Automated, Single-use Purification Platforms in Biopharmaceutical Production

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A: Disposable Filtration Platforms

The use of disposable bioreactors and single-use purification platforms for Normal Flow Filtration (NFF), Tangential Flow Filtration (TFF) and Preparative Chromatography have received significant attention in recent years. Up to very recently, the use of single-use purification platforms was limited almost exclusively to manually operated systems. The transitioning from manual to automated single-use purification systems has been limited primarily by the availability of appropriate sensors and detectors and other in-line system components. The need for gamma-stable, pre-calibrated sensors and pre-sterilized, pre-validated system components has given rise to new and innovative technologies (1, 2).

The utilization of disposable systems in biopharmaceutical production, including single-use purification platforms, has been extensively reviewed (3). Reasons for the increased use of disposable systems have been reported as:

1. Eliminating system cleaning requirements.
2. Decreased risk of product cross-contamination.
3. Reduce time to get facility up and running.
4. Faster campaign turnaround time.
5. Reduce capital investment in facility and equipment.
6. Greater assurance of sterility

Manually operated, single-use purification platform have been enthusiastically embraced by the biopharmaceutical industry. However, fully automated, single-use purification platforms have, until recently, not been commercially available. In addition to reliable, single-use platform components, the availability of automated, sensor-based liquid handling capability are a critical pre-requisite for platform automation. Automated data acquisition, documentation and communication are also critically important in automating, single-use purification platforms. This last requirement is aided by platform OPC connectivity capable of real-time data transfer to a dedicated data historian. The use of historical data for model predictive control (MPC) greatly enhances process knowledge and can provide significant economic benefits.

It is useful to summarize the advantages that can be achieved through automation of single-use purification platforms:

Managing Process Variability: Reducing batch process variability is the overarching goal of any automation. This is typically achieved through careful monitoring and control of purification parameters. For example, comparing the relative standard deviation of various process parameters, i.e. pressure, temperature, pH, conductivity, collected during a batch process provides insights into critical parameters and their relative contribution to overall process variability.

Improved Data Quality: The goal of reducing batch process variability is closely linked to data quality generated by platform sensor and detectors. The notion: “garbage in, garbage out” certainly applies here. In this context, data quality refers to accuracy (i.e. deviation from true value) and precision (i.e. reproducibility). Improved data quality can be achieved by bench-marking sensor outputs for a given purification process and product. Data sets of subsequent batches are compared to the process-dependent bench-marks and their associated performance limits.

In addition, the spectrum of process variables defining the purification process must be accessible to monitoring and control. At a minimum, this may require the installation of appropriate sensor and detectors for continuous monitoring of pressure, flow rates and temperature. In addition, the quality of the purification process itself must be monitored with in-line chemical sensors, i.e. pH, conductivity or UV detectors. For example, sensors of this type can provide early recognition of filter break-through events that otherwise may remain undetected during batch processing.

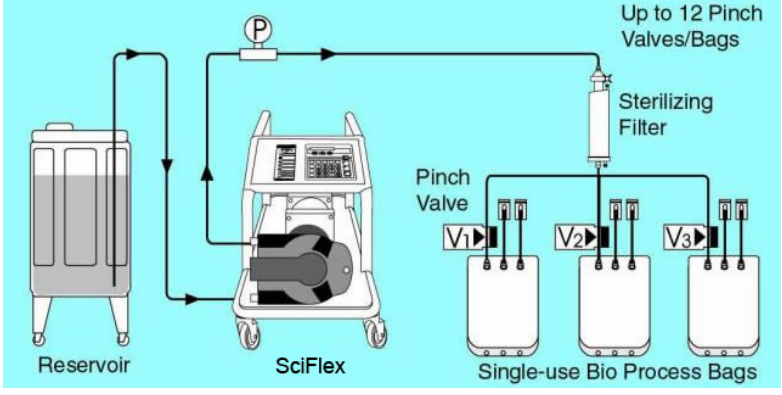
Improved Process Knowledge: Consistent high product quality can only be achieved through continuous process and product improvements guided by improved process knowledge. By comparing bench-mark data for a given purification process with historical data collected under different conditions, new process insights can be gained which lead to improved process tuning, thereby reducing process variability and improve product quality.

