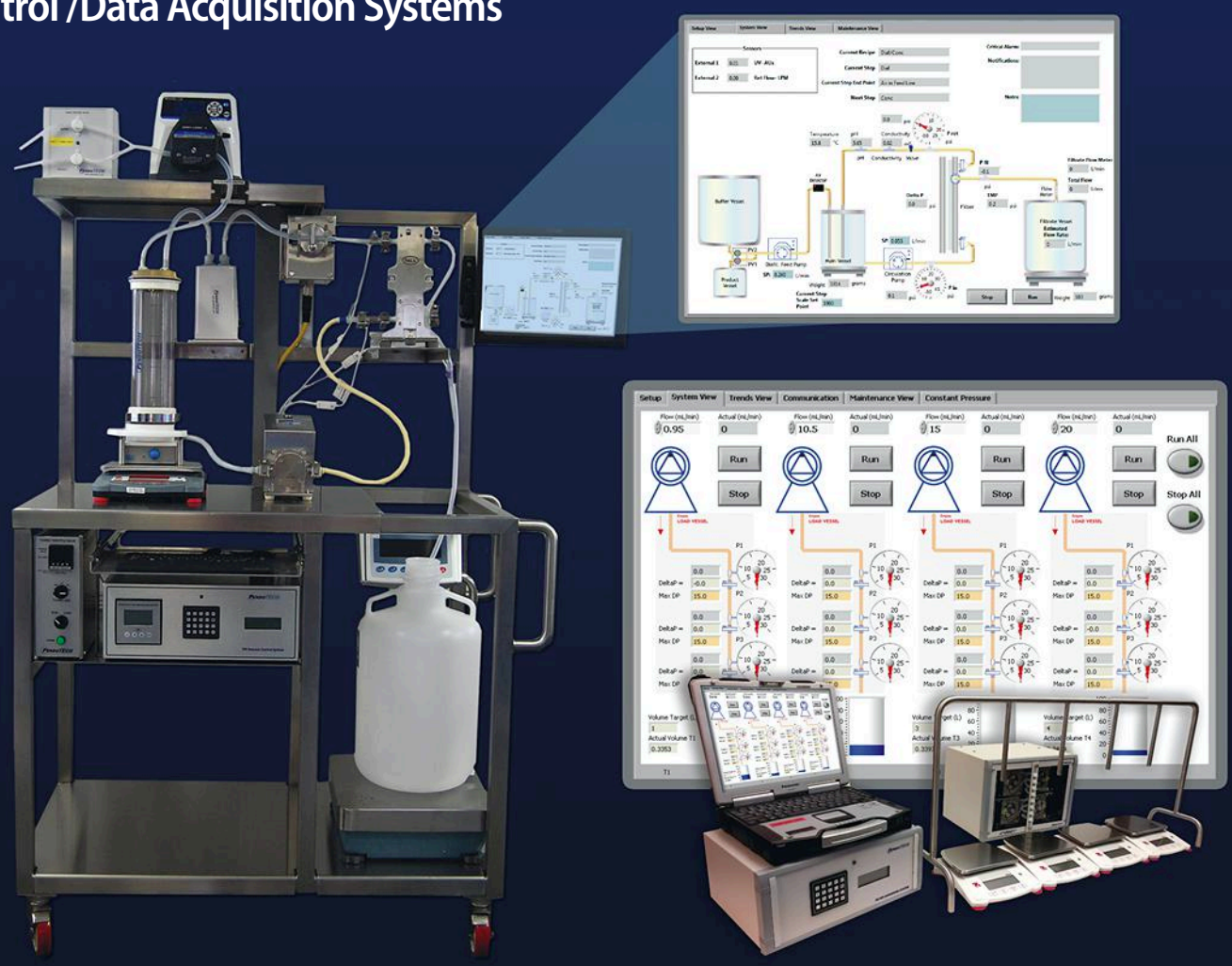
BioProcess™
SPECIAL REPORT

PENDOTECH
Adding Value To Your Process

Single–Use Sensors and Control and Data Acquisition Tools to Streamline Bioprocess Development

Single-Use In-Line Sensors

Control /Data Acquisition Systems



PendoTECH Process Control Systems...

Use your valuable time to analyze data, not collect it

Normal Flow Filtration ↓ **Tangential Flow Filtration**

Screening

(~1L or less)

Screening System

TFF Benchtop System

5 Station TFF Screening System

Process Development / Scale-up

(~ 1 to 5L)

VF-DF System

TFF Compact Cart

Pilot

(~ >5L)

VF-DF Pilot Cart

TFF Pilot Cart

In-Line Single Use Sensors

Pressure

Conductivity

Temperature

UV Absorbance

Turbidity

PENDOTECH

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Welcome and Contents

Process development and biomanufacturing in the biopharmaceutical industry have evolved extensively over the past 10 years. More tools are available to study process variables to enable more efficient and productive processes, speed development, and reduce costs. High-powered microcontrollers are embedded in laboratory devices to carry out complex tasks. Recently, users have started working with microcontrollers such as Raspberry Pi for personal projects.

As personal computer power has accelerated multiplefold, leading to high processing power and compact, high-capacity memory readily available for process development engineers, scientists, and suppliers, laboratories are generating large amounts of data. Data analysis tools previously restricted to high-power computer workstations can be accomplished with simple PCs. Powerful, efficient, and user-friendly software can automate laboratory processes and trend, log, and analyze the data they generate.

PendoTECH process control systems for downstream process development were developed and improved over the past decade to take advantage of advances in PC and microcontroller technology. Collaborating and listening to customers was key to designing the initial product features and implementing improvements over time. Networked PCs and high-level control systems allow data sharing with common databases such as the PI historian system from OSIsoft, further speeding analysis of data coming from many different process systems and analyzers concurrently.

Manufacturing trends in single-use technology have promulgated the industry with evolution and refinement of the technology and organizations such as the Bio-Process Systems Alliance (BPSA) to facilitate dialog between users and suppliers and assist in addressing concerns. In many cases, disposables have become the most logical option. The advantages of eliminating cleaning/sterilization validation and associated costs related to large capital infrastructure have led to increasing and rapid implementation of single-use processes in facilities.

In the growing areas of cell and gene therapy processing, single-use technology is vital to handling large numbers of small batches and biohazardous materials. High-performance product-contact films to make process bags and bioreactors, tubing, filters, columns, connectors, and sensors are key pieces of the puzzle of creating a single-use process. PendoTECH has focused extensive product development efforts over the years on new types of sensors and an expanding size range to adapt to different process types and scales. Sensors are used in many critical applications to monitor processes and enable decision making. A range of sensor monitors with features required to both read them and transmit their data have been qualified for use by many companies, even for lengthy continuous processes. Qualification data and technical reports are being released to help users implement these sensors into their processes.

Our entire staff is committed to creating unique products with the utility to increase efficiency and solve process problems, to manufacture those products to high quality, and to deliver them to market through efficient internal operations in our ISO 9001:2015 registered quality system.

—Jim Furey

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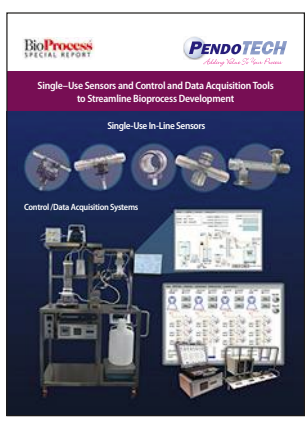


Using In-Line Disposable Pressure Sensors to Evaluate Depth Filter Performance

Lee R. Bink and James Furey

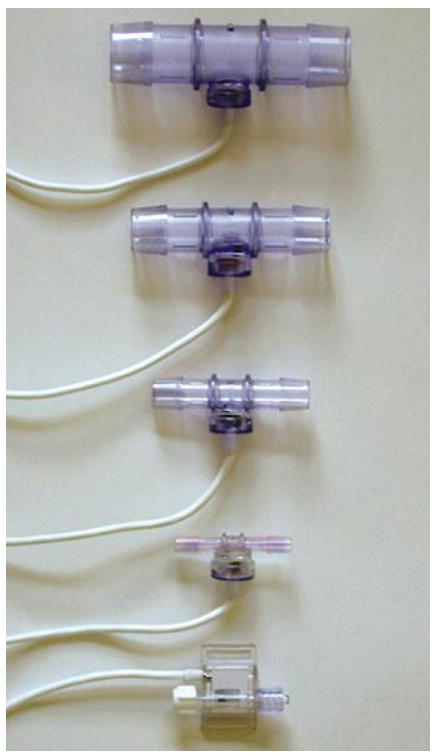
Development of a recovery process for a fed-batch mammalian cell culture product involves several objectives: process scalability, robustness, maximizing product yield, elimination of subsequent purification steps, and low cost of goods. In an effort to achieve those objectives, we developed a three-stage primary recovery process to remove biomass and clarify the feed stream for downstream column chromatography (Figure 1). The initial stage involves removal of whole cells and larger cellular debris using a continuous disc-stack centrifuge. Depth filtration is the second stage, removing smaller particulates based on size exclusion and adsorption. The third stage consists of 0.2-µm filtration, which removes potential bioburden. To assist in process development, we are investigating innovative approaches to achieve better process control and maximize processing efficiency. Monitoring pressure in the depth filtration process step is one potential area for improving throughput and efficiency.

Depth filtration coupled with 0.2-µm filtration adequately removes cellular debris and contaminants from a feed stream. It offers two different methods for removing biomass and other contaminants. The depth filter medium is a porous mix of diatomaceous earth and cellulosic fibers that removes small particles (<1 µm) by size exclusion. That medium may also contain positively charged adsorptive binding sites. They can also effectively remove smaller charged particles that are too small to be removed by size exclusion but can impair subsequent column chromatography operations.

Because of inherent differences in cell lines, a purification process needs to be developed individually for each mammalian cell culture. There is a shortage of relevant data on process feed streams that can accurately predict the reliability of depth filtration. But pressure differential (pressure drop across a depth filter) is an important means of monitoring the overall performance of a depth filter during use. After initial sizing for a particular clarification process, depth filter performance still needs to be closely monitored to prevent premature fouling, which can be caused by unforeseen impurities or less-than-optimal process parameters, such as a high flow rate. This would require additional depth filter area to clarify the remaining cell culture harvest.

No correlation currently exists between the characteristics of a cell culture (e.g., cell viability, cell density, viscosity) and the ability of a depth filter to successfully clarify it. Changes in media feeding rates and other growth parameters play a role in the characteristics of a cell culture to be clarified. So even with proper filter sizing for a given volume of a particular culture, depth filter performance can still suffer if key variables are not monitored closely. Monitoring the pressure differential is a way to ensure that a cell culture harvest is not prematurely plugging the porous medium of a depth filter.

Photo 1: Pressure sensors



Traditional analog, stainless steel pressure gauges have been used to record both pre- and post-depth filter pressure. A major drawback of such gauges is their need for frequent calibration and cleaning verification and validation. Another drawback is the need for an in-line stainless steel sanitary "tee" to install each pressure gauge into a flow path. That causes a short deadleg with a hold-up volume, which can lead to inaccurate readings. For mammalian cell culture operations, pressure readings have to be taken by operators and recorded manually. Frequent data collection can

Figure 1: Schematic presentation of a cell culture harvest clarification train

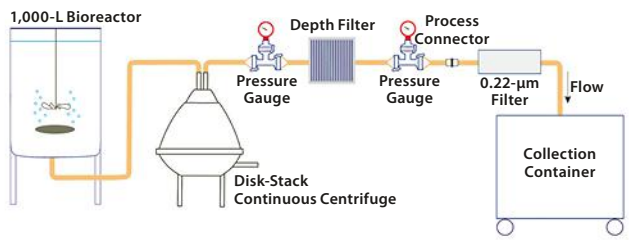


Figure 2: Inlet pressure profiles of depth filter and 0.2-µm filter with the pressure differential using traditional stainless steel pressure gauges (manually collected data)

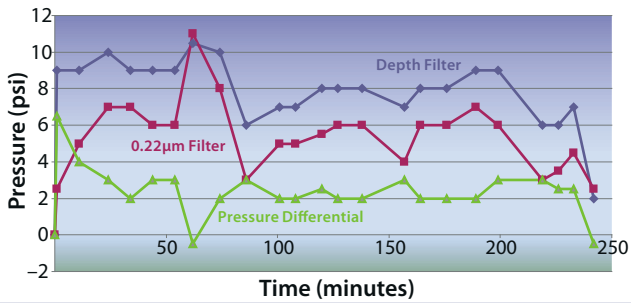


Figure 3: Schematic presentation of a cell culture harvest clarification train with PendoTECH pressure sensors and PressureMAT system

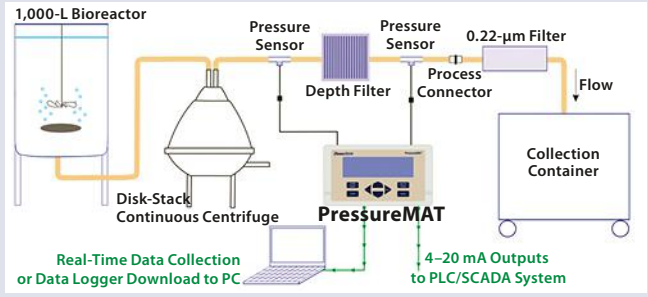
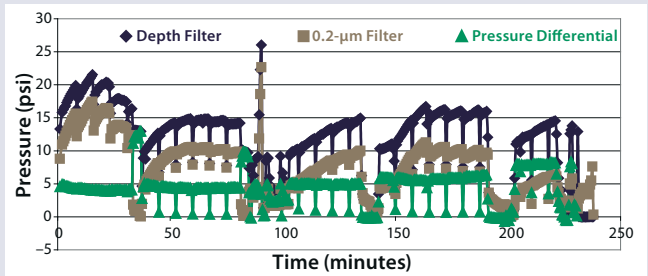


Figure 4: Filter pressure profiles and pressure differential using PendoTECH pressure sensors and PressureMAT system; data points were transmitted continuously, then collected on a laptop computer.



put a strain on limited manpower and may also lead to transcription errors.

Single-Use Pressure Sensors and Data Collection

At Centocor, we were interested in PendoTECH single-use pressure sensors as an alternative to stainless steel pressure gauges to be used in conjunction with the company's PressureMAT monitor, alarm, and transmitter system. The combination can be used to record and transmit pressure information to a data collection system. Each pressure sensor has an in-line, flow-through design, and sizes are available from Luer to 1-in. hose barb fittings (Photo 1), eliminating the need for sanitary tees and their associated hold-up volumes. This reduces the number of process components (e.g., gaskets, clamps, tubing adapters), and the disposability of the pressure sensors reduces demand on cleaning verification/validation.

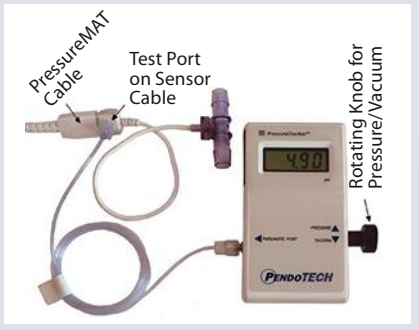
The pressure sensors use an innovative microelectromechanical (MEM) chips. MEM technology integrates mechanical elements, sensors, actuators, and electronics on a common silicon substrate through microfabrication technology (1). These chips are manufactured using a silicon piezoresistive sensing element in a Wheatstone bridge circuit, through which an applied pressure gives a proportional output voltage. Before installation into finished devices, the chips undergo an accuracy test and integrity testing that includes a pressure stress test to >100 psi.

The plastic material used to mold the sensor body is either polycarbonate or polysulphone that, along with the other fluid contact materials used in the sensor, meets USP Class VI requirements. The sensors are manufactured in a clean room at an ISO13485-certified, FDA-registered facility. Each individual device undergoes several tests to determine electrical integrity, to confirm the absence of leaks, and to ensure proper calibration within a tight specification range. These sensors are qualified for use ≤75 psi, with burst testing conducted up to 150 psi. Gamma irradiation has been qualified for ≤50 kGy, so the sensors can be preassembled with ready-to-use tubing, filter, and bag assemblies. The accuracy specification of 30 psi (2 bar), +/-3% is sufficient for most clarification process operations (2). All these factors ensure that PendoTECH pressure sensors provide highly accurate pressure monitoring.

