Intelligent BioProcessing Systems

# Significant Increases (35%) in Dead End Filtration Throughput using Non-traditional Liquid Handling Procedures

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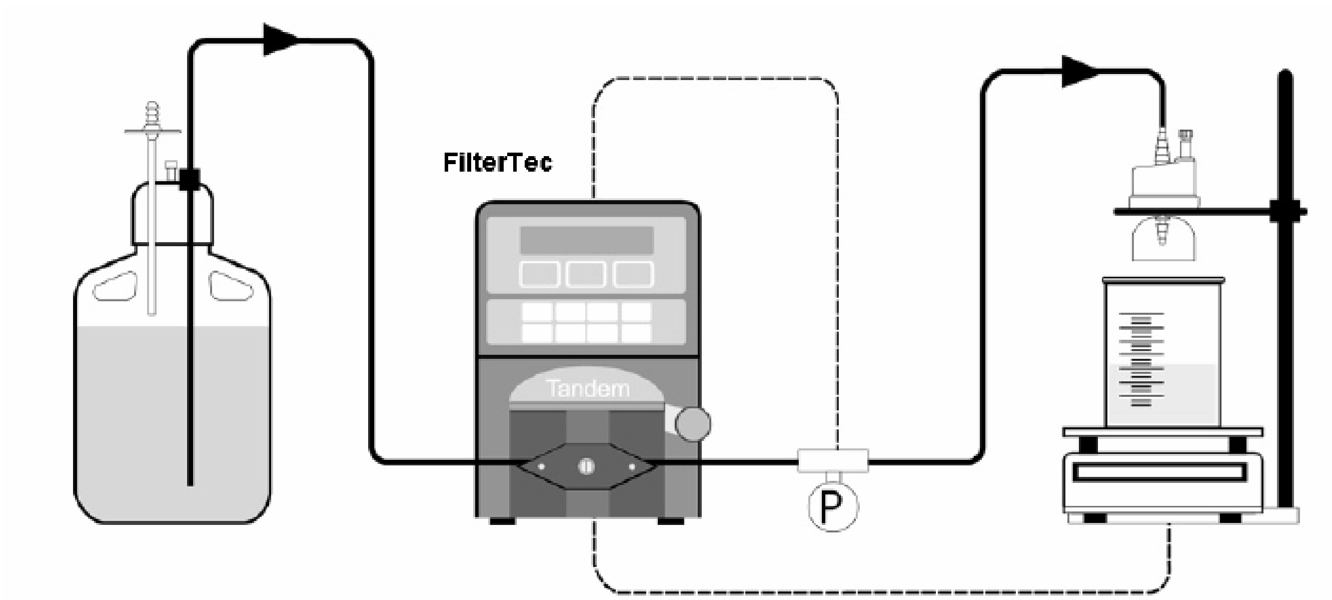
**Abstract:** Dead End Filtration (DEF) of dilute protein solutions is typically carried out with either a constant pressure or constant rate fluid delivery system. As will be shown, both approaches fall short of utilizing the available filter capacity. Significant DEF yield enhancements of up to 35% can be achieved by utilizing a novel, automated liquid handling approach: Initially, a constant pump rate fluid delivery is implemented until a user-defined pressure limit is reached. At this point the fluid handling system automatically switches to a constant pressure delivery, i.e. modulating the pump output, until a user-defined lower flow limit is reached and the pump stops. Comparative DEF throughput data are presented and discussed.

**Introduction**: The FilterTec***** system, recently introduced by SciLog Inc., is intended for developing and documenting filtration procedures as well as for automating routine DEF applications. The system consists of a smart pump module capable of interfacing with up to three (3) disposable pressure sensors and also interfaces with an electronic scale for quantifying filtration yields and collection rates.




**The FilterTec comes with the following application software for optimizing and implementing:**

1. **Constant Pressure Filtration:** Automated Vmax. data acquisition Documentation and graphical presentation of Vmax. data.
2. **Constant Rate Filtration:** Automated data collection and graphical presentation of constant rate data.
3. **Constant Rate/ Constant Pressure Filtration:** Implements user-defined pump rate while monitoring system pressure and filtrate collection. When a user-defined pressure limit is reached, the FilterTec switches to constant pressure fluid delivery. The system stops when a lower flow limit has been reached.
4. **Step-Scan Filtration:** Provides time-programmable pressure and/or pump rate scans. User-defined scan rates, e.g. pressure scan from 0 to 30 psi over a 10 minute time interval followed by a constant pressure (30 psi) interval. This operational mode is a very useful investigative tool for optimizing filtration procedures.

*US Patents: 5,947,689; 6,350,382; 6,607,669; Other Patents Pending

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## Experimental:

The data presented herein were generated with the FilterTec system using a Tandem 1081 peristaltic pump head driven by a 160-RPM motor. #14 Tygon pump tubing was used for pump rates less than 35 ml/min; #16 pump tubing was used for pump rates between 35 ml/min. and 100 ml/min. A #15 pump tubing with a Tandem 1082 peristaltic pump head was used for pump rates between 100 and 250 ml/min.