The concept of continuous process and product improvement is well established and its benefits are recognized across many industries. The biopharmaceutical industry has historically relied on standard operating procedures (SOP) and manual batch-production records (BPR) to meet production as well as regulatory requirements. Concerns regarding regulatory non-compliance seemed to make continuous process improvements an incompatible objective.

However more recently, the biopharmaceutical industry has been increasingly relying on integrated digital automation systems (DAS) and electronic batch-production records to meet regulatory requirements. The batch management software and batch historian provide the data for the electronic batch-production record. With DAS technology in place, continuous process and product improvement becomes an achievable objective. Within the biopharmaceutical industry, the Delta V (Emerson Process Management) platform has become the preferred process control system. The Food and Drug Administration (FDA) is actively encouraging biopharmaceutical companies do adopt newer technologies to meet regulatory requirements.

Automated, Single-use Platform for Normal Flow Filtration (NFF): Normal Flow Filtration, aka Direct Flow Filtration, is extensively used in the filtration of biopharmaceutical solutions, including filtration of dilute solutions of proteins, monoclonal antibodies and vaccines. The solution is pumped through a membrane or depth filtration device of a selected porosity and surface area. In selecting appropriate filter porosity, a single-step separation of certain undesirable solution constituents can often be achieved. In a typical NFF setup, the process solution enters the filtration device at a constant pump rate. The undesirable constituents are trapped by the filter and accumulate on the inlet filter surface; resulting in a progressive increase in the filter differential pressure. The NFF process is terminated when the differential pressure reaches a maximum allowable value designated by the filter manufacturer. Any further increase beyond this pressure limit can potentially result in rupture of the filter and leakage of undesirable material across the filter matrix.

Figure 1: Automated Filtration & Bag Filling



SciLog has developed a range of innovative liquid handling solutions specifically geared at automating Normal Flow Filtration while improving overall filtration yield (4). For membrane as well as depth filters, significant NFF yield enhancements of up to 35% can be achieved by utilizing a novel, automated liquid handling approach (**Figure 1**). Initially the process solution is pumped, at constant rate, through the NFF device until an application-defined pressure limit is reached, e.g. 20 psi. At this point, the inlet membrane surface is typically covered by a filter cake or gel layer. The filter inlet pressure is monitored with an in-line pressure sensor located down-stream from the filter element. At the pressure limit the liquid handling system automatically switches to a constant pressure delivery mode. In this mode, the pump maintains the safe pressure limit by modulating the pump output until a user-defined lower pump rate limit is reached and the pump action automatically stops.

Figure 2: Normal Flow Filtration (NFF) with Constant Rate - Constant Pressure Fluid Delivery
Sartobran 300 Filter, Area: 300cm² Porosity: 0.45 µm + 0.20 µm