EACH pressure sensor has an in-line, flow-through design, and sizes are available from Luer to 1-inch hose barb fittings (Photo 1), eliminating the need for sanitary tees and their associated hold-up volumes. That reduces the number of process components (e.g., gaskets, clamps, tubing adapters). The DISPOSABILITY of the pressure sensors reduces demand on cleaning verification/validation.

Each pressure sensor chip circuit requires a narrow range of applied voltage. The circuit voltage output directly proportional to pressure is not a traditional field output signal such as 4–20 mA or 0–10 V, which gives a higher resolution for analog-to-digital

Figure 5: PressureChecker assembly



conversions. The PressureMAT system serves as a voltage source and processes the output signal from the sensor into a pressure reading. It is therefore required as an intermediate device to integrate the sensors into a control system for building a feedback control loop. Once the sensors are inserted into a flow path, data will be transmitted to the PressureMAT system at intervals as frequent as 1 data point per second. Pressure data can be viewed on the PressureMAT monitor display. Outputs (4–20 mA) from the PressureMAT transmitter can be brought into a data handling system, which facilitates data recording and processing.

A Case Study

We clarified cell culture harvest from a 1,000-L bioreactor using an LAPX404 disc-stack centrifuge from Alfa Laval (www.alfalaval.com), followed by depth filtration using Pod A1HC filters and 0.2-µm filtration with Express SHC filters, both from Millipore Corporation (www.millipore.com). The cell culture in defined media was harvested after 14 days, showing a viable cell density of 2.96 cells/mL and cell viability of 68%. This process was continuous, with no break tanks in between the steps (Figure 1). Flow rate throughout the clarification train remained constant at 5 L/min. We collected filtrate in 200-L collection containers, each with a 0.2-µm filter attached. As each collection bag was replaced during culture clarification, the 0.2-µm filter was also replaced to reduce the number of interruptions in the overall process and reduce potential bioburden or endotoxin contamination of the final filtrate.

Comparing Figures 2 and 4, it's clear that the combined PendoTECH pressure sensor and PressureMAT system are SUPERIOR to the analog stainless steel pressure gauges alone. This new approach offers frequent, accurate, and automatic pressure readings that provide a complete picture of depth filter performance. The information assists in process monitoring, process improvement, and trouble-shooting.

Before obtaining the sensors, we set up stainless steel pressure gauges in front of both filters (Figure 2). We recorded pressure readings manually and calculated the pressure differential as the difference between readings from those two gauges. The stability of the pressure differential at a constant flow rate indicates almost no change on the depth filter's performance throughout the process.

To test the pressure sensors and PressureMAT system, we installed half-inch sensors into the flexible tubing directly upstream of both the depth filter and the 0.2-µm filter without tee connectors. Cell culture was clarified from a 1,000-L bioreactor using the same process train described above. A similar (but not identical) feed stream was used in this particular case study.

Cell culture in defined media was harvested after 16 days, showing a viable cell density of 3.56 cells/mL and viability of 63%. Pressure data were collected using the PendoTECH single-use pressure sensors and PressureMAT system (Figure 3). We took pressure readings (Figure 4), every 30 seconds during processing. The "valleys" in those pressure readings (about every 50 minutes) represent replacement of a 0.2-µm filter and a 200-L filtrate collection bag. Small pressure decreases seen at ~7-minute intervals are due to the discharge of solids from the centrifuge bowl. Each time the centrifuge enters a discharge period, the feed flow is stopped until that discharge period ends, so the pressure differential dropped to zero at those times.

Because the pressure differential remained unchanged, we attributed the increase in pressure readings from both filters during the filling of each individual bag to increased fouling of the 0.2-µm filter. The pressure differential remained constant at ~5 psi for the first 120 minutes of processing, at which point 600 L of cell culture harvest were filtered. At the end, the pressure differential reached 8 psi. Regardless of that minor increase, the pressure differential did not approach the maximum allowable value for this depth filter (20 psi).

A spike in pressure at ~85 minutes was due to a piece of kinked tubing. The PressureMAT monitor's audible alarm, which can be triggered by a predetermined high or low pressure value, alerted the operator to the flow-path obstruction. Without that alarm, it could have gone unnoticed. The alarm set point can also trigger an internal relay that can be easily wired to automate process control by shutting off a pump or opening a valve.

Comparing Figures 3 and 5, it's clear that the combined PendoTECH pressure sensor and PressureMAT system are superior to the analog stainless steel pressure gauges alone. This new approach offers frequent, accurate, and automatic pressure readings that provide a complete picture of depth filter performance. The information assists in process monitoring, process improvement, and trouble-shooting. In addition, the automated pressure monitoring and recording function can free up operators for other tasks, reducing the labor demand of these operations.

Implementation, Scale-Up

PendoTECH single-use pressure sensors are available in a wide range of sizes. They are easily adaptable to filter screening experiments with small disc filters or scale-up to high flow rates with sensors for 1-inch tubing size. Each sensor is tested during manufacturing to be in calibration, but there is no ability to directly calibrate the sensors or PressureMAT monitor. If a demand for verifying proper functioning of the monitor and the output of a sensor exists, particularly for a GMP process, it is feasible to test the sensors and the system without interfering with the flow path. Each pressure sensor has a test port on its connector cable that can be used to access the atmospheric reference side of the pressure-sensing chip. The test port is a female Luer port, and by applying a calibrated vacuum source to this port, it will give a pressure reading with the same absolute value on the monitor.

For demands on validating the pressure readings, PendoTECH has developed the National Institute of Standards and Technologies (NIST) traceable PressureChecker device to perform this operation (Figure 5). It has an internal cylinder with an external adjustment knob that can create vacuum and pressure to the sensor test port. This testing can be done without flow-path interferance and serves to verify proper functioning of a newly installed sensor. It has a pressure sensing chip simulator embedded that references the internal cylinder. In addition, the PressureMAT monitor cable can be connected to the simulator port and the PressureMAT can be tested to verify it is functioning properly.

Advantage: Process Control

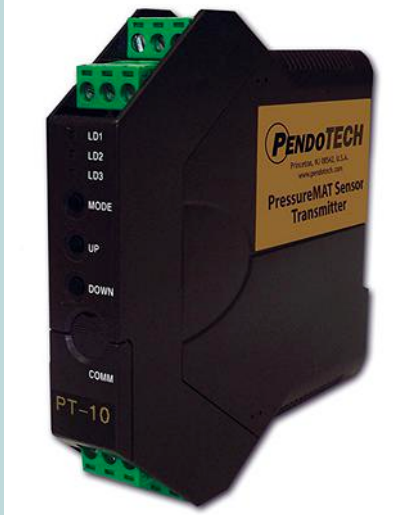
At Centocor, we found PendoTECH single-use pressure sensors and the PressureMAT monitoring system to provide many advantages. Disposables eliminate the demand for cleaning verification or validation, saving time and cost. Set-up is easy, and the configuration does not create deadlegs in process streams. This system could also provide for automation and feedback control functions.

PendoTECH Single-Use Pressure Sensors: Since this article was written, PendoTECH has developed a broader range of sensor options including 0.75-in and 1.00-in sanitary flange sensors, flange to barb sensors, and a 0.125-in hose-barb sensor for small-scale processes. Also, these sensors have been introduced into many good manufacturing practice (GMP) processes, and tools now exist to accommodate and support such work. For further information, download the *PendoTECH Single Use Pressure Sensors: Calibration, Accuracy, and Implementation* technical report at www.pendotech.com/products/disposable_pressure_sensors/Technical_Note-Accuracy_and_Implementation%20of%20PendoTECH_Pressure_Sensors_Rev0.pdf.

The PressureMAT system offers 4–20 mA outputs that can be interfaced with a distributed control system (DCS). This feature would allow end users to connect it with other instruments (e.g., pumps and scales) that might not be directly connected to the system, but that would offer additional control over a particular process. Analog inputs configured in DCS can also be sent to data historian programs for data collection purposes.

These pressure sensors provide accurate and reliable data that can be automatically recorded into data historians and control systems, allowing operators to focus on other tasks. This is ideal for pilot plants and multiproduct facilities, where product turnover and equipment changeover are frequent. This new technology is being tested and evaluated in development operations, and its application in routine manufacturing operations will be further evaluated later. The system also can be used on other applications in which pressure monitoring is needed, such as for filter capacity testing, determining chromatography column pressure flow curves, and controlling tangential flow filtration operations.

PressureMAT Sensor Transmitter Card: Since this article was first published, PendoTECH has introduced the simplified PressureMAT sensor transmitter card for applications to transmit pressure-sensor readings to process controllers (e.g., programmable logic controllers, PLC) on a custom skid. This is more cost and space effective if the display and alarms of the PressureMAT monitor mentioned herein are not required.



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1 What Is MEMs Technology? *MEMs and Nanotechnology Clearinghouse.* 11 May 2009; www.memsnet.org/mems/what-is.html.

2 PendoTECH. 11 May 2009; www.pendotech.com/pressure.

*At the time of original publication, corresponding author **Lee R. Bink** was an assistant scientist at Centocor R&D (www.centocororthobiotech.com). Corresponding author **Jim Furey** was general manager and a consultant in single-use process technology implementation at PendoTECH, 1-609-799-2299, jim@pendotech.com, www.pendotech.com. This article first appeared in the February 2010 issue of* BioProcess International.

Streamlining Downstream Process Development

An Integrated and Automated System for Normal-Flow Filter Screening Experiments

Brian Kluck, Binh Thai, Jim Furey, and Dennis Annarelli

Normal-flow filtration is used throughout downstream processes for biologics including depth, sterile, and viral filtration applications. Because of its ubiquity in large-scale biomanufacturing, using the most efficient normal-flow filter media area and type can lead to significant cost savings. To determine the most effective media type and area, developers use a scaled-down process model is used in bioprocess laboratories to minimize material requirements. Constant–flow-rate filter evaluations involve direct scale-down parameters that match manufacturing-scale process conditions. This type of evaluation can be time consuming, labor intensive, and complicated because of a lack of specialized laboratory-scale equipment. Clearly an integrated and semiautomated system would facilitate the efficient evaluation of filter media for manufacturing-scale bioprocesses.

Genentech Inc. and PendoTECH collaborated on developing an integrated filter-sizing system that addresses several disadvantages of the equipment commonly used. Those disadvantages included poor integration of multiple components from different sources (including pressure sensors, signal conditioning, data acquisition, balances, and pumps), continuous operator monitoring, and decreased portability of most systems due to their numerous components. In addition, peristaltic pumps have been the primary scale-down model for constant-flow filter-sizing applications. Such pumps require frequent calibration and can have difficulty maintaining flow rates at high back-pressures. Screening multiple media types from different vendors can require a range of flow rates because of varying scaled-down areas available. That requires multiple peristaltic pumps to supply needed flow rates, further reducing system portability and increasing laboratory space requirements for process development.

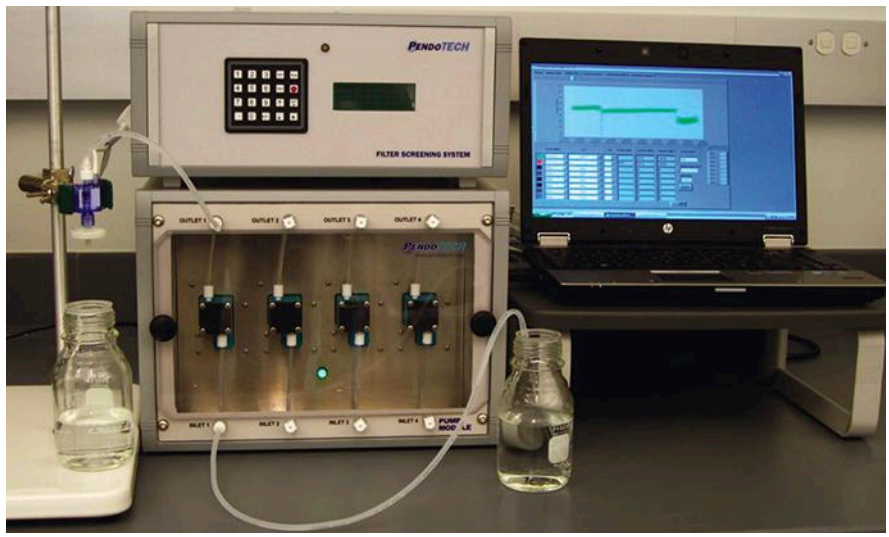


Photo 1: PendoTECH normal-flow filter screening system

System Development and Specs

Several requirements shaped development of our filter-sizing system to address those disadvantages of existing equipment used for such applications. Genentech desired the following characteristics of its new laboratory-scale system to efficiently perform normal-flow filter sizing and screening experiments:

- Can be easily purchased from one vendor with direct technical support
- Can control four independent filtration trains
- Can monitor and record three pressures from each train
- Should automatically stop a filtration train when a target volume or maximum filtration pressure is reached
- Uses high-precision pumps that eliminate the need for table-top balances to measure flow rate
- Operates at pressures ≤40 psig and flow rates of 1.0–20 mL/min

• Is controlled by a simple, graphical user interface (GUI) with user-friendly data retrieval and manipulation

• Offers real-time trending of process values

• Can be moved (portability) easily.

PendoTECH had worked on many systems for filter sizing and offers customized systems for process control solutions and/or data acquisition through its PendoKIT program. We used that program as the building block for developing our control system. It was designed to simultaneously record data from as many as 12 pressure sensors (through high-resolution analog inputs), control four pumps (through relay control and analog outputs or digital control), record data from four optional balances (through Recommeded Standard 232), and connect to a PC through two data ports (also RS232). Pressure sensors, pumps, and balances were arranged to enable four independent filtration trains, each capable of controlling one pump, measuring three pressures, and reading one balance if needed.

PendoTECH follows the good automated manufacturing practice (GAMP) lifecycle in development of its automated systems to manage the process from user requirements to system build. Based on Genentech's needs and additional requirements from the vendor, we finalized a design specification and put the system into production after extensive real-world testing. The standardized normal-flow filter screening system design included a control module and pump module (Photo 1).

The entire control module is housed in one box with electrical connectors on its back panel designed to interface with sensors, pumps, and other instruments. At the core of the control module is a dedicated, programmable, high-performance microcontroller that uses firmware stored in radom-access memory (RAM) with a battery back-up. Embedded firmware in the control system stores process parameters entered from the GUI, controls pumps, reads sensors and scales, and monitors process conditions. This control system has a keypad and liquid crystal display (LCD) primarily used to enable nonstandard configurations.

We included a PC-based GUI (Photo 2) in our specification for user friendliness, and it allows complete control of the pump module. The GUI operates from a local PC and can be operated from outside a laboratory through remote-desktop applications. Through the GUI, users specify key process parameters such as maximum filtration train pressure, maximum differential pressure across each filter, and volumetric end-points for each filtration train. When a given set-point is reached, the corresponding pump automatically stops. For example, an audible alarm sounds when a pressure limit is reached, and the GUI highlights the affected filter and stops its corresponding pump automatically. The GUI records experiment-specific information such as filter names, filtration train names, and experimental notes. It can display real-time experimental data such as flow rate, differential pressure, flux, and filter permeability. Full manual pump control is also possible through the GUI. These functions (and more) are distributed across six easily accessed tabs that users can select.

