002/BIT.10510.