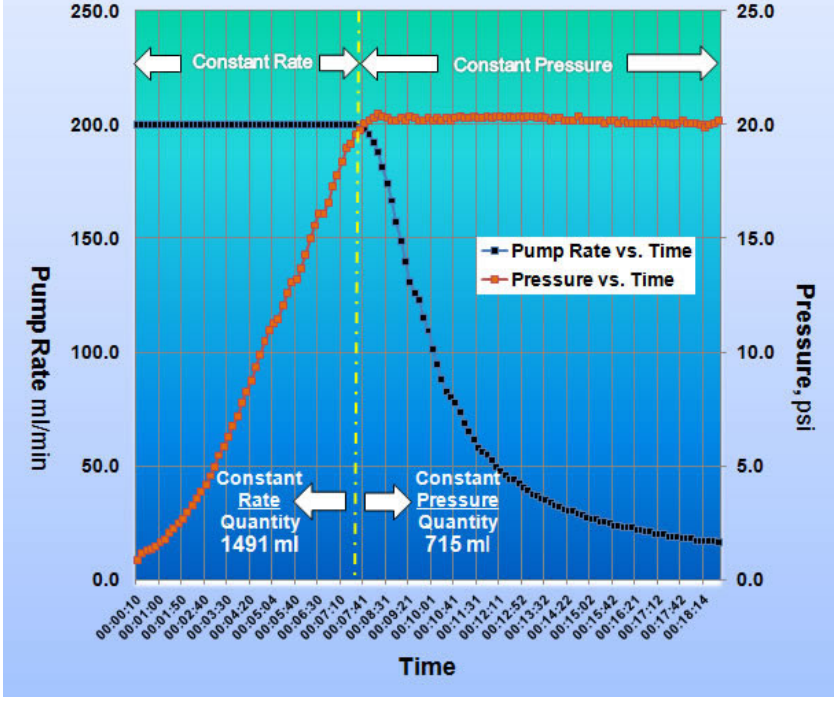


Figure 2 provides an overview of the pump-rate versus pump-pressure relationship with respect to time for a dilute protein test solution. The quantity of filtrate collected during the constant pump-pressure mode is approximately 50% of the filtrate quantity collected during the constant rate mode or 30-35% of the total collected filtrate.

SciLog has developed laboratory (FilterTec & FilterTec Plus, see www.scilog.com) as well as production scale (SciFlex NFF & Batch) equipment capable of automating, quantitative filtration in the manner outlined above. The SciLog systems are ideally suited for automated filtration of biopharmaceutical solutions and filling of the resulting filtrate into single-use storage bag (**Figure 3**). For multiple bag-filling operations, a valve control module provides automated, serial access to disposable storage bags.

Figure 3: SciLog SciFlex Bag Filling System

It is important to recognize that the SciLog liquid handling approach delivers significant, additional filtrate that otherwise would not be available. The increase in the filtrate amount is largely due to the higher degree of filter capacity utilization of the SciLog method when compared to other NFF procedures. Second, the SciLog liquid handling method is inherently safe, i.e. self-limiting and operates in an automated manner with minimal operator input.

Third, when the process solution reservoir is placed on an electronic scale (optional) the pump controller meters the solution by weight based on the scale reading. Dispensing or metering by weight can typically provide accuracy levels of 99.5% or better, i.e. the dispensing inaccuracy is less than 0.5%.

The increased filtration yield of the SciLog liquid handling approach is application and product dependent. However, simple laboratory-scale flow rate experiments (FilterTec) will provide scalable filtration yield data for a given solution and filter. The SciLog systems contain application software to implement optimized filtration parameters. This can be achieved by implementing either a constant or a programmable pump rate/pressure fluid delivery. For example, it is frequently advantageous to start the NFF process at low pump rate or pressure and increase the pump rate or pressure over time until an application-specific pressure limit has been reached. From this point on, the fluid delivery will automatically maintain the pressure limit by modulating the pump output. At constant filter back pressure, the pump rate will decay over time because of progressive filter clogging as shown in **Figure 2**. The filtration process stops once a user-defined lower pump rate limit has been reached and the resulting filtrate collection rate has become negligible.

The SciLog NFF method is particularly advantageous in the quantitative filtration of high-value products, i.e. monoclonal antibodies, proteins and vaccines. SciLog's automated NFF procedure provides maximum filtration yield while maintaining safe filtration conditions at all times. The use of an electronic scale together with NFF system OPC connectivity provides full documentation of process parameters and yield data.

In addition, the SciLog liquid handling approach is well suited for automated, closed-loop NFF applications where solution is removed from a single-use bioreactor, filtered and stored in single-use bags for future processing. SciLog provides a range of custom design, manufacturing and service support capabilities.