Whereas the control module is the "brain" of this filter-sizing system, the

Photo 2: System tab of the graphical user interface

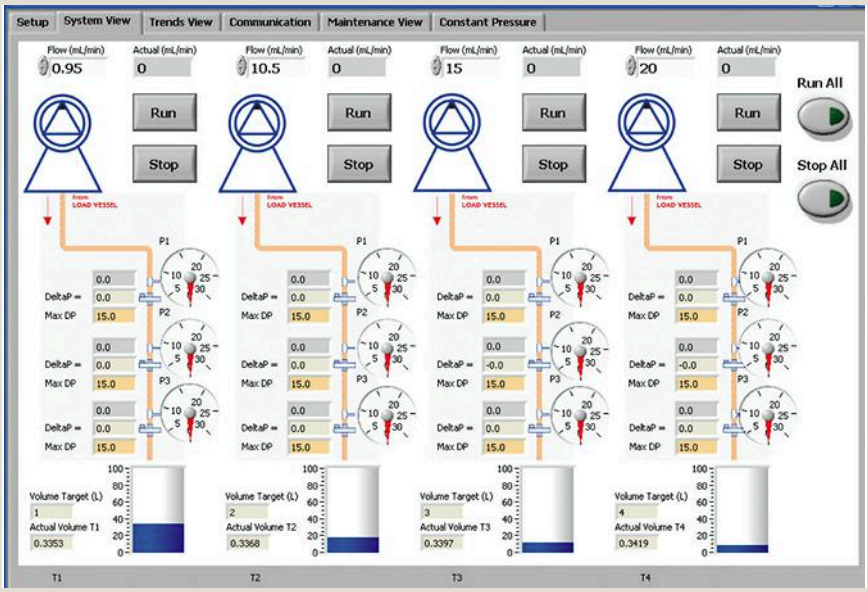
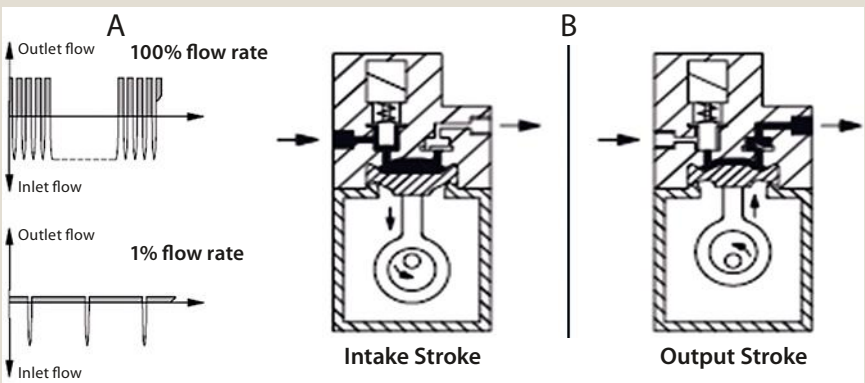


Figure 1: KNF Neuberger diaphragm pump dispensing method (KNF Neuberger, www.knf.com); technology for long-term precision



A flexible diaphragm is moved up and down by an eccentric connected to the motor shaft. During its downward movement, liquid is sucked through the inlet valve into the chamber; by its upward movement, liquid is pushed through the outlet valve. The pump's working chamber is hermetically separated from the motor to protect the liquid from contamination. The stepper motor is controlled by an electronic module. The intake stroke is carried out at maximum speed, and the output stroke is varied so liquid can be dispensed evenly — resulting in a quasicontinuous, low-pulsation flow.

INTEGRATION of pump and control modules — along with precise pressure sensors — provides for complete control of experimental parameters.

pump module is its "heart." To maintain portability of the integrated system, this pump module interfaces seamlessly with the control module and houses four high-precision diaphragm pumps. They are all digitally controlled using serial connections that allow precise flow control at low flow rates, which eliminates the need for bench-top balances to measure dispense volumes.

Although peristaltic pumps are typically used in filtration studies, we learned that diaphragm pumps provided similar results for reduced shearing of the protein feedstocks used in filtration experiments. A stepper-motor–driven diaphragm pump from KNF Neuberger provided the required accuracy and precision along with low shear in a compact design needed for our pump module. This pump also has low pulsation because of its unique operating method, in which the intake stroke is much faster than a varied output stroke so liquid can be evenly dispensed (Figure 1). The added accuracy, precision, and small footprint of this diaphragm pump simplified our system design. Flow rate and volumes can be automatically calculated by the control system rather than measured with bench-top balances, which were required with peristaltic pumps because of their inaccurate flow rates over the duration of filtration experiments.

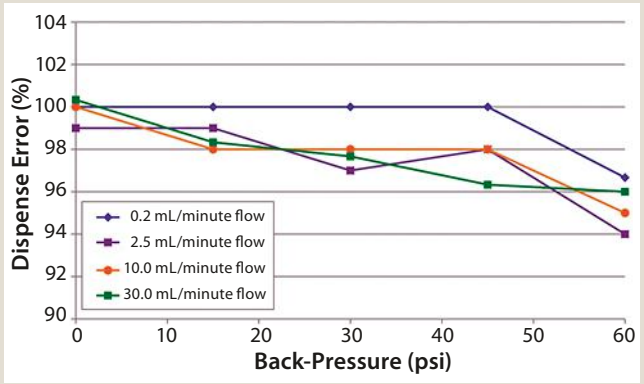
The operating range of the diaphragm pumps in our pump module is 0.2–30.0 mL/min. They are designed for minimum flow loss when pumping against higher pressures. Pump hold-up volume is ~1.0 mL with inlet and outlet tubing. All product-contacting internal pump components are fluoropolymer based, which provides a broad range of chemical compatibility.

PendoTECH manufactures pressure sensors ranging from luer size to 1-in. diameter that can be used with this normal-flow filter screening system or stand-alone monitors. These sensors are economical enough to be disposed of after biohazardous studies, but also robust enough to be reused during development work. Although most disposable pressure sensors come in polycarbonate housings, these also come in polysulfone for caustic sanitization.

A Fully Integrated System

Integration of pump and control modules — along with precise pressure sensors — provides for complete control of experimental parameters. Tests can be run without constant human monitoring to prevent excessive pressures or exhaustion of feedstock. If any filtration train reaches its pressure and/or volume set-point and stops, the other filtration trains will continue to operate without interruption. Reduction in the number of physical components shortens set-up time and makes the equipment

Figure 2: Comparing PendoTECH pump module flow-rate and back-pressure curves; three minutes test duration for each flow rate and back-pressure setting (applied from external air supply)



easily portable to remote locations. Because this system is fully integrated, it captures all data in one comprehensive but easy-to-analyze file. With a consistent format of such data files, we can use templates and rapidly produce relevant graphs for data analysis.

The GUI is unique to this system. It consolidates all experimental set-up, pump control, data trending, system communication, and maintenance functions into six tabs. Beginning an experiment with four unique filtration trains, twelve filters, and twelve pressure transducers can be accomplished by clicking a single button. The ability to run four independent trains from the GUI enables rigorous head-to-head comparison of filters using a common feedstock or multiple filter configurations using a range of feedstocks.

Pump Module Development and Comparability: Peristaltic pumps are typically used for filter sizing and screening experiments in downstream process development. However, tubing durability, pump-head variation, and flow decay at increased pressures can lead to inaccurate filter sizing and screening results. Because of the limitations of such pumps for these applications, we considered several different types of pumps in development of our pump module.

We compared the performance of diaphragm, piston, rotary-piston, and peristaltic pumps using monoclonal antibody (MAb) solutions. These solutions were filtered through a 0.22-µm polyvinylidene fluoride (PVDF) filter using each pump type, and only the diaphragm pump consistently matched the performance of the peristaltic pump in terms of solution filterability. We chose the diaphragm pump for filtration of protein feedstocks because it offered several performance advantages intrinsic

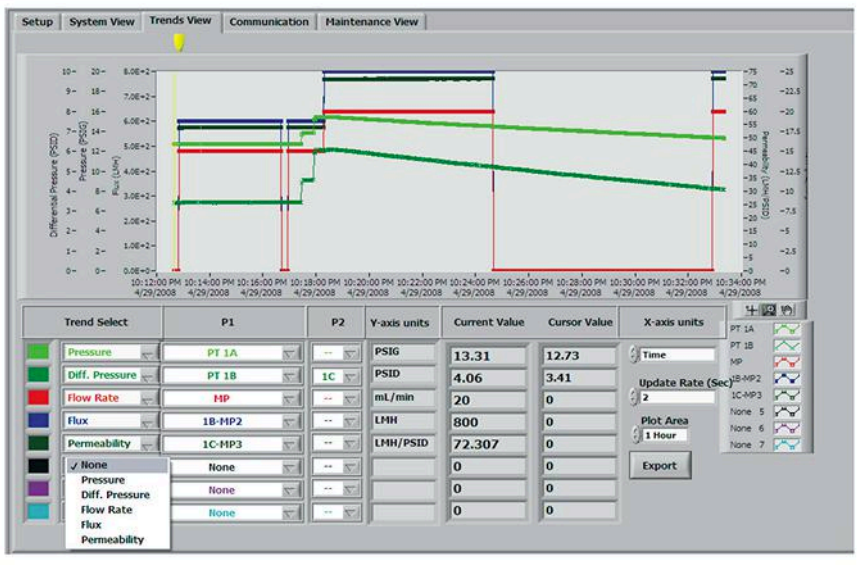
The ability to run four independent trains from the GUI enables rigorous head-to-head COMPARISON of filters using a common feedstock or multiple filter configurations using a range of feedstocks.

Updates

Since this article was published, a range of other pump options have been introduced to accommodate user requirements, including a peristaltic pump module and a range of standalone pumps with individual pump control (see setup, right) in a complete system setup with the peristaltic pump module. Software includes an advanced pump calibration method enabling users to verify calibration in real time. This has enhanced integration of the different pump options. The software allows for pump totalization, but scales on each train have become the preferred method to verify the amount of liquid filtered.

Data Management and Control Software: Data can be viewed in real time through an internal Trends View tab (see screenshot, right) and are written to a permanent file. Also, a locked PDF is produced at the end of each experiment. Now the system allows for personal computers (PCs) running graphic user interface (GUI) software to serve data (an server option) to objects for process control (OPC) clients such as the PI historian system from OSIsoft.

A popular additional option allows for capturing additional data into the system through spare analog inputs mentioned in the article. PendoTECH offers an in-line turbidity monitor system and in-line temperature sensors for scientists who wish to study the impact of temperature on filtration. Finally, an automatic report generator has been added to the software for generating customizable reports.



to the pump design. KNF Neuberger's diaphragm pump head features low pulsation at a range of pump speeds as well as precise flow control and dispense volumes over a wide range of flow rates and backpressures. We conducted further studies to show that its product filterability impact was comparable to that of the peristaltic pump typically used in filter sizing.

Figure 2 shows the accuracy and performance of our diaphragm pump module at a range of flow rates and back-pressures. The pump module delivers accurate flow performance across a wide range of flow rates against pressures typically observed for MAb solution filtration experiments (~40 psi).

To demonstrate the comparability of our diaphragm pump module with peristaltic pump performance, we filtered MAb feedstocks through several filter types using both pumps. Their performance was comparable as measured by the filter-fouling profiles in Figures 3a–3c. This evaluation demonstrates comparable performance for both pumps. Although we observed comparable fouling profiles in our study, laboratory-scale pumps may not demonstrate filterability profiles for certain product solutions comparable to manufacturing-scale pumps. As with any scale-down pump, comparability studies should be performed to ensure accurate scale-up.

Table 1 shows the precision of our pump module for all four pump trains. Although the pump-head orientation allows for purging of entrapped air, we established a 30-mL/min (maximum flow rate) pump head priming procedure to ensure consistent dispense-volume performance. After sufficient priming, all four pump trains achieve very low dispense errors and deliver precise dispense volumes.

Control System Flexibility

Peristaltic Pump and Bench-Top–Scale Application: To accommodate the wide range of normal-flow filtration applications, our control module has numerous built-in capabilities beyond its default configuration. If needed, the control system and GUI can be used to control peristaltic pumps remotely. A user enters the maximum flow rate for

Table 1: Average pump module dispense errors

Train	Average Dispense Error
1	1.50%
2	0.25%
3	1.50%
4	0.33%

Experiment Details: 5-mL/min flow rate; 150-mL target volume; 10-psid applied back pressure; $n = 4$ experiments per train

Table 2: PendoTECH normal-flow filter screening system overview

Feature	Value
Filtration trains	4 (independent)
Pressure transducers	12 (3 per train)
Flow-rate range	0.2–30.0 mL/min.
Hold-up volume	~1 mL
Max. operating pressure	60 psi
Additional sensor inputs	8 (2 per train)

Figure 3A: Parvovirus filter; 0.84-mL/min flow rate; cation-exchange–purified MAb B

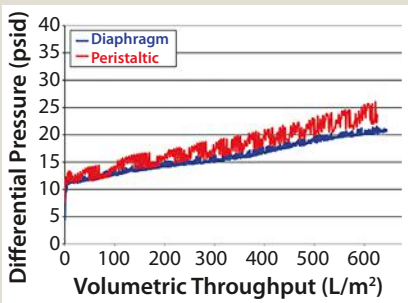


Figure 3B: Sterile filter; 2-mL/min flow rate; affinity-purified MAb C

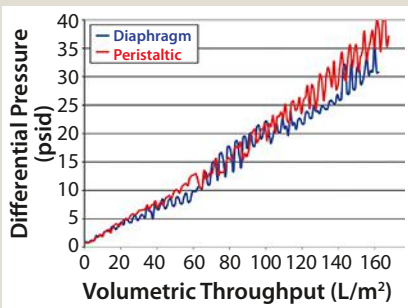
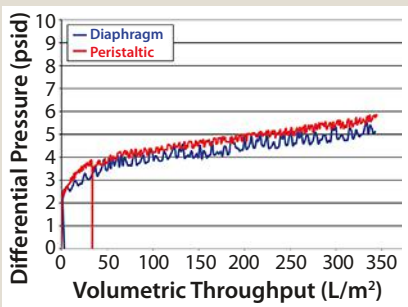


Figure 3C: Depth filter; 10-mL/min flow rate; centrate MAb D



Genentech (a member of the Roche Group) and PendoTECH COLLABORATED on developing an integrated filter-sizing system to address several disadvantages of the equipment commonly used.

a pump–tubing combination into the control system and sets the analog output signal based on those entered settings and maximum flow. All experimental set-ups and pump operations are identical to those used with the pump module. Users can enter a measurement of total volume filtered as an optional setting. Total volume is calculated either by flow totalization (as when the pump module is used) or by filtered volume measurement with bench-top scales that can be connected to the back of the control system.

In-line Sensors and Devices: Built-in connection ports on the back panel of the control system enable use of other devices with mA or voltage outputs. Turbidity sensors, RTDs, pH and conductivity probes, proximity switches, level sensors, liquid sensors, bubble detectors, flow meters, or other devices can be interfaced with this system. Eight total analog inputs (two per train) can be used in the range of 0–10 V or 4–20 mA. With that feature enabled, data from the inputs are displayed by the GUI system view and logged to the main data file with all other process data.

Feedback Flow Control: In addition to constant flow control, this system can run pumps under feedback control, in which a signal relays pressure data to the control system to vary the pump speed once it reaches a designated pressure set-point. This feature can be useful for achieving a maximum volumetric throughput before filter fouling in validation studies, for example. Selecting the "Pressure Control" button in the experimental set-up tab on the GUI enables this feature, and each train can be configured with a different pressure set-point.

Constant Pressure Application: For additional versatility, a "Constant Pressure" tab on the GUI screen allows for experiments with intentional filter fouling carried out under constant pressure. Taking advantage of the ability to read four scales, the system automatically plots time and volume data at a fixed pressure and performs real-time calculations during the experiment.

Improving Process Development

Genentech and PendoTECH worked together to develop and produce an integrated and semiautomated system that aids in efficient evaluation of filter media for manufacturing-scale processes. This normal-flow filter sizing system is widely adopted across the global Genentech network, where it has been used for a broad range of filter sizing and screening applications. Implementation of the system has increased productivity in process development. It has been invaluable for early and late-stage process development as well as supporting commercial downstream processes.

At the time of original publication, ***Brian Kluck*** *was an engineer II in process technical development, and* ***Binh Thai*** *was an engineer II in device development at Genentech Inc., 1 DNA Way, South San Francisco, CA 94080; thai.binh@gene.com. Corresponding author* ***Jim Furey*** *was general manager, and* ***Dennis Annarelli,*** *PhD, was technical manager at PendoTECH, 66 Witherspoon Street, Suite 256, Princeton, NJ 08542; 1-609-799-2299, fax 1-609-784-7889, jim@pendotech.com; dennis@pendotech.com.* *This article first appeared in the June 2011 issue of* BioProcess International.

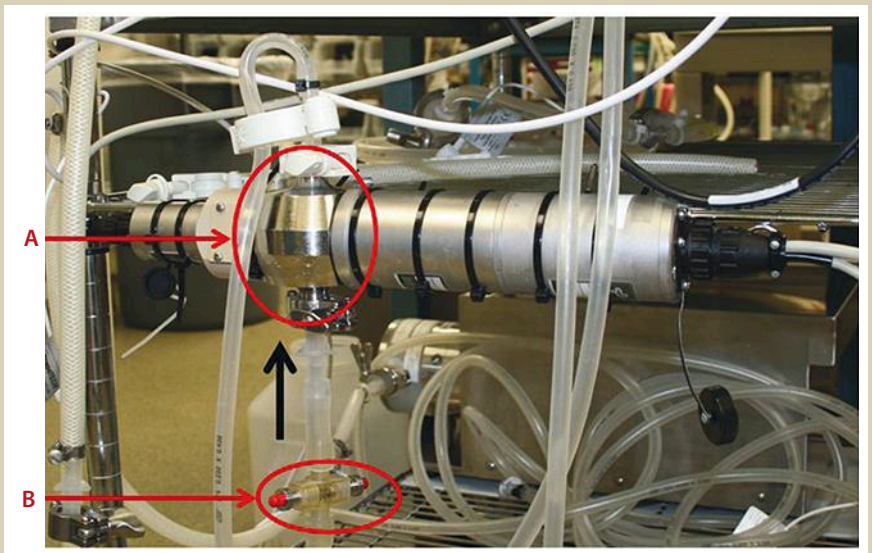
Evaluation of a New Single-Use UV Sensor for Protein A Capture

Paul Renaut and Dennis Annarelli

As the adoption of single-use systems continues to expand beyond bags and tubing to complete process steps, a full range of sensing technologies will be needed to complement the resulting varied single-use applications. Single-use sensors must meet or exceed the performance of traditional sensing technologies in areas such as accuracy, response time, ease of use, control system integration, process compatibility, regulatory requirements, and cost. Single-use flow-through process sensors are currently available for pressure, temperature, flow, and conductivity. Here, we report results from a comparative study of a new single-use flow-through UV absorbance sensor against traditional UV detection equipment in a protein A capture chromatography application.

Photo 1: Assembled UV flow cell, optical couplers, fiber optic cables, and photometer/transmitter

Figure 1: UV flow cells installed on column outlet tubing showing (A) traditional UV absorbance flow cell and (B) PendoTECH single-use UV absorbance flow cell; black arrow indicates direction of flow.



Elements of UV Detection

Several types of chromatography steps are routinely used to separate components during downstream processing in biopharmaceutical manufacture. Protein A affinity chromatography with UV spectroscopic detection is one of the most important types, partly because of its strong affinity for antibodies.

UV detection is particularly useful in bioprocess applications because biomolecules can absorb specific wavelengths of light between 200 and 400 nm. In particular, proteins usually show strong absorbance around 280 nm because of the presence of aromatic amino acids in their structures. According to the well-known Beer's Law, the amount of

PendoTECH system's COMPACT DESIGN is evident with the single-use flow cell connected (using tubing with hose barbs) to the process.

Figure 2: Process chromatogram comparing UV absorbance at 280 nm from traditional monitor system (blue) and PendoTECH single-use system (red); equilibration, load, wash, elution, and postelution cleaning steps

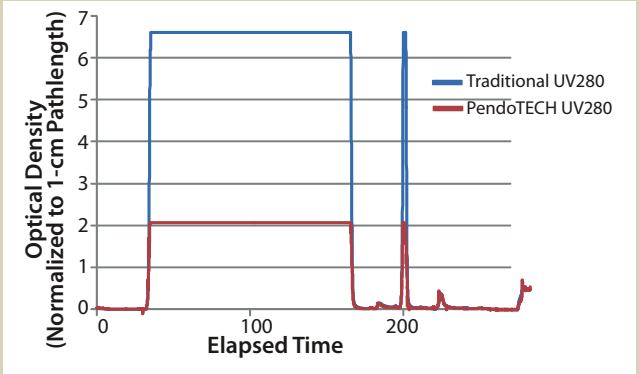


Figure 3: Process chromatogram comparing UV absorbance at 280 nm from traditional monitor system (blue) and PendoTECH single-use system (red); wash, elution, and postelution cleaning steps with a zoomed-in view on the baseline at lower absorbance values

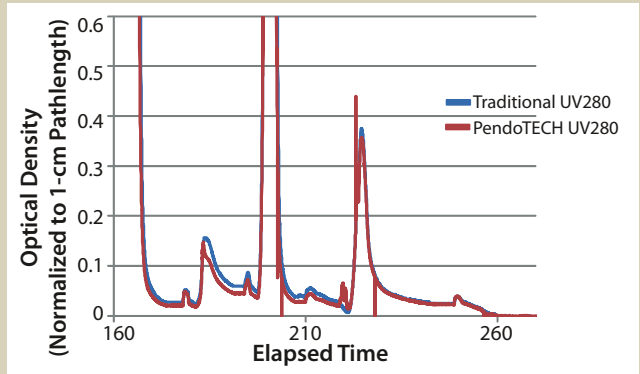


Table 1: Comparison of system design specifications

Attribute	PendoTECH System	Traditional System
Material for flow-cell optical windows	Silica glass	Sapphire
Light source	Light-emitting diode	Low-pressure mercury lamp
Detector	Silicon photodiode	Silicon photodiode
Repeatability	±0.5% of range	±0.5% of range
Linearity	±0.5% of range	±1% of range
Maximum zero shift	<2% of range	Not specified
Long-term output drift	<5% of range per month	Not specified

light absorbed is directly proportional to the concentration of an absorbing molecule in the path length of the light. The path length through which the light travels is also important. For long path lengths, more of a light-absorbing molecule is present in the light's path. A detector measures the ratio of incoming (incident) light energy to the energy that has passed through a sample as a measure of the absorbance of a material. A simple UV chromatogram compares time with absorbance in a flow-through system as molecules in a solvent move through a UV light beam.

In-line monitoring of UV absorbance during bioprocess chromatography is commonly applied for

- determination of when to start and end collection of a product-related peak
- confirmation of proper process execution during batch record review (by comparing a chromatogram with a reference chromatogram)
- identification and characterization of potential process disturbances during process investigations
- batch-to-batch process trending.

Often, accuracy at low-absorbance values (<0.5 OD) is most critical because peak collection start and end points typically occur at low-absorbance values. In certain applications, however, accuracy at higher absorbance values may be of interest such as when comparing peak heights or areas corresponding to impurity-related and product-related peaks for process trending. A UV system must have proper specifications to ensure that the instrument will have both the required sensitivity and range for a particular application.

A New UV Sensor

PendoTECH has developed a single-use, flow-through UV sensor with a path length of 1 cm and hose barb connections to attach tubing to a process stream (Photo 1). Reusable couplers for focusing UV light are screwed into the sensor. Attached to the couplers are fiber-optic cables that in turn are attached to a compact light source/detector photometer. The photometer is built with an LED light source of a customer-specified single wavelength from 240 to 1,000 nm.

For this application, the PendoTECH UV monitoring system provides a suitable single-use flow cell ALTERNATIVE to traditional equipment that are accompanied by fixed-asset stainless steel flow cells.

The photometer is powered by 24V from a wall supply and has a tare button to blank-out solvent background absorbance. The photometer also functions as a transmitter with a 4–20 mA analog signal scaled to 0–2 AU (absorbance units).

The single-use flow cell is made from USP Class VI, polysulfone free of animal-derived components. The cell windows through which UV light passes into a process stream are high-purity fused silica glass. The flow cell can be sanitized with dilute NaOH and can be gamma irradiated.

Comparative Studies

We placed the PendoTECH sensor/photometer/transmitter equipped with a 280-nm light source slightly upstream of a traditional UV-absorbance detector with a stainless-steel flow cell for a direct comparative trial of both approaches (Figure 1). PendoTECH system's compact design is evident with the

The SINGLE-USE flow cell is made from USP Class VI polysulfone free of animal-derived components. The cell windows through which UV light passes into a process stream are high-purity fused silica glass. The flow cell can be sanitized with dilute NaOH and can be gamma irradiated.

single-use flow cell connected (using tubing with hose barbs) to the process. The process application used for the evaluation was protein A chromatography for capture of protein from clarified mammalian cell culture fluids.

From both detector systems, transmitter outputs of 4–20 mA were connected to a National Instruments 9207 A/D converter for capture and recording of data on a PC using LabView software (National Instruments). The optical path length for the comparator traditional flow cell is 0.5 cm. To normalize absorbance results from the different detector path lengths, we converted absorbance into optical density (on a 1-cm path length basis) by using the following equation: Optical density = (instrument absorbance units, AU) ÷ (instrument optical path length, cm). Figures 2 and 3 provide example process chromatograms comparing results obtained from both monitoring systems.

The traditional UV absorbance measurement system has a maximum scale of 3 AU (OD = 6). The PendoTECH UV system maximum scale is 2 AU (OD = 2). Hence, the OD for the PendoTECH instrument is observed to go off scale in Figure 2 at a lower OD value than the traditional sensor.

Updates

Since this article was written, PendoTECH has broadened its UV flow-cell product line to include both a 5-mm path-length flow cell and a 2-mm path-length flow cell. PendoTECH has also introduced a dual-wavelength photometer. Additionally, using the technology to measure turbidity has become commonplace with the introduction of an 880-nm photometer. Finally, the technology has been applied to fluorescence applications.

Results: A Comparative Monitoring System

Aside from the different maximum-scale OD values, the process chromatograms are comparable (Figures 2 and 3). At low-absorbance values, the data from either monitor are quite similar (Figure 3), with only minor differences that may be attributable to small offsets in calibration and data acquisition signal conditioning. Peak collection in this process is based on UV absorbance value. The points for start and end of eluate peak collection are essentially the same for both monitor systems. For this application, the PendoTECH UV monitoring system provides a suitable single-use flow cell alternative to traditional equipment that are accompanied by fixed-asset stainless steel flow cells.

Further Reading

Furey J, Clark K, Card C. Adoption of Single-Use Sensors for BioProcess Operations. *BioProcess Int.* 9(5) 2011: S36–S42.

Schmid F-X. Biological Macromolecules: UV-Visible Spectrophotometry. *Encyclopedia of Life Sciences.* Macmillan Publishers: New York, NY, 2001.

Scott C. Ten Years of Chromatography. *BioProcess Int.* 10(6) 2012: S32–S36.

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Measuring Pressure at Very Low Levels with High Accuracy in Single-Use Systems

Improved Performance and Single-Use System Testing

Dennis C. Annarelli, Jim Furey, Benjamin Less, and Joshua Huang

Measuring pressure in single-use systems (SUS) has become an integral part of both upstream and downstream bioprocess operations. Articles have been published on filtration applications (**1**), and integration into other SUS has been widely adopted. Additionally, information is available on low-pressure applications such as how to prevent overpressurization in single-use bioreactors (**2**).

However, as users and applications both become more sophisticated, improved performance is sought for low-pressure applications (<1 psi) such as in single-use bioreactors. The reasons are two-fold: First, more information can be gleaned through improved accuracy in measuring a SU process condition. Second, with improved performance, useful applications such as leak testing and pressure hold can be better evaluated for implementation in SUS by using a sensor installed on a given system for in-process monitoring.

Single-use pressure sensors such as PendoTECH sensors have a specification of ±2% of reading. After a 10-minute warm-up of the electronics for best performance, followed by a tare relative to atmosphere, a sensor will read 0.00 psi when using a standard PendoTECH PressureMAT monitor. However, normal electrical variations within optimized, standard monitors that can read to 75 psi (to cover all applications) can contribute to a value referred to as "zero offset stability" or ZOS of ±0.03 psi. In a filtration application, a ZOS contributes negligibly to the error of a reading, and in many cases a user decides to not even display the second decimal place of 1/100ths. However, in a low-pressure application, where readings on the order of 0.1 psi are designed to be measured accurately, a ZOS error of 0.03 psi constitutes a high percentage of error. Therefore, improved resolution is desirable.

Using a pressure-hold test to detect leaks is a well-established practice. In the bioprocess arena, it is

Figure 1: Test set-up with PressureMAT HR

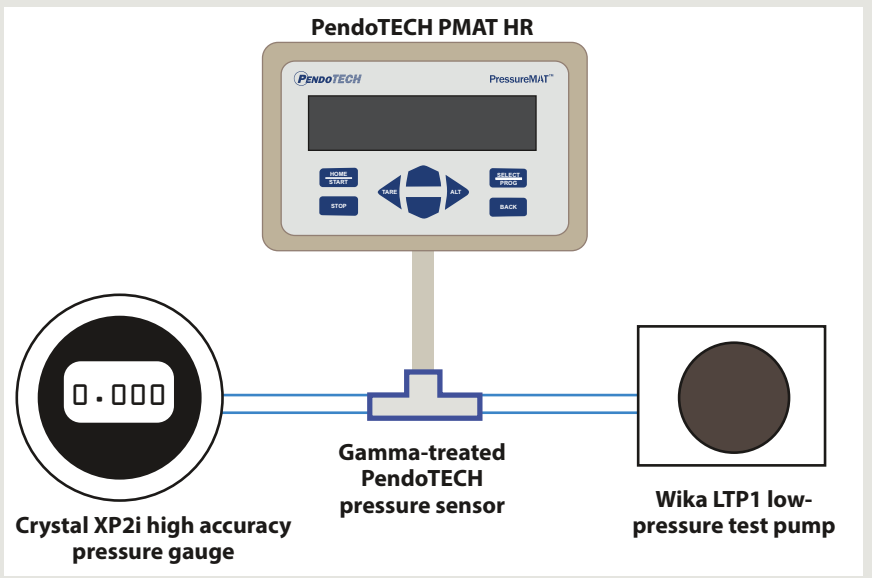
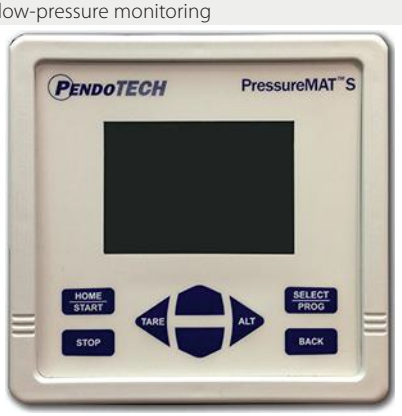


Photo 1: Single-channel PressureMAT unit for low-pressure monitoring



Materials and Methods

Monitor
PendoTECH PressureMAT HR model

Sensors
PendoTECH PRESS-S-000 single-use pressure sensors

Pressure Source
Wika LTP1 low-pressure test pump

Calibrated Pressure Gauge
Crystal XP2i digital pressure test gauge with 15-psi positive pressure and –14.5-psi vacuum at full scale

Irradiation Source
Steris Isomedix services

Irradiation Levels
27.5–33 kGy (low) and 40–45 kGy (high)

Table 1 (A and B): Results from low-pressure high-accuracy testing, Lot 1131340

A Lot 1131340 — Irradiation Level 27.5–33.0 kGy (low)

Gauge Pressure (psi)	Sensor # 1	2	3	4	5	Group Average	Standard Deviation	Relative Std. Dev.
–0.500	–0.502	–0.501	–0.500	–0.498	–0.501	–0.500	0.0015	0.30%
–0.250	–0.253	–0.250	–0.251	–0.250	–0.251	–0.251	0.0013	0.52%
–0.100	–0.101	–0.101	–0.100	–0.100	–0.100	–0.100	0.0005	0.55%
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.0000	0.00%
0.050	0.050	0.049	0.050	0.050	0.051	0.050	0.0004	0.90%
0.100	0.100	0.099	0.100	0.100	0.101	0.100	0.0004	0.45%
0.150	0.149	0.150	0.149	0.150	0.151	0.150	0.0005	0.37%
0.200	0.200	0.200	0.199	0.200	0.201	0.200	0.0004	0.22%
0.250	0.250	0.249	0.249	0.249	0.251	0.249	0.0005	0.22%
0.500	0.500	0.500	0.500	0.498	0.502	0.500	0.0009	0.18%
1.000	1.000	1.004	0.999	0.997	1.005	1.000	0.0025	0.25%
2.000	2.007	2.006	2.003	1.999	2.012	2.003	0.0035	0.18%

B Lot 1131340 — Irradiation Level 40.0–45.0 kGy (high)

Gauge Pressure (psi)	Sensor # 1	2	3	4	5	Group Average	Standard Deviation	Relative Std. Dev.
–0.500	–0.501	–0.500	–0.501	–0.499	–0.502	–0.500	0.0008	0.17%
–0.250	–0.251	–0.250	–0.251	–0.249	–0.252	–0.250	0.0008	0.33%
–0.100	–0.101	–0.101	–0.101	–0.099	–0.101	–0.100	0.0009	0.89%
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.0000	0.00%
0.050	0.049	0.049	0.050	0.051	0.050	0.050	0.0008	1.68%
0.100	0.099	0.099	0.100	0.101	0.100	0.100	0.0008	0.84%
0.150	0.149	0.149	0.150	0.150	0.150	0.150	0.0005	0.37%
0.200	0.200	0.200	0.200	0.201	0.200	0.200	0.0004	0.22%
0.250	0.250	0.249	0.250	0.251	0.251	0.250	0.0007	0.28%
0.500	0.500	0.500	0.501	0.501	0.502	0.500	0.0005	0.11%
1.000	1.002	1.000	1.002	1.000	1.004	1.001	0.0011	0.21%
2.000	2.007	2.004	2.007	2.004	2.010	2.004	0.0029	0.14%

the basis of some filter-integrity test methods for measuring a rate of diffusion through a wetted membrane and release testing on single-use bags. When a single-use sensor is used for a pressure-hold test to leak-test a complex and costly setup such as a single-use bioreactor, a ZOS of 0.03 psi could limit applicability.