B: Disposable Sensor Technology

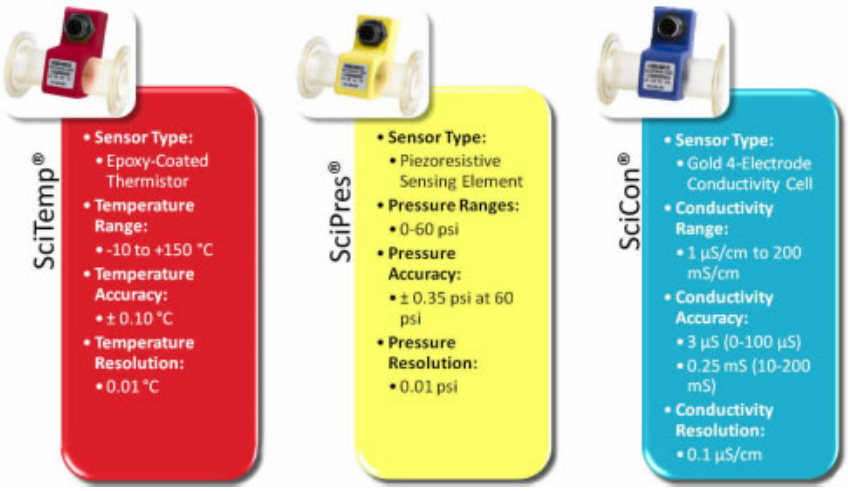
Sensors designed for incorporation into disposable purification platforms, i.e. single-use Tangential Flow Filtration (TFF), single-use Normal Flow Filtration (NFF) platforms or disposable chromatography platforms must meet a number of challenging requirements.

Single-use platforms for downstream purification typically consist of an integrated assembly of filter elements or columns, flexible tubing, plastic connectors and bags, segments of peristaltic pump tubing as well as sensors. Such assemblies are designed and pre-assembled for a specific purification process and a given, maximum process volume. Special, aseptic plastic connectors are used to hook up to external, single-use bioreactors and/or buffer solutions. In the final configuration, all elements of the purification platform are pre-sterilized, assembled and operated as a closed system.

Single-use purification assemblies are typically custom-manufactured and gamma-irradiated at 30-40 kGy to insure sterility of the integrated assembly. Alternative sterilization approaches are available utilizing "Steam-Thru" connectors as well as steam sterilized, disposable purification assemblies. Ethylene oxide sterilization appears to be used less frequently because of potential contamination by residual ethylene oxide.

SciLog Inc. has recently introduced a family of disposable in-line sensors (**Figure 4**) for monitoring conductivity (SciCon), pressure (SciPres) and temperature (SciTemp). These pre-calibrated sensors come in five different sizes, ranging from Luer, 3/8" Barb, ½" Barb, ¾" TC to 1.0" TC and accommodate the industry's need for system scalability. The performance characteristics of the SciLog sensors are summarized:

Figure 4: SciLog Pre-Calibrated, Disposable Sensors



Sensor Calibration: Maintaining sterility of single-use purification platforms is of the highest importance. Thus field calibration of sensors and insertion of sensors into a pre-sterilized purification manifold is not an acceptable option because of the obvious contamination concerns. A primary, post-use sensor calibration is equally unacceptable because of uncertainty of the in-use sensor accuracy and precision. However, secondary post-use sensor performance verification may be useful to metrology departments provided the pre-use sensor performance is known and has been fully characterized.

In this context, sensor characterization will answer the questions related to sensor performance after gamma-irradiation, steam-sterilization (Temperature >121° C) under pressure (~20psi) and exposure to high concentrations of NaOH (1.0 Normal). These are the conditions that sensors will be exposed to when integrated into down-stream, single-use purification platforms. The SciLog's family of sensors has been designed to provide accurate data after exposure to such harsh and challenging conditions. SciLog's approach of sensor pre-calibration, sensor characterization and full sensor performance disclosure has been well received by the biopharmaceutical process community.