Some background on the basis of the pressure-hold test takes us back to Boyle's Law and Charles's Law from the 17th century and 18th century, respectively. They were later combined and stated as the Ideal Gas Law. It describes the behavior of a gas under certain conditions and is represented by the equation

$$PV = nRT$$

where the letters denote absolute pressure, volume, amount (in moles), ideal gas constant, and absolute temperature of the gas.

In a pressure-hold test, the system's volume and temperature are held constant. That means that as the pressure decreases, n also drops as gas leaves the constant volume container. That can be readily converted to a leak rate by converting the ΔP in the system to ΔV at room conditions and applying the time over which the ΔP occurred and the known system volume:

$$\text{Rate} = (\Delta P \times V_{\text{system}})/(\text{test time} \times P_{\text{atm}})$$

That equation yields units of volume per unit time. There are two important factors to consider when applying this method to critical applications: The measured pressure is relative to atmospheric conditions at the time a sensor was tared, and temperature is directly proportional to pressure; any change will be interpreted as change in pressure.

For a short test time the effect of a change in atmospheric pressure would be minimal, but for a longer leak-test time, atmospheric pressure changes may come into play. For instance, a barometric pressure change of 20 mbar during impending weather could be interpreted as an increased pressure in the system being tested (or mask a pressure drop). Regarding shipping of a container under slight positive pressure and a monitor with the tare stored to a higher elevation, the container will experience an increase in pressure dependent upon altitude. For example, at 5,000 ft of elevation, the absolute atmospheric pressure is 12.2 psi (14.7 psi at sea level).

The impact of a change in temperature can be illustrated simply by using the ideal gas law. A vessel might be placed close to an air-vent outlet so that the building climate-control system could alter that vessel's air temperature beyond normal temperature variations. Using an absolute temperature of 293 K, a 3 °C shift can contribute a 1% test error.

So the question is whether a single-use sensor that is already being used on a SUS can measure pressure with sufficient accuracy to optimally measure process performance and be sensitive enough to measure a leak rate. To address the low-pressure application requirement, PendoTECH developed a modified

Table 1 (C and D): Results from low-pressure high-accuracy testing, Lot 1132283

C Lot 1132283 — Irradiation Level 27.5–33.0 kGy (low)

Gauge Pressure (psi)	Sensor # 1	2	3	4	5	Group Average	Standard Deviation	Relative Std. Dev.
−0.500	−0.499	−0.500	−0.500	−0.499	−0.501	−0.500	0.0005	0.11%
−0.250	−0.250	−0.250	−0.250	−0.250	−0.250	−0.250	0.0000	0.00%
−0.100	−0.100	−0.100	−0.100	−0.100	−0.100	−0.100	0.0000	0.00%
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.0000	0.00%
0.050	0.050	0.050	0.050	0.049	0.050	0.050	0.0004	0.90%
0.100	0.099	0.100	0.100	0.099	0.100	0.100	0.0005	0.55%
0.150	0.150	0.150	0.150	0.149	0.150	0.150	0.0004	0.30%
0.200	0.200	0.200	0.200	0.199	0.201	0.200	0.0004	0.22%
0.250	0.249	0.250	0.250	0.249	0.250	0.250	0.0005	0.22%
0.500	0.500	0.500	0.501	0.499	0.501	0.500	0.0007	0.14%
1.000	1.000	1.001	1.002	0.999	1.002	1.000	0.0011	0.11%
2.000	2.002	2.003	2.008	1.999	2.008	2.002	0.0035	0.18%

D Lot 1132283 — Irradiation Level 40.0–45.0 kGy (high)

Gauge Pressure (psi)	Sensor # 1	2	3	4	5	Group Average	Standard Deviation	Relative Std. Dev.
−0.500	−0.500	−0.498	−0.501	−0.499	−0.501	−0.500	0.0011	0.23%
−0.250	−0.250	−0.248	−0.250	−0.250	−0.250	−0.250	0.0009	0.36%
−0.100	−0.101	−0.100	−0.100	−0.100	−0.100	−0.100	0.0004	0.45%
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.0000	0.00%
0.050	0.049	0.051	0.051	0.050	0.050	0.050	0.0008	1.67%
0.100	0.100	0.101	0.101	0.100	0.100	0.100	0.0005	0.55%
0.150	0.150	0.150	0.151	0.150	0.150	0.150	0.0004	0.30%
0.200	0.199	0.200	0.201	0.201	0.201	0.200	0.0008	0.42%
0.250	0.249	0.250	0.251	0.251	0.251	0.250	0.0008	0.33%
0.500	0.499	0.499	0.501	0.501	0.501	0.500	0.0010	0.20%
1.000	1.000	0.998	1.005	1.002	1.003	1.001	0.0026	0.26%
2.000	2.003	1.999	2.008	2.005	2.008	2.003	0.0037	0.18%

version of its PressureMAT monitor for single-use sensors. This version is designated as the "HR" model for "high resolution." By contrast with the standard monitor that can read to 75 psi, the HR models have been modified and optimized for low-pressure measurements to 7.5 psi (~0.5 bar). That is more than adequate for low-pressure applications, and the ZOS is reduced to <0.003 psi. An important point is that HR models function with standard PendoTECH single-use pressure sensors, and only the PressureMAT monitor electronics have been modified and optimized for the low-pressure reading. The HR models have the same features of standard models such as 4–20 mA analog outputs for integration to a user's control system, alarm relay outputs, and serial data output for connection to a PC-based data acquisition system.

A study was undertaken to verify the accuracy of the combined PMAT HR with standard PendoTECH single-use pressure sensors after they had been exposed to two typical levels of gamma irradiation. The results of that study follow (see the Materials and Methods box).

Irradiation

Thirty sensors from three different lots were randomly selected and subjected to two levels of gamma irradiation: 27.5–33 kGy (low) or 40–45 kGy (high). Five sensors from each lot were treated at the low level and five at the high level. Sensors were sent for irradiation in their standard individually packaged pouches. The sensors were then shipped to PendoTECH in their sealed bags for testing with the PMAT HR. Figure 1 shows the testing setup.

Testing Process

Using the Wika LTP1 low-pressure test pump in conjunction with the Crystal XP2i high-accuracy pressure test gauge, the sensors were subjected to vacuum at –0.50, –0.25, and –0.10 psi and then pressurized to 0.05, 0.10, 0.15, 0.20, 0.25, 0.50, 1.00, and 2.00 psi. The pressure displayed on the PendoTECH HR pressure monitor was recorded at each pressure or vacuum level, and results were compared with that from the Crystal gauge.

Results

Table 1 (A–F) reports the testing results after applying a known pressure/vacuum to each sensor from all lots exposed to both irradiation levels. The averages, standard deviations, and RSDs are calculated and reported for sensors grouped by lot and irradiation level. These results show that group averages, measured to three decimal places, are nearly identical to the applied pressure. The accuracy specification of the sensors is ±2% of reading plus the 0.003 psi ZOS. Looking at the data in Table 1, all of the data points on the 30 sensors tested meet that accuracy specification. The statistics also demonstrate the precision of the performance.

For potential use in a pressure-hold/leak-test application, the contribution of the 0.003 psi ZOS can be calculated by the following example. Populate the formula based on testing a SUS with an approximate bag volume of 1,000 L for converting pressure hold to leak rate from

$$\text{Rate} = (\Delta P \times V_{system})/(\text{test time} \times P_{atm}) =$$
$$(\pm 0.003 \text{ psi} \times 1{,}000 \text{ L})/$$
$$(10 \text{ min} \times 14.7 \text{ psi}) = \pm 0.020 \text{ L/min}$$

So during a potential test, you could expect to see a potential fluctuation of ±0.003 psi corresponding to a value of ±20 mL/min during the test that would not be interpreted as a leak. This rate value would go up or down proportionately with larger or smaller

Updates

Since this article was written, PMAT-HR models have become a common device for monitoring pressure in bioreactors and other low pressure applications. Additionally, PendoTECH pressure sensors are demonstrated to be drift free and to maintain their accuracy over up to 90 days of continuous use.

Data show that two typical levels of gamma irradiation make no significant difference in the accuracy or consistency of results. The increased resolution of the PressureMAT HR by 10× over the standard monitor REDUCES THE ERROR of a low-pressure process measurement and improves its capability for leak testing in SUS.

Table 1 (E and F): Results from low-pressure high-accuracy testing, Lot 1131350

E **Lot 1131350 — Irradiation Level 27.5–33.0 kGy (low)**

Gauge Pressure (psi)	Sensor # 1	2	3	4	5	Group Average	Standard Deviation	Relative Std. Dev.
−0.500	−0.500	−0.500	−0.500	−0.500	−0.503	−0.500	0.0000	0.00%
−0.250	−0.251	−0.250	−0.250	−0.250	−0.253	−0.250	0.0004	0.18%
−0.100	−0.990	−0.100	−0.101	−0.101	−0.103	−0.100	0.0007	0.71%
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.0000	0.00%
0.050	0.050	0.051	0.050	0.049	0.048	0.050	0.0007	1.41%
0.100	0.100	0.101	0.101	0.099	0.099	0.100	0.0008	0.83%
0.150	0.150	0.151	0.151	0.150	0.149	0.150	0.0005	0.36%
0.200	0.200	0.200	0.202	0.200	0.198	0.200	0.0009	0.45%
0.250	0.250	0.251	0.252	0.250	0.248	0.251	0.0009	0.36%
0.500	0.501	0.500	0.501	0.500	0.499	0.500	0.0005	0.11%
1.000	1.002	1.003	1.004	1.002	1.000	1.002	0.0015	0.15%
2.000	2.006	2.006	2.009	2.009	2.005	2.006	0.0037	0.18%

F **Lot 1131350 — Irradiation Level 40.0–45.0 kGy (high)**

Gauge Pressure (psi)	Sensor # 1	2	3	4	5	Group Average	Standard Deviation	Relative Std. Dev.
−0.500	−0.500	−0.500	−0.501	−0.501	−0.500	−0.500	0.0005	0.11%
−0.250	−0.250	−0.250	−0.250	−0.251	−0.251	−0.250	0.0004	0.18%
−0.100	−0.100	−0.100	−0.100	−0.100	−0.100	−0.100	0.0000	0.00%
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.0000	0.00%
0.050	0.051	0.050	0.050	0.051	0.050	0.050	0.0005	1.09%
0.100	0.100	0.100	0.100	0.101	0.100	0.100	0.0004	0.45%
0.150	0.151	0.150	0.150	0.151	0.150	0.150	0.0005	0.36%
0.200	0.201	0.201	0.200	0.201	0.201	0.201	0.0005	0.27%
0.250	0.251	0.251	0.250	0.252	0.251	0.251	0.0008	0.33%
0.500	0.500	0.501	0.501	0.503	0.501	0.501	0.0012	0.24%
1.000	1.003	1.002	1.003	1.006	1.002	1.003	0.0022	0.22%
2.000	2.009	2.007	2.008	2.017	2.010	2.008	0.0061	0.30%

containers. This ZOS factor would need to be considered for any test design. And relative to the single-use systems having a constant volume (V), a settling time should be considered before the final tare to achieve rigid position of the somewhat flexible components.

High Accuracy at Low-Pressure

Leak-testing/pressure decay on single-use systems with air is feasible by applying the ideal gas law as long as a pressure sensing device can measure to the accuracy required for a low-pressure system such as in single-use bioreactors and storage bags. The commercially available inSITE inflation and integrity test system from Advanced Scientifics, Inc. can automate that type of test. The data presented here support that the PressureMAT HR monitor with the PendoTECH single-use pressure sensors provides excellent experimental results for high-accuracy pressure measurement in the range of −0.500 to 2.000 psi. Furthermore, the data show that two typical levels of gamma irradiation make no significant difference in the accuracy or consistency of results. The increased resolution of the PressureMAT HR by 10× over the standard monitor reduces the error of a low-pressure process measurement and improves its capability for leak testing in SUS. And finally, at low-pressure levels, to prevent false results during any type of pressure hold test, atmospheric pressure changes or localized temperature variations must be taken into consideration.

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*At the time of original publication, corresponding author **Dennis C. Annarelli** was technical manager, and **Jim Furey** was general manager at PendoTECH , 174 Nassau Street, Suite 256, Princeton, NJ 08542; dennis@pendotech.com. **Benjamin Less** was an engineering intern at PendoTECH when this testing was completed. And **Joshua Huang** was an engineering intern at PendoTECH and a student at Rutgers University. This article first appeared in the March 2015 issue of* BioProcess International.

In-Line Turbidity Sensors for Monitoring Process Streams in Continuous Countercurrent Tangential Chromatography

Dmitriy Fedorenko, Jasmine Tan, Oleg Shinkazh, and Dennis Annarelli

A strong connection between turbidity and total suspended solids (TSS) has been linked in the past to measuring well-defined particles in processes. Optical-density probes have seen wide adoption in the biotechnology industry for monitoring cell growth within a bioreactor, whereas in-line turbidity sensors have been used to monitor filter performance. Turbidity measurements offer a rapid quantification of suspended solids but have not been used in the biotechnology industry for chromatographic resins. In this study, turbidity measured with equipment developed by PendoTECH was used with novel continuous chromatography technology developed by Chromatan for accurate measurements of chromatography resin slurry concentrations.

CCTC

Continuous countercurrent tangential chromatography (CCTC), developed by Chromatan Corporation, has overcome many limitations of batch columns without the drawbacks of multicolumn systems (**1**). The CCTC platform is a true moving-bed technology that runs at steady state and eliminates the use of columns. The system's novel design enables

Figure 1: PendoTECH turbidity flowcell placed at the resin outlet of the CCTC; resin passing through the flowcell indicates a signal breakthrough, and the system is set to recirculation once the resin in the system has reached steady state. The steady-state "peak-free"product stream can be measured in-line with PendoTECH UV flowcells.