Pre-calibrated Sensors: In order to implement a sensor calibration, the SciLog sensor is exposed to a set of known calibration conditions. The sensor response is stored together with the calibration value. For example, the standard factory calibration procedure for the SciLog pressure sensor (SciPres) calls for the sensor to be calibrated at a 0.00 psi applied pressure and at a 30.00 psi applied pressure utilizing digital, NIST traceable pressure gauges. Based on the sensor response a sensor Calibration Factor (CF) and sensor Zero Offset (PZ) are calculated and stored in a proprietary sensor memory together with a unique sensor ID. For each SciPres sensor, a sensor-specific Calibration Certificate is issued and accompanies the sensor shipment. The sensor ID code contains the following sensor–specific information:

Sensor Specific Information:

CX-ZLLNNNN-MMYY

CX = Pressure Sensor Connector
C1=Luer,
C2= 3/8"Barb,
C3 = ½"Barb,
C4= ¾" TC,
C5=1.0" TC
Z = Sensor Material Code,
Z=1, Polysulfone,
Z=2, Polypropylene
L = Sensor Production Lot Number
N = Sensor Serial Number
M = Month of Sensor Calibration
Y = Year of Sensor Calibration

On request, SciLog provides a Sensor Material Traceability Service which includes Sensor Extractable Reports, Material Lot Certification, as well as Product Certificates of Compliance.

Sensor Performance: For special sensor applications, SciLog offers a customer specific sensor calibration service. For example, oxygen gas addition to single-use bioreactor bags requires careful pressure monitoring. Depending on bioreactor bag size, excessive gas pressures in the 1-5 psi range may cause sudden rupture of the bioreactor. Users of single-use bioreactors frequently request gamma-stable pressure sensors that are calibrated at 5.00 psi and capable of monitoring low-level pressures, in the 1.00 to 5.00 psi range, with 1.5% accuracy.

A similar problem exists when overfilling single-use bags causing an excessive build-up of internal bag pressure. If not relieved, the pressure build-up can cause bag rupture. Incorporating the SciPres sensors into bag inlet will not only monitor the bag pressure but also stop filling operation when a user-defined pressure level has been exceeded. SciPres monitor has digital alarm outputs that can be used for this purpose.

Although the SciPres pressure sensors have been safety-tested up to 90 psi, the standard operating range is limited to 60 psi. The SciPres Sensor Calibration Certificate includes the specific calibration points as well as the sensor-specific calibration factors (CF). The specific cal factor variations from the lot average CF represent the variances within the manufacturing tolerances of the proprietary solid-state sensing element. The sensing element is covered by a medical grade silicone gel diaphragm (USP Class VI) capable of withstanding a 100-hour exposure to sodium hydroxide solution (1.0N), see Sensor Characterization.

When connected to SciPres monitor, the sensor-specific CF value is used in a monitor-based correction algorithm that compensates for the slight sensor non-linearity. The pressure values displayed by the monitor as well as the pressure values communicated by the monitor's analog (4-20mA) and digital outputs represent accurate, CF-corrected pressure values.

Sensor Performance Characterization: All SciLog sensors, including the SciPres pressure sensors, have been performance-tested under three different conditions. The sensor test protocols as well as test results are summarized below:

Post-Gamma Sensor Response: 14 randomly selected SciPres pressure sensors were calibrated at 0.00 psi and 30.00 psi prior to gamma-irradiation at 38.4 kGy. Post-gamma sensor accuracy was determined at 0.00 psi and 30.00 psi of applied pressure, see **Table I**.