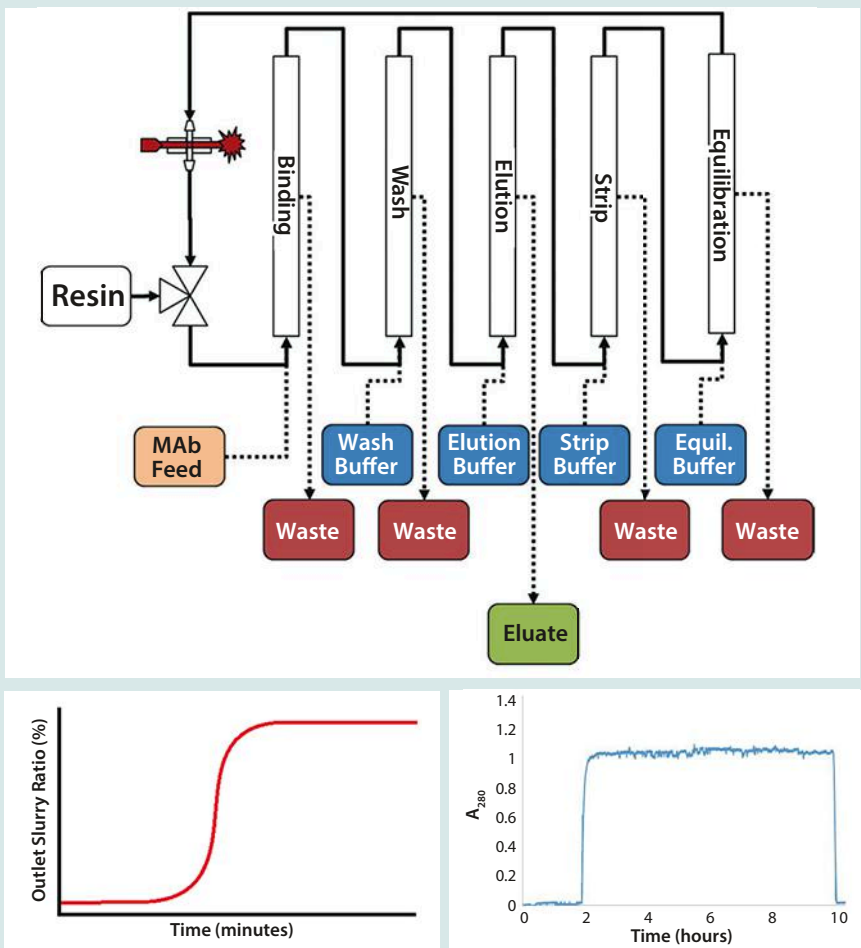


Figure 2: End-to-end integrated bioproduction using CCTC in development at Chromatan

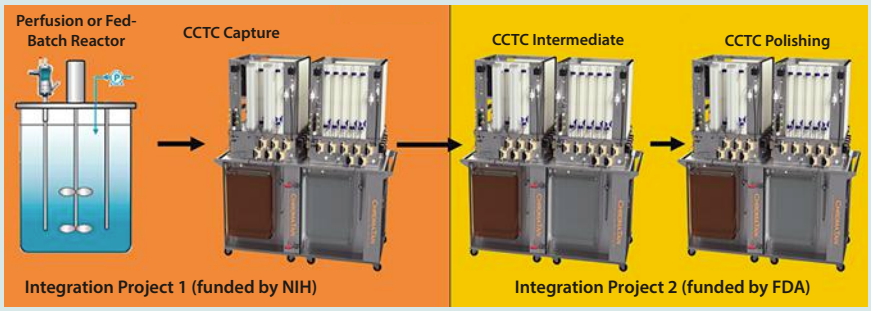
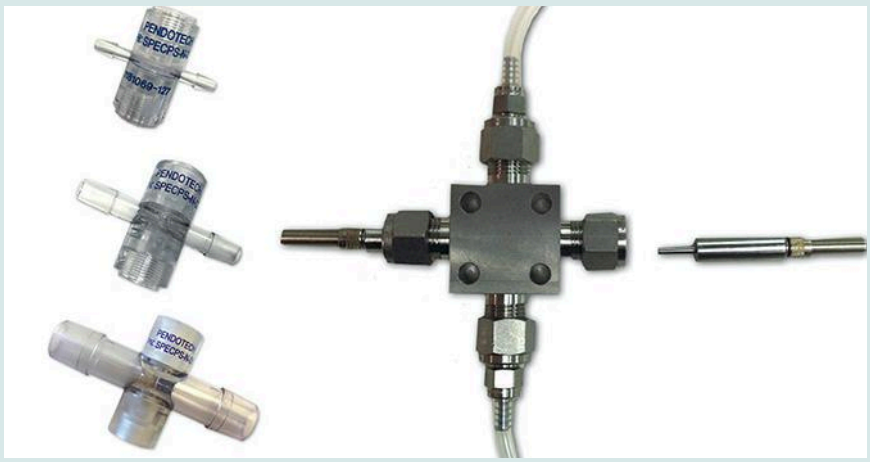


Figure 3: Turbidity flowcell options from PendoTECH — fixed pathlength single-use flow cells (left) and adjustable pathlength flowcell (right).



CCTC is uniquely suited for processing SENSITIVE MOLECULES because of its ability to control buffer and micromixing conditions independently for all chromatographic steps.

short residence times, consistent product concentration and quality, and easier implementation of advanced process analytical technology (PAT) and process control. In addition, CCTC is uniquely suited for processing sensitive molecules because of its ability to control buffer and micromixing conditions independently for all chromatographic steps. That eliminates product-related concentration and buffer gradients that always accompany column-based separations.

The patented CCTC system includes all of the traditional chromatographic process steps: binding, washing, elution, stripping, and equilibration. Those are comparable with traditional batch chromatography, but they are conducted simultaneously on a moving slurry rather than with the step-wise batch approach of a packed column (Figure 1). Each step comprises a cascade of stages that consist of a single static mixer connected in series to a hollow-fiber membrane. Countercurrent configuration of these stages enables enhanced impurity removal and higher yield for each chromatographic step. The static mixers provide sufficient residence times for adsorption/desorption to occur. The microporous hollow-fiber membranes retain the large resin particles while allowing all dissolved compounds (proteins and salts) to diffuse across the membrane into the permeate.

The CCTC system has been shown to operate continuously 5–15× higher productivity than with batch columns, with the product eluting at steady state. The steady-state "peak-free" product stream can be measured in-line for product quality attributes. The steady-state nature of the process also enables seamless integration with other continuous/in-line unit operations. More details are provided in Dutta et al. (2).

As a consequence of these benefits, The National Institutes of Health (NIH) has funded a US$1.75 million Fast-Track Phase 2 SBIR to support the integration of CCTC and a perfusion bioreactor into a single steady-state bioproduction platform. The FDA also has financed a $2.5 million contract to develop and commercialize a fully integrated continuous downstream process that includes capture, intermediate, and polishing CCTC steps for antibody purification (Figure 2).

As a major strategic deliverable, both integration projects require significant developments of in-line process analytical technologies (PAT) and continuous process monitoring. Single-use sensors for process parameters such as pH, conductivity, flow rate, and pressure have been developed and tested by the industry. However, because of the true moving-bed nature of CCTC, it also has become necessary to develop a robust in-line measurement for accurate determination of chromatography resin concentration in the CCTC slurry.

PendoTECH

PendoTECH offers in-line single-use UV absorbance and turbidity measuring and monitoring systems that collect data from bioprocess fluid streams. Three single-use polysulfone optical flowcells with pathlengths of 2 mm, 5 mm, and 1 cm with hose-barb process connections can be installed in-line to any process stream. Reusable couplers for focusing light are screwed into the flowcell. Fiber-optic cables connect the couplers to the light source and to the detector in the compact photometer. The output from the photometer is a 4–20 mA signal scaled 0–2 AU. The systems use an LED to generate a single

Figure 4: Poros 50HQ (●) and Purolite (■) resins were measured with a benchtop Hach 2100Q turbidity meter. Purolite could be measured directly for slurry ratios 2.5–20% v/v with a strong correlation between the NTU and the slurry ratio. The Poros resin was too turbid and needed to be diluted to a slurry ratio <0.35% v/v.

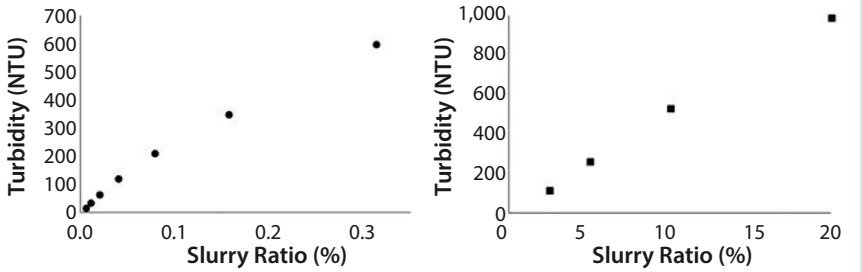
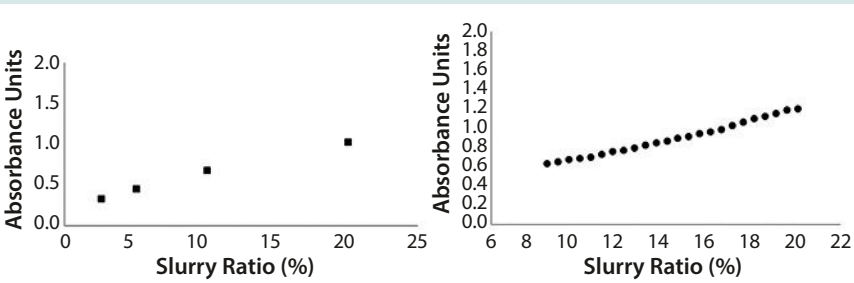


Figure 5: Purolite (■) and Poros 50HQ (●) resins were monitored with a PendoTECH flowcell at 880 nm with pathlengths set to 2 mm and 0.5 mm, respectively. The Purolite resin had a linear range for slurry ratios 2.5–20% and an R^2 of 0.99. The Poros resin had a linear range for slurry ratios 6.5–20% and an R^2 of 0.99.



wavelength of light, customizable by PendoTECH from 240 nm to 1,000 nm. Additionally, PendoTECH offers a stainless steel flowcell with adjustable pathlengths of 0.05–2.0 mm, allowing end users to set the pathlength for a linear response to the process stream. Figure 3 shows the flowcell offerings.

Past work has demonstrated that using the principles of the Beer-Lambert law, a flowcell pathlength can be selected to measure a process stream accurately in its linear range (3). A 2-mm pathlength flowcell has been used with a 280-nm light source to measure the elution stream of a protein in a capture process in the CCTC. This in-line sensor provides accurate concentration and yield measurements at one-second intervals, enabling PAT.

Materials and Methods

Resin Preparation: Two resin types were tested: an agarose backbone 22-µm resin from Purolite; and POROS 50HQ, a polystyrene divinylbenzene (PSDVB) backbone 50-µm particle-size resin from Thermo Fisher. PSDVB is used as a standard for calibrating turbidity meters. The agarose resin is significantly less turbid. Using these materials enabled investigations of the in-line turbidity at a wide range of slurry ratios and absorbances.

Stocks of both resins were exchanged into 1 × PBS and prepared into 20%, 10%, 5%, and 2.5% v/v slurry ratios. The slurry ratios of all the stocks were measured in duplicate by gravity settling for 24 hours in 12.5-mL Koehler K61141 centrifuge tubes.

Offline Turbidity Meter: A Hach 2100Q turbidity meter, a nephelometer with a 90° detector angle from incident light and 1-inch pathlength, was calibrated with Stabical Formazin primary standards from 10 to 800 NTU and used to measure the primary stocks of agarose and PSDVB resin slurries. The 2100Q has a maximum reporting turbidity of 1,000 NTU. Traditional nephelometers can provide an accurate measurement of turbidity up to 2,000 NTU depending on the excitation light source and detector configuration.

This in-line sensor provides ACCURATE concentration and yield measurements at one-second intervals, enabling process analytical technology.

The agarose measured with the 2100Q demonstrated a strong linear relationship for the measured slurry ratios 2.5%–20% v/v slurry ratio and 120–1000 NTU, as shown in Figure 4, with an R^2 of 0.99.

Although Formazin is the only recognized primary standard for calibrating turbidity meters because of its relative stability, size distribution, and consistent 90° light scatter, PSDVB also has been used as a primary standard for calibrating turbidity meters. However, the PSDVB slurries saturated the detector. The 2.5% slurry stock of PSDVB was diluted into range, and a dilution curve from 0.004% to 0.31% v/v slurry was generated from 20 to 600 NTU (Figure 4). ISO 7027-compliant nephelometers are limited in this regard and provide only a linear measurement that can be used for quantification of suspended solids such as resin only from 0 to 40 NTU, necessitating a large dilution factor. In a practical sense, however, the large dilution factor could introduce a significant source of error that might interfere with efficient loading of resin into the CCTC system.

PendoTECH Turbidity Meter: A test solution was agitated with an overhead mixer set to 400 rpm, and a peristaltic pump delivered the fluid through the flowcell in an upflow configuration and back to the beaker. The agarose resin had a linear relationship for transmittance in a 2-mm pathlength for the slurry ratio range of 2.5–20% with an R^2 of 0.99 (Figure 5). The PSDVB resin was beyond the linear range for a 2-mm pathlength and slurry ratios >10% v/v. Further testing was performed with shorter pathlengths. Both resins demonstrated

a stable equivalent reading for flowrates 5–40 mL/min at the same slurry ratios.

A 20% stock of PSDVB was prepared and serially diluted with an identical setup to the agarose resin, but with a shorter pathlength. The PSDVB resin had a linear relationship for transmittance in a 0.51-mm pathlength for the slurry ratio range of 5–20% with an R^2 of 0.99 (Figure 5). Additionally, the PSDVB testing demonstrated that tuning into a linear range is readily accomplished with the PendoTECH stainless steel flowcell by adjusting the pathlength. The experiment also demonstrated strong sensitivity to slurry ratio and can be used to quantify the exact concentration of resin being circulated within the CCTC system.

Integration with CCTC

Flowcells and photometers offered by PendoTECH enable in-line monitoring of process streams for CCTC in both single-use and reusable formats. This study demonstrates that in addition to monitoring product titers, optical sensors also can be used for measuring and controlling CCTC resin concentrations.

The flowpath in Figure 1 shows an integrated PendoTECH optical sensor at the resin-loading manifold. The location of the sensor helps determine resin cycle time in the CCTC, which is used to calculate resin volume and CCTC productivity. The sensor also measures outlet slurry ratio, providing in-line measurement of timing to reach steady-state. Once the outlet resin concentration reaches the inlet, the CCTC system automatically switches the flow path into a closed loop resin circulation mode (by-passing the resin tank). With the known cycle time, resin also can be unloaded once the full lifetime has been reached and replaced with fresh resin if necessary.

Additionally, monitoring the exact concentration entering the binding step allows for precise resin loading. This has a strong effect on output yield and product quality especially for ion-exchange operations. Unlike batch columns, in which concentration gradients and product peaks are a direct consequence of the sequential batch operation, the CCTC can target exact loading and elution conditions throughout the process without compromising yield or product quality.

Overall, the PendoTECH single use flow cells and customizable photometers open a wide range of POSSIBILITIES for in-line process control and PAT. Turbidity and UV sensors enable the CCTC platform to provide accurate measurements of titer and yield in the product streams as well as accurate resin concentration measurements for system startup and efficient resin cycling.

Overall, the PendoTECH single-use flowcells and customizable photometers open a wide range of possibilities for in-line process control and PAT. Turbidity and UV sensors enable the CCTC platform to provide accurate measurements of titer and yield in the product streams as well as accurate resin concentration measurements for system startup and efficient resin cycling.

Chromatan and PendoTECH believe that joining together to develop such technologies is essential for development and eventual adoption of continuous single-use manufacturing at the current good manufacturing practice (CGMP) scale. We look forward to initiating and continuing such collaborations with one another, as well as with other customers and suppliers.

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A UF–DF Screening System for Bioprocess Development

Efficient and Cost-Effective Process Fit and Scale-Up to Manufacturing

Atul Bhangale, Yan Chen, Anurag Khetan, and Jim Furey

Ultrafiltration and diafiltration (UF–DF) of therapeutic proteins are performed in either tangential or crossflow mode using membrane filters. UF–DF plays a critical role in both downstream and upstream processes for the biopharmaceutical industry (**1**). In upstream production processes, classical tangential-flow filtration (TFF) or alternating tangential-flow (ATF) systems are used in high–cell-density perfusion for protein expression by cell culture (**2**). TFF is used in downstream processing for UF–DF and concentration of therapeutic proteins. TFF unit operations are common in protein purification because of their scalability and amenability to continuous processing (**3–5**). Typical TFF process development involves optimization of transmembrane pressure (TMP), permeate flux, feed flowrate, buffer compositions, and the interdependence of all those parameters. Optimization requires a broadly defined design space and many experiments needing significant time and resource investments.