Table I: Post-Gamma Sensor Response
Gamma Irradiation @ 34.8 kGy

SciPres, 3/4" TC Sensor ID	*Pre-Gamma Test* Applied Pressure		*Post-Gamma Test* Applied Pressure	
	NIST 0.00 psi	NIST 30.00	NIST 0.00 psi	NIST 29.99 psi
S4-290006-0408	0.00	30.00	-0.01	29.94
S4-290008-0408	0.00	30.00	0.00	29.97
S4-290011-0408	0.00	30.00	0.00	29.97
S4-290013-0408	0.00	30.00	-0.01	29.92
S4-290017-0408	0.00	30.00	-0.02	30.01
S4-290018-0408	0.00	30.00	0.00	29.96
S4-290020-0408	0.00	30.00	-0.01	29.95
S4-290021-0408	0.00	30.00	0.00	29.96
S4-290022-0408	0.00	30.00	0.00	30.03
S4-290023-0408	0.00	30.00	-0.03	29.92
S4-290024-0408	0.00	30.00	-0.02	29.92
S4-290025-0408	0.00	30.00	0.00	30.01
S4-290026-0408	0.00	30.00	0.00	29.95
S4-290027-0408	0.00	30.00	0.00	29.95
Group Average	0.00	30.00	-0.01	29.96
*Group SD**			0.01	0.03

SD = Standart Deviation

Test Results: All sensors survived gamma-irradiation and tested accurately well within SciLog's established accuracy limit of +/- 0.35 psi. The post-gamma data shows a group average of 29.96 psi (for 14 SciPres, ¾"TC) with a standard deviation of +/- 0.03 psi.

Post-Autoclaving Sensor Response: Six randomly selected SciPres sensors (1/2" Barb) were tested after repeated exposures (3x) to autoclave conditions, see **Table II**. The initial, factory calibration responses are tabulated under "Pre-Autoclave" heading. The ambient air vent as well as the electrical connector of SciPres sensor was covered with autoclave tape and place into a Tuttnauer EZ9 Autoclave. The following

autoclave conditions were maintained during the three test trials:

Sterilization Temperature:	257°F (125°C)
Sterilization Time:	30 minutes
Sterilization Pressure:	19 psi
Drying Time:	30 minutes

After each trial, the SciPres sensors were allowed to cool to room temperature and pressure tested at 0.00 psi and 30.00 psi. The SciPres response data are listed under the "Post Trial" columns. All trials were carried out with the original factory sensor calibration. No sensor re-calibrations were made before or during the three trials.

Table II: Post-Autoclaving Sensor Response

Test Conditions: *Sterilization Temperature: 257ºF (125ºC)*
Sterilization Time: 30 Minutes
Sterilization Pressure: 19 psi
Drying Time: 30 Minutes

SciPres 0.50" Barb Sensor ID	Pre-Autoclave psi NIST 0.00	Pre-Autoclave psi NIST 30.00	Post Trial 1 psi NIST 0.00	Post Trial 1 psi NIST 30.02	Post Trial 2 psi NIST 0.00	Post Trial 2 psi NIST 30.00	Post Trial 3 psi NIST 0.00	Post Trial 3 psi NIST 30.02	Sensor Average 30.00 psi	Sensor SD* 30.00 psi
S3-220221-1007	0.00	29.99	0.01	30.06	0.02	30.04	0.04	30.04	**30.05**	**0.01**
S3-220227-1007	0.00	29.99	0.00	30.06	0.02	30.05	0.04	30.06	**30.06**	**0.01**
S3-220229-1007	0.00	29.99	0.02	30.10	0.03	30.06	0.04	30.07	**30.08**	**0.02**
S3-220230-1007	0.00	29.99	0.01	30.06	0.30	30.24	NR	NR	**30.15**	**0.13**
S3-220234-1007	0.00	29.99	0.00	30.05	0.10	30.22	0.05	30.16	**30.14**	**0.09**
S3-220240-1007	0.00	30.00	0.02	30.06	0.79	30.06	NR	NR	**30.06**	**0.00**
Group Average	***0.00***	***29.99***	***0.01***	***30.07***	***0.21***	***30.11***	***0.04***	***30.08***		
Group SD*		***0.00***		***0.02***		***0.09***		***0.06***		

* SD = Standard Deviation NR = No and/or Erratic Respone, Not Included in Averages

Test Result: All of the tested SciPres sensors survived autoclave conditions. For the tested sensors (6), the group average ranged from 29.99 to 30.11 with an average standard deviation of +/- 0.06 psi. However, sensor accuracy became increasingly compromised after two autoclave cycles. For accurate performance, SciPres sensors are limited to two autoclave cycles.

Sodium Hydroxide Exposure Test: Six randomly selected SciPres sensors (Luer) were tested after a 100-hour exposure to 1.0 N sodium hydroxide solution at 22°C and at 10 psi line pressure, see **Table III**. The sodium hydroxide solution was continuously re-circulated (150ml/min) through the sensors using a peristaltic pump. At 25 hour intervals, the exposure test was briefly interrupted, the sensor were flushed with distilled water and tested at 0.00 psi and 30.00psi.

Test Result: The data show a stable sensor accuracy and precision over the 100-hour exposure to 1.0 N sodium hydroxide solution. For the four timed exposure tests, the group averages ranged from 30.06 to

30.08 psi with an average standard deviation of +/- 0.06 psi. All sensor responses were monitored with a digital, NIST-traceable gauge.

Table III: Pressure Sensor, NaOH Exposure Test

Test Conditions: 100 Hour Exposure of SciPres to 1.0 N Sodium Hydroxide at 22° C, 10 psi Pressure

SciPres, Luer Sensor ID	Start psi 0.00	psi 30.00	25 hr Test psi 0.00	psi 30.00	50 hr Test psi 0.00	psi 30.00	75 hr Test psi 0.00	psi 30.00	100 hr Test psi 0.00	psi 30.00	*Sensor Average 30.00 psi*	*Sensor SD* 30.00 psi*
S1-240058-1007	0.00	30.05	0.00	30.07	0.00	30.06	0.00	30.08	0.00	30.00	***30.05***	***0.03***
S1-240057-1007	0.01	30.19	0.00	30.20	0.02	30.18	0.02	30.22	0.00	30.21	***30.20***	***0.02***
S1-230238-1007	0.02	30.02	0.01	30.03	0.00	30.03	0.00	30.02	0.00	30.03	***30.03***	***0.01***
S1-240061-1007	0.00	30.03	0.01	30.07	0.00	30.06	0.00	30.06	0.00	30.05	***30.05***	***0.02***
S1-230107-1007	0.01	30.05	0.00	30.08	0.00	30.06	0.00	30.05	0.00	30.06	***30.06***	***0.01***
S1-230110-1007	0.00	30.05	0.00	30.04	0.01	30.03	0.00	30.03	0.00	30.02	***30.03***	***0.01***
Group Average ***Group SD****	***0.01*** ***0.01***	***30.07*** ***0.06***	***0.00*** ***0.00***	***30.08*** ***0.06***	***0.01*** ***0.01***	***30.07*** ***0.05***	***0.00*** ***0.01***	***30.08*** ***0.07***	***0.00*** ***0.00***	***30.06*** ***0.07***		

* SD = Standard Deviation

Sensor Accuracy & Precision: The SciPres sensor accuracy, based on performance data sensor lot of 250 sensors, has been established to be +/- 0.35 psi for the 0 – 60 psi applied pressure range. “Out-of-Box” sensor performance has been tested. For 12 randomly selected, factory pre-calibrated SciPres sensors, the “Out-of-Box” accuracy was 30.07 psi +/- 0.07 psi (NIST test pressure: 30.00 psi) with test results ranging from 29.96 to 30.19 psi.

Sensor Monitors: The SciPres laboratory monitor is designed accommodate up to three SciPres sensors simultaneously. The monitor displays the three pressure sensors as P1, P2, and P3. In addition, the monitor will also display the differential pressure DP = (P1-P2) or the TFF trans-membrane pressure TM = [(P1+P2)/2] - P3. Furthermore, four analog outputs (4-20mA) are available: P1, P2, P3, DP or TM. The four analog output signals have an 18 bit resolution. Four TTL output alarms provide user-selectable, Hi/Lo pressure limit settings for P1, P2, P3, DP or TM.