With speed to first-in-human initiatives leading to shrinking timelines in early process development, the demand for enhanced throughput has increased significantly in recent years. Expedited timelines and minimal material availability in early phases complicate process development of TFF. For increased throughput on

Expedited timelines and minimal material availability in early phases are known to COMPLICATE process development of tangential-flow filtration.

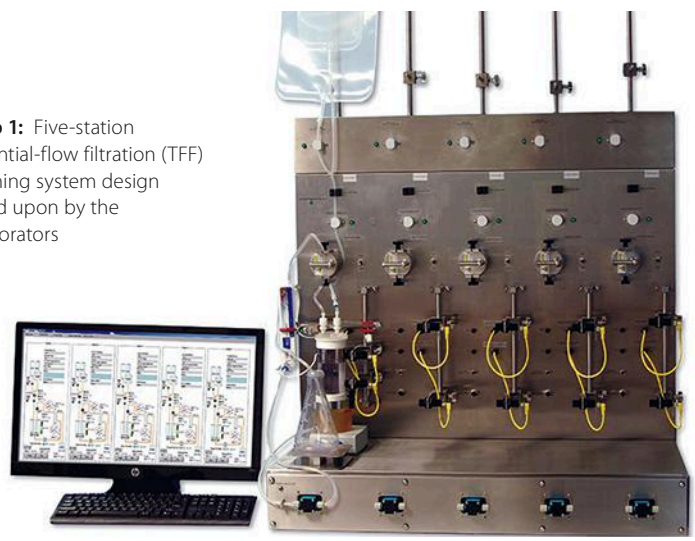


Photo 1: Five-station tangential-flow filtration (TFF) screening system design agreed upon by the collaborators

Table 1: Key objectives and system requirements for UF–DF; GUI = graphical user interface

Scale	Screening	Intermediate-Scale Process Development	Manufacturing
Key objectives	Screening membranes and buffer conditions; precise process-data acquisition	Process optimization; characterization; improvement of process yield, consistency, and performance validation	Reproducible product yield; quality and purity; robust process control; precise data acquisition
Process volumes	0.1–0.5 L	0.5–5.0 L	5.0–50 L
Key system features	High-throughput, walk-away automation; scalability; user-friendly GUI; wide operating range	Scalability; precise data acquisition; reproducibility; safety controls and notifications; low hold-up volume; simple design; easy clean-up and installation; superior in-process controls	Highest level of safety controls; low hold-up volume; minimal maintenance; easy installation and validation; superior in-process controls

Table 2: Designed process capabilities

	Tested Operating Range	100-cm² Membrane	Accuracy
Feed flow	240–480 LMH	40–80 mL/min	±5% of setting
Permeate flux range	25–75 LMH	4.2–12.5 mL/min	±0.1 mL/min
Transmembrane pressure	10–30 psi	10–30 psi	±1 psi
Inlet feed pressure	0–40 psi	0–40 psi	0–40 psi

Table 3: Feed pump and permeate scale sensitivity

	Range	Lowest Detection Limit
Feed pump	1.0–100 mL/min	1.0 mL/min
Permeate scale	0–1,000 g	0.01 g
	0–3,000 g	0.1 g

chromatography-based unit operations, options are available such as high-throughput chromatography and robotic screening tools such as TECAN systems. However, few such tools are available commercially for filtration unit operations. The few systems on the market require users to adapt to device requirements, leaving very little flexibility to work. It is important that such tools provide as smooth a flow as possible, with accurate information for scaling up TFF processes to intermediate (for further process development/verification) and then manufacturing scales (Table 1).

Early phase process development verifies initial platform fit assessment of a downstream process and helps companies determine whether downstream process modifications are needed. Early phases of process development often require rapid decisions made regarding choice of membrane types and UF–DF process conditions. Although the objectives for early phase development primarily focus on determining platform suitability of process conditions for a given molecule, late-stage UF–DF development focuses on more detailed process characterization and risk assessment. That includes improving process yield, throughput, and consistency as well as performance validation (reproducibility, sensitivity, and lot-to-lot variations) with a representative scale-down model. For a suitable scale-down model, it is critical to distinguish between system- and process-related variations in UF–DF. For process development or characterization, superior control of system parameters helps developers differentiate between system- and process-related responses.

Early phases of process development often require RAPID decisions made regarding choice of membrane types and UF–DF process conditions.

Here we report the design and development of a fully automated TFF multistation consisting of five independently operated TFF stations with advanced system controls that can run in parallel for screening UF–DF process conditions (Photo 1).

Benefits of Laboratory-Scale Process Automation

A typical laboratory-scale TFF process is semiautomated with a few pumps, some dial pressure gauges, a scale, and manual valves. Integrating system parts from multiple suppliers poses a challenge. Data logging requires an operator to monitor and export process data manually to third-party software for analysis, often with the challenge of merging data from

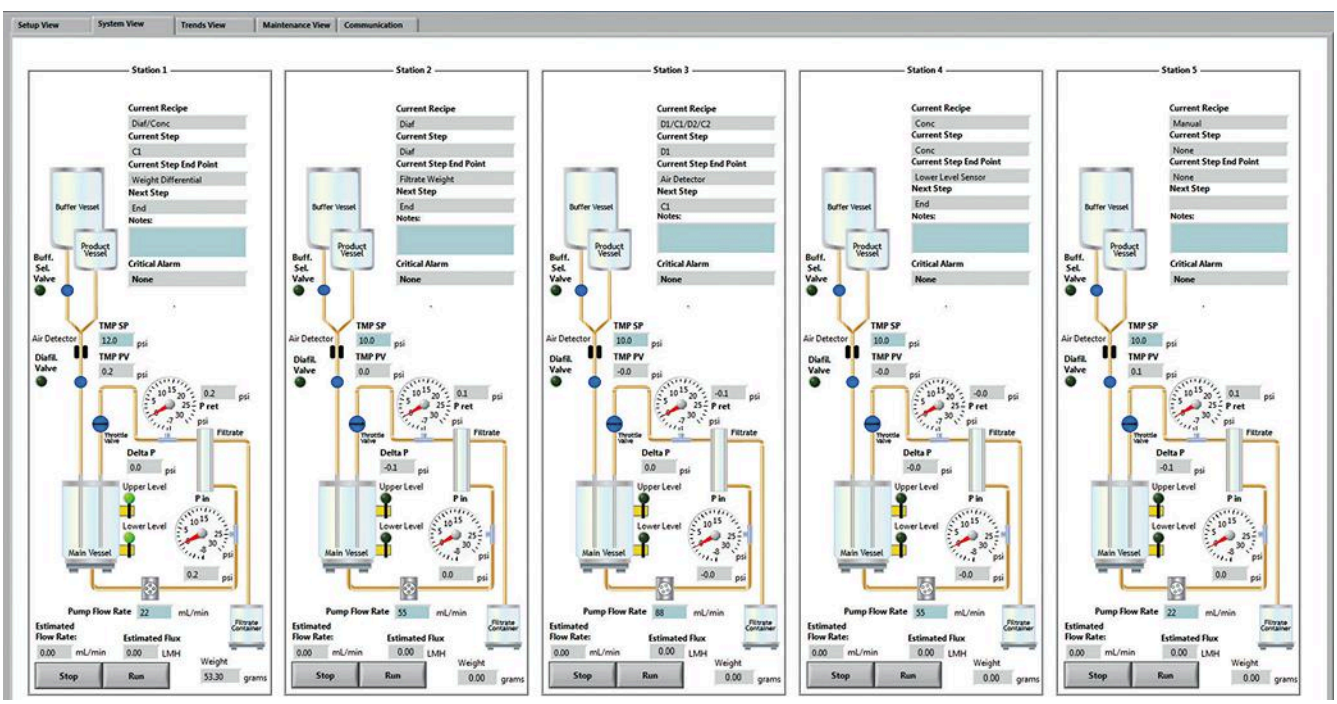


Photo 2: Five-station TFF screening system's graphical user interface (GUI) with process schematic and system control ability

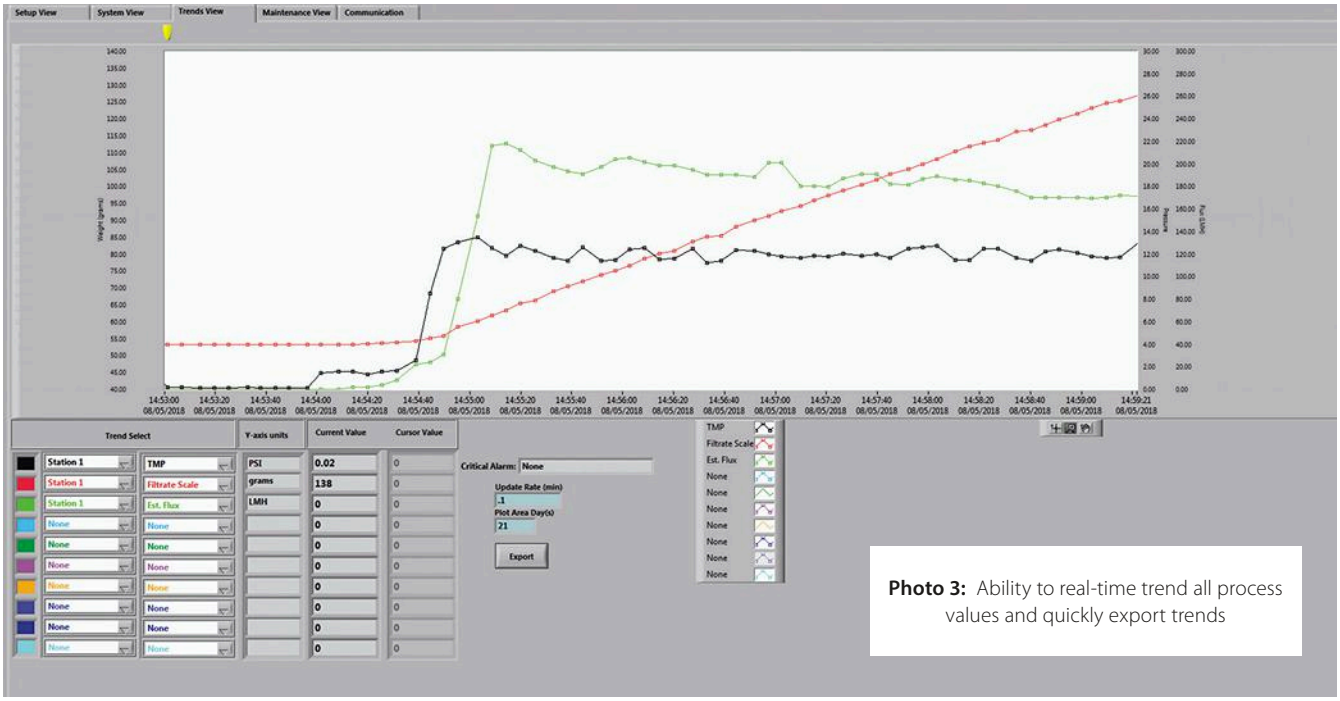


Photo 3: Ability to real-time trend all process values and quickly export trends

different sources that have uncoordinated time stamps. In a process that can have a run-time of several hours, varying a range of conditions with multiple runs can consume many personnel resources, especially if run manually. Therefore, automation is critical to enable process optimization while minimizing key personnel resources.

We believe that to create a more efficient development operation, we need a system with automated process steps that have defined endpoints, control of key process-independent variables throughout a process, calculation of key process values (e.g., ΔP, TMP, and flux), alarm settings that shut down the pump, data-logging and real-time trend viewing. By its nature, such an automated system would provide benefits of minimal user interaction. With automated data-logging, user focus can be placed on analysis rather than data collection.

Creating the TFF Screening System

In discovery and early phases of biologics process development, low expression titers typically limit availability of therapeutic protein. Bristol-Myers Squibb (BMS) wanted an automated TFF system for screening at these early phases when only small amounts of proteins are available. We believed it should have the ability to generate data from small-scale processes that could provide reliable information for scaling up. To address these challenges and add increased throughput capability for fast and efficient process development, BMS collaborated with PendoTECH (an industry supplier of downstream process development systems) to develop a multistation medium-throughput TFF screening system.

In a process that can have a run-time of several hours, varying a range of conditions with multiple runs can consume many personnel resources, especially if run manually. Therefore, AUTOMATION is critical to enable process optimization while minimizing key personnel resources.

Here we report on development of a fully automated system with features as listed above. A parallel TFF screening system consisting of five independently operated stations was designed and built based on BMS requirements for typical early phase TFF process-development goals. The multistation parallel TFF system described herein can provide ≤70% reduction in material requirements, speed up design-space development for nonplatform molecules, and potentially reduce development times by 50–80% (Photo 2).

Defining User Requirements: Before establishing the requirements of a new TFF screening tool, we conducted a thorough market survey of available options and their capabilities and weighed their relative merits and limitations. Most systems did not support parallel screening and required too many manual interventions because they lacked high-level automation. To address the disadvantages of existing equipment, several requirements shaped development of the parallel TFF screening system.

Along with the process capabilities outlined in Table 2 and 3, the following characteristics were desired for this new laboratory-scale system to efficiently perform TFF process development experiments:

It should control five independent TFF trains, monitoring and recording

Table 4: Comparing UF–DF system capabilities at different scales

Scale Feature	Screening	Intermediate-Scale Process Development	Pilot-Scale Technology Transfer	Clinical Manufacturing
Starting batch size	0.1–0.5 L	0.5–5.0 L	5.0–50 L	50–200 L
Parallel processing	Yes	No	N/A	N/A
Flexibility in process volume	Yes	Yes	Yes	Yes
Automated recipe	Yes	Yes	Yes	Yes
Permeate flowmeter*	N/A	Yes	Yes	Yes
Retentate flowmeter	N/A	Yes	Yes	Yes
Process excursion capability for optimization	N/A	Yes	N/A	N/A
Disposable fluid path	N/A	N/A	Yes	Yes
On-line conductivity	N/A	Yes	Yes	Yes
On-line pH	N/A	Yes	Yes	Yes
On-line temperature	N/A	Yes	Yes	Yes

* rather than estimating by permeate scale

process parameters from each one (Photo 3). It should enable "walk-away automation," automatically stopping a TFF train when a set UF–DF goal is reached. We needed flexibility for processing volumes and high-precision diaphragm pumps to minimize shear stress on proteins. Operating pressure should extend up to 40 psi and flow rate range 1.0–100 mL/min. A simple graphical user interface (GUI) and user-friendly data retrieval and manipulation would facilitate operator efficiencies, as would real-time trending of process values. And the system would need a small footprint to minimize operational cost by making the most efficient use of laboratory bench space.

A User–Supplier Collaboration: BMS and PendoTECH had a history of about eight years working closely together in a customer–supplier relationship involving both new and existing products, so we had an established working relationship with key personnel in place. As required for a successful collaboration, we first established timelines and milestones, defining and aligning our strategic interests. Device development began with a feasibility evaluation to develop a system that must meet at minimum these key features: work with small volumes of product and use both existing and new smaller area filters in development; be fully automated and able to perform parallel processing in a small footprint; and include safety alarms and check parameters. All product development programs involve risks and unknowns. So we scheduled technical reviews and decision points throughout our program for project refinements and deciding next steps based on information gained.

BMS collaborated with PendoTECH to develop a multistation medium-throughput TFF screening system. The system described HEREIN can provide ≤70% reduction in material requirements, speed up design-space development for nonplatform molecules, and reduce development times by 50–80%.

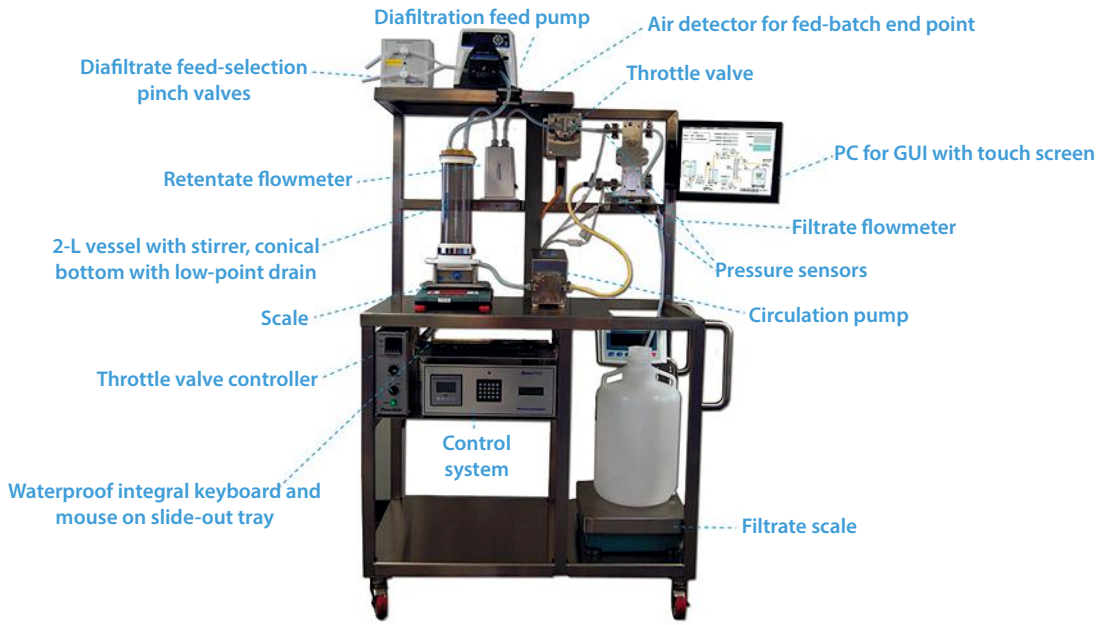
Feasibility evaluations were performed to investigate key technical aspects of the project. One of those was whether PendoTECH's microcontroller platform could handle the complex processing required to keep a TFF process running without errors at the speeds required. At the heart of many of the company's electronic products is a microcontroller platform and associated expertise for programming complex tasks in the "C" language.

User-Requirement Specifications

- Five to 10 parallel tangential-flow filtration (TFF) process stations
- 3 ft^2 of bench space for five stations
- Use of level sensors for diafiltration/concentration points on main retentate vessel (versus a scale) so the level can be precisely controlled on a range of vessel volumes without concern for sensitivity issues and to remove space requirement for a scale. Vessel options of either 125 mL, 500 mL, or 1 L were selected for the product design space.
- Filtrate scale load cell with 0.01-g resolution and ≤1,000 g, with an option to increase up to 3,000 g with 0.1-g resolution to estimate filtrate flow/flux accurately by the change in weight over time
- Integration of automated throttle valve for transmembrane pressure control
- Simultaneous concentration and diafiltration
- Multiprocess recipe options for
  - —Concentration
  - —Concentration–diafiltration–concentration
  - —Fed-batch–concentration–diafiltration–concentration
  - —Run pump only for liquid transfer

Process-control systems often are defined partly by the number of inputs and outputs they can handle. For this system, each station has 11 inputs/outputs (I/Os), and a multistation multiplies that by five for 55, with two data ports to communicate with a GUI) running on a personal computer, thus yielding a total of 57 I/Os. The Freescale Semiconductor (now part of NXP Semiconductors) microcontroller at the heart of PendoTECH's platform has been proven in many critical control applications such as factory monitoring systems and automobiles. Thus, selecting this control platform takes advantage of an I/O-rich architecture supported by a fast microcontroller processor, all in an industrial and compact form. A multitasking real-time operating system programmed in native "C" language provides the necessary flexibility to code complex and robust

Figure 1: Process development system in cart for robust process development



control algorithms for concurrently supporting five independent TFF processes. Even though the popular programmable logic controller (PLC) is used for many industrial control projects and has an advantage of nearly unlimited I/O capabilities, the microcontroller's ideal features in factors of size, cost, and programming flexibility made it the superior choice for this project.

After feasibility testing of the microcontroller platform was successful, the project could move into its next phase. Microcontroller programming was completed and the control hardware configured. We tested a crude breadboard set-up with one station connected to observe its control performance — the other four stations had their processing running with no equipment interfaced. That testing was successful, so the formal user requirements could be defined (as listed in the "User-Requirement Specifications" box).

The project was budgeted and resources committed to finish system building, and it was decided for multiple reasons to limit the system to five stations at least initially. Further requirements were added to the detailed design:

- To meet the challenge of fitting in 3 ft^2 of bench space, a vertical design layout was required for each station.
- To eliminate the need for a diafiltration pump, gravity feed with a valve would be the ideal approach.
- Technical points included confirming the number and type of I/Os required, using a flow restrictor to control diafiltration feed without a pump, having two feed bags for fed batch operation, designing the GUI and load cells, liquid-level sensors, and interfacing with different vessels.

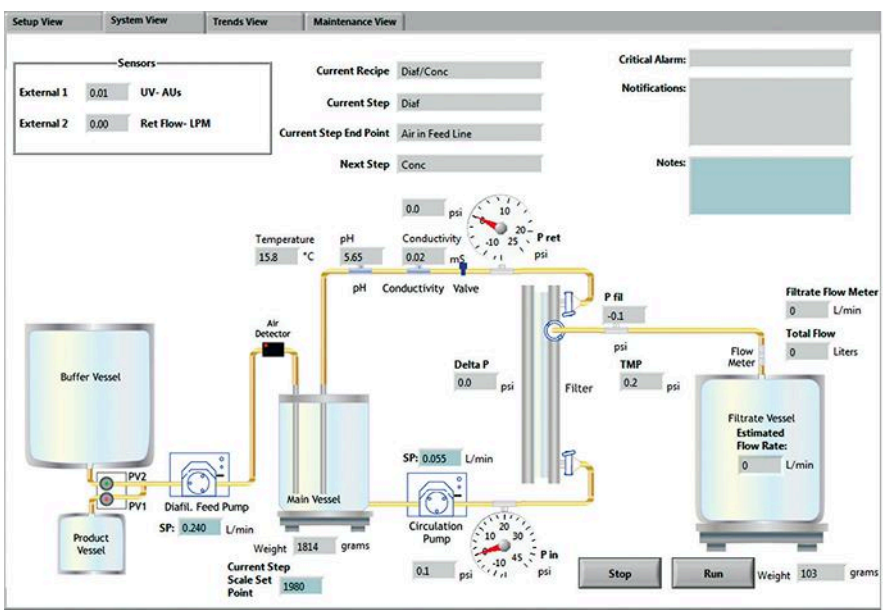


Photo 4: Intuitive GUI for process/pilot development system for scale-up

Performance of Key Components

Programming and Software Development: After software and GUI development and shakedown/debugging were completed (Photo 2), system use could begin. PendoTECH implemented changes and/or corrections based on feedback provided by BMS after initial use.

Microcontroller: With fine-tuning of the final programming, the microcontroller was highly efficient for reading inputs, sending outputs, monitoring alarms, and controlling recipe steps.

Photo 5: Process development system — excursions of ≤40 conditions in flow, transmembrane pressure (TMP), and concentration

Pumps: For high-pressure capability, low-flow design, and a low-pulsation pump stroke, the final pump selected for use with this system was KNF Neuberger's SIMDOS10 diaphragm pump. Because only a console model was available, PendoTECH worked with KNF engineering staff to develop an original-equipment manufacturer (OEM) panel-mounted model for use with the system that would fit within the compact-form design constraint.

Diaphragm pumps have check valves that control pump-chamber inlet and outlet flow during their pump strokes. It is important that those check valves seal during each pump stroke because performance can degrade with a drop in flow rate from the actual set point. To prevent particulates or foreign matter from interfering with check-valve sealing, the pump inlet tubes are outfitted with a 35-µm filter that can be replaced periodically. Even though that is the standard pump option, a modular design with pump controls and power delivered to the pumps from the base of the system enables easy integration of other pumps (e.g., peristaltic) for lower pressure processes.

Throttle Valve: UF–DF typically operates with one of three control strategies: constant retentate pressure, constant transmembrane pressure, or constant filtrate flux. A throttle valve controls TMP and retentate pressure to a user-entered set point. This is a key independent variable for process design and replaces a manual pinch clamp.

The throttle valve enables automation. As process conditions change, such as increased product viscosity during concentration, the valve can adjust automatically to control TMP. This prevents process upsets or alarms and enables processes to run automatically at the higher concentrations desired for many modern final formulations.

Another key feature of this throttle valve is that it prevents users from struggling to set it manually in the correct position that will meet desired pressure set points. With a 0.125-in. (3.2-mm) inner diameter (size 16) targeted for use with this TFF system, the travel distance for a valve to affect flow is about 20% of the tube diameter (~0.64 mm). The valve has a very complex "stepper motor" that can move in very small increments and thus enable precise control to user-entered set points. The valve runs an autocalibration procedure when the system is turned on to reset the home position of its motor positioner.

Load Cells for Permeate Weight: Load-cell performance is critical for measurement of permeate weight, which is used to estimate flow and measure diafiltration volumes. A basic industrial load cell was outfitted to a compact platform that minimizes bench space and cost. This load cell is connected to the system and tared to zero as needed through the GUI. In addition, a simple calibration wizard can be executed by users at any time based on a 500-g weight. That ensures accuracy of data collected during the experiments that follow.

For three different BMS molecules, we have conducted 70 UF–DF runs in fed-batch mode, 45 concentrations to 60–75 g/L, and 45 concentration/diafiltration studies over a period of 45–50 days without any technical errors or loss of information. All 160 runs yielded COMPLETE RECOVERY of all process material.

System Design, Hardware Expandability, and Future Enhancements: There is large convergence of technology on this TFF system: solenoid valves, ultrasonic air detectors, capacitance-level sensors, a Wheatstone bridge load cell, compact Luer pressure sensors, a miniature diaphragm pump, and a valve with stepper-motor control. One objective in the system design was to minimize the amount of external wiring required. With so many external components required to control and monitor TFF processes, either panel-mount receptacles or wires running through water-tight grommets would be placed as close as possible to their point of use to streamline the length and organization of external cabling. Future enhancements should include addition of a possible on-line means of conductivity measurement and a low-flow flowmeter (0.1–15 mL/min) to enable permeate recycling with flow measurement for flux excursions. Photo 1 shows the final hardware design and a representative real time trends of the UFDF process parameters.

Performance of the TFF Screening System

Over the past two years, many scientists and engineers in BMS's early

phase purification development group have used this system extensively. As complex as the system is, its GUI has been described as easy to use. Mechanically, the level sensors are easy to adjust on their slide bars to desired concentration points. During this time, the five-station TFF screening system has demonstrated robust reproducibility. For three different BMS molecules, we have conducted 70 UF–DF runs in fed-batch mode, 45 concentrations to 60–75 g/L, and 45 concentration/diafiltration studies over a period of 45–50 days without any technical errors or loss of information. All 160 runs yielded complete recovery of all process material. The system was cleaned in place and sanitized with 0.2-N sodium hydroxide after each run. UF–DF studies were performed with maximum inlet feed pressure of 25 psi and maximum feed flow rate of 480 L/m/h. Tubing and the inline 35-µm filter were replaced with each change of molecule, and the permeate scale calibration was confirmed every 10 runs. The throttle-valve's home position sensor was calibrated while replacing the system tubing according to its auto-calibration procedure.

Scale-Up to Platform, Pilot, and Manufacturing

Table 4 compares typical UF–DF system capabilities. The multistation UF–DF screening system has shown excellent process scalability to intermediate and pilot scale. The UF–DF process parameters obtained from the multistation TFF system were scaled up successfully to a bench-scale operation with a membrane of 0.1–0.5 m^2. The larger scale TFF skid used a PSG Quattroflow Q150 diaphragm pump that can operate ≤3 L/min for bench-scale operation, and a larger model can be used for process operations ≤99 L/min. A PendoTECH TFF process-control system that has been on the market for years operates only one independent UF/DF process at a time (Figure 1, a cart version of this system; Photo 4, the system GUI). It offers the same automation and control features as the screening system while adding features such as on-line pH, conductivity, and temperature sensing; retentate flow-control capability; options for on-line UV monitoring; and pump and process-scale flexibility.

With a new feature added to the TFF process control system, a robust design of experiments (DoE) can be conducted. To determine optimal flux and operational conditions, the GUI's Process Excursion tab allows users to enter up to 10 different process conditions for feed flow and TMP. That can be set for up to four different concentrations, thus fully automating a matrix of 40 different process conditions. That allows for executing a range of UF–DF process conditions required to perform statistical DoE in a relatively short time. Each stage in Photo 5 would take place with the system in 100% recycle mode, with permeate redirected back to the retentate vessel and creating a roughly steady-state condition.

A Pivotal Advancement

The multistation UF–DF tool developed through collaboration between BMS and PendoTECH is being used extensively by the BMS biologics process development team. The system has been pivotal particularly in accelerating early phase UF–DF process development and characterization activities. The capability to execute a completely automated statistical DoE study with minimal manual intervention is a first on the bioprocessing market (Photo 5). As new biopharmaceutical products work their way through the development pipeline, such novel process development tools will enable this industry to meet its goals of speed, flexibility, quality, and cost as laid out by the BioPhorum Operations Group (BPOG) in a number of publications (**6–8**).

Acknowledgments

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PendoTECH TFF Process Control Systems for Process Development

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- Measure of pressure with PendoTECH sensors that are robust for extensive re-use
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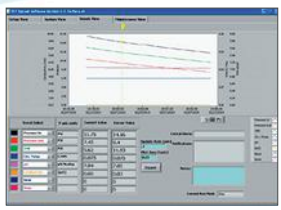

Trends View

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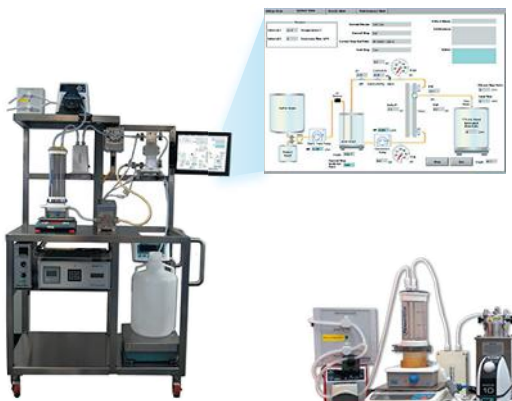
- Complete batch control with 6 built-in programmable recipes
- No limitation on process scale- from milliliters and up
- Integrates with different types of pumps, scales, pressure sensors and flowmeters
- Use any brand of filter
- Built in conductivity, pH & temperature measurement
- Other features to custom-configure a system

Scalable from Development to Pilot



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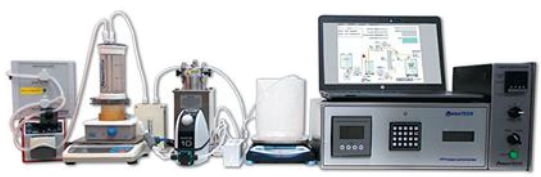
Process Development Cart



Small Scale Benchtop Setup

5 Station TFF Screening System

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- Provides ability to conduct parallel experiments in a compact footprint
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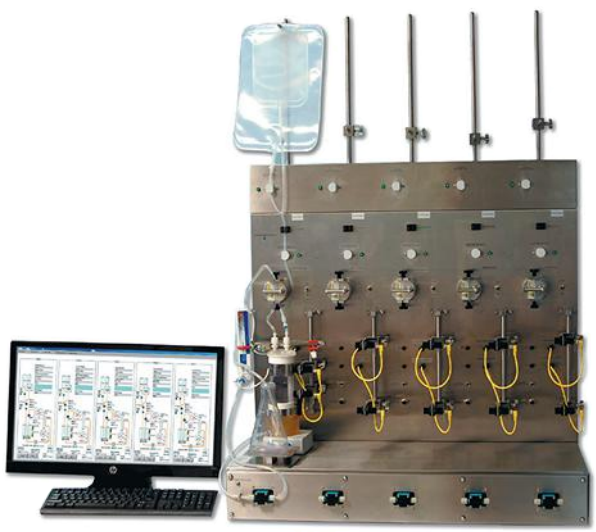
- High pressure pumping with diaphragm pump that can provide up to 60psi/4 bar of pressure with other pumps such as peristaltic available
- Compact load cells used for filtrate volume measurement and are used to estimate filtrate flow and flux
- Different vessel size options



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