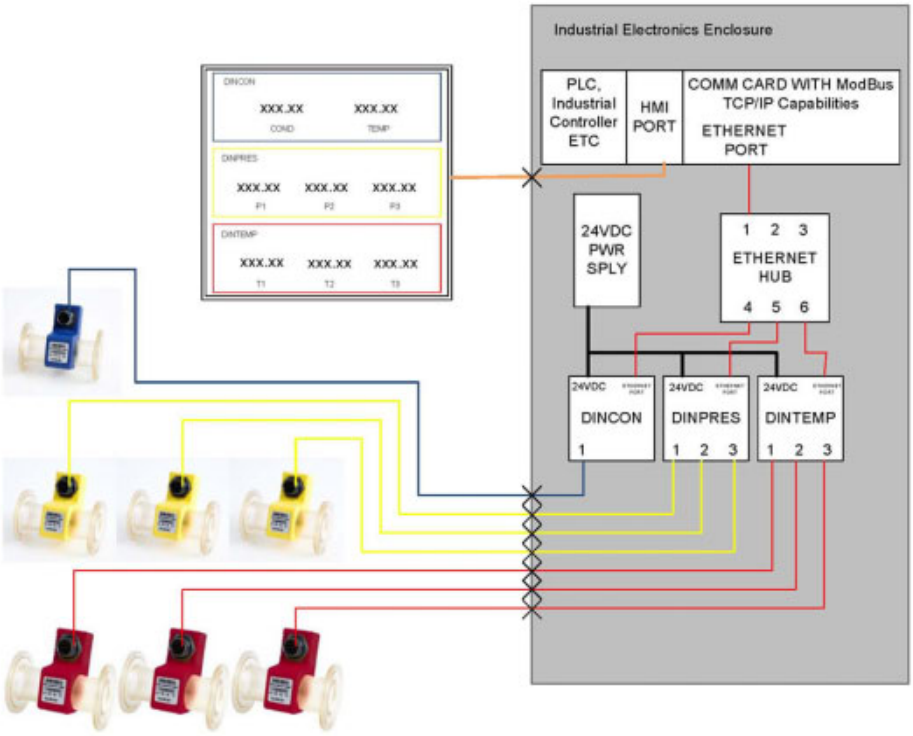
In addition to the factory installed sensor pre-calibration, all SciLog sensors allow a two point, custom re-calibration of the sensor. In the case of the SciPres monitor, a custom calibration menu is selected while a known pressure is applied to the connected SciPres sensor. The custom calibration factor (CCF) represents a correction to the installed factory calibration factor (CF) for a user-defined pressure range. The original calibration factor can be re-installed by selecting the cal-reset menu.

SciLog has developed three digital sensor monitors (DIN-Con, DIN-Pres and DIN-Temp) that communicate over ModBus (RTU or TCP/IP) communication protocol, see **Figure 5**. These digital modules allow hook-up of multiple SciLog sensors and are intended for Systems Integrators (SI) and Value Added Resellers (VAR). The SI or VAR will incorporate these modules to an Industrial Control System to communicate to a central controller. The digital output of the DIN modules is extracted from the module and placed directly into the host computer or controller that is requesting the sensor information. The host computer or controller must be capable of communicating with the SciLog DIN modules via RS-232, RS-485, USB or Ethernet physical layers.

The full benefit of single-use purification platforms, i.e. Normal Flow Filtration, Tangential Flow Filtration and Chromatography, can only be achieved through automation. Automated, sensor-based fluid handling systems and connectivity (OPC) to a digital automation system, e.g. Delta V, are critical steps to realizing the full benefits of single-use purification platforms in GMP production.

SciLog has made a commitment to providing pre-calibrated, single-use sensor technology and associated data acquisition modules to the biopharmaceutical industry. SciLog's scalable sensors provide stable performance after exposure to gamma-irradiation, steam-sterilization and extended exposure to 1.0 N sodium hydroxide solution. The SciLog's family of sensors is designed and manufactured to provide accurate data after exposure to such harsh and challenging conditions. SciLog's approach of sensor pre-calibration, sensor characterization and full sensor performance disclosure has been well received by the biopharmaceutical community.

Figure 5: Ethernet Communications Over ModBus TCP/IP



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