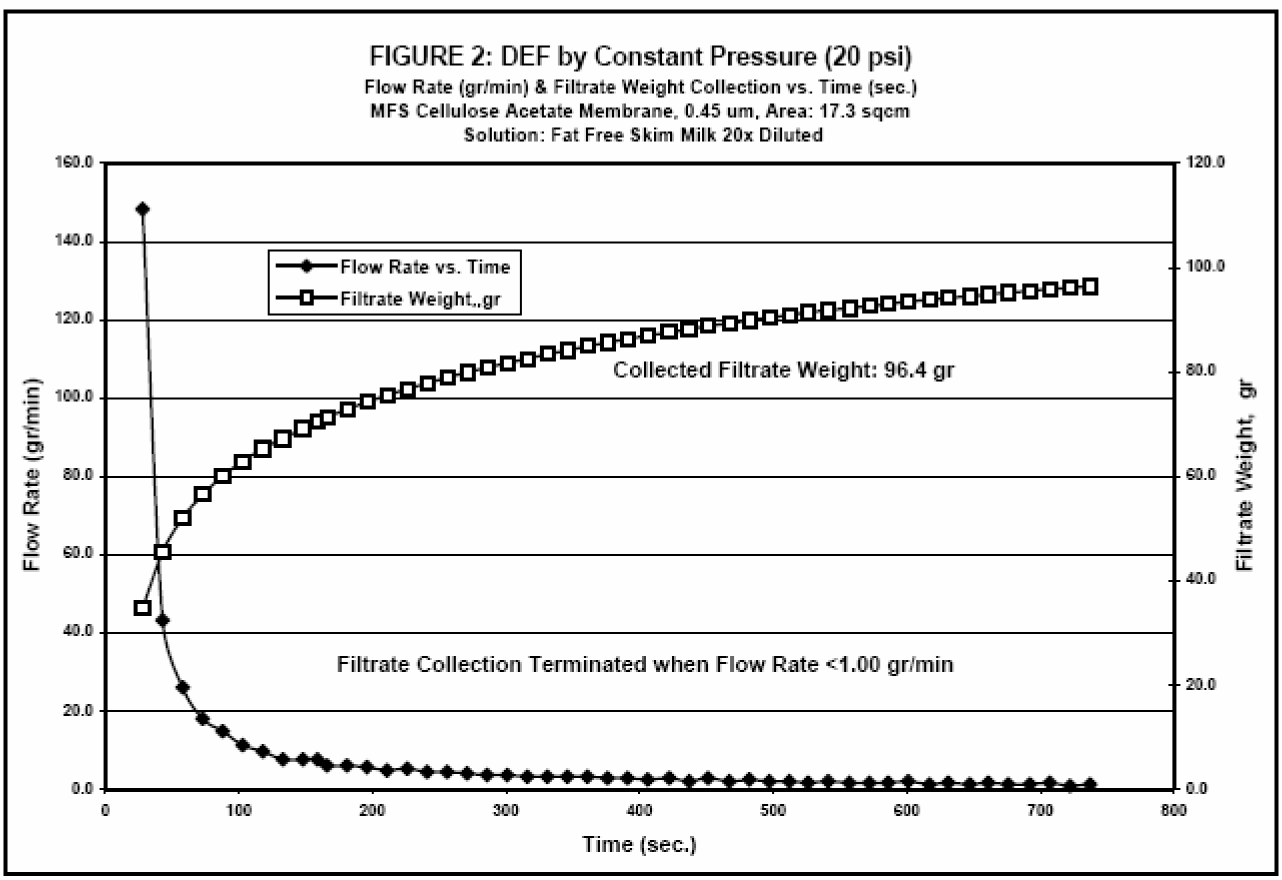
For small-scale filterability studies, Cellulose Acetate Membrane Filters (pore size 0.45 µm, 47 mm diameter) were used. A 50 ml aliquot of distilled, de-ionized water was pumped through the filter prior to introducing any surrogate protein solution.

For larger-scale filterability studies, Sartobran 300 Cellulose Acetate Filter Capsules were used. Composite filter porosity: 045 µm + 0.20 µM, filter area 300 sqcm. An 800 ml aliquot of distilled, de-ionized water was pumped through the filters prior to introducing any protein solution.

A surrogate dilute protein solution was prepared by diluting (20x) Fat-free Skim Milk The undiluted milk contained 9% protein the dilute solution was prepared fresh daily.

**Filtration Throughput under Constant Pressure (20.0 psi) Condition:**

A pressurized container was used to generate Constant Pressure filtration data. The filtrate was collected in a container located on an electronic scale. Figure #2 summarizes the Flow Rate and Filtrate Collection Weight data under constant pressure condition. At a constant 20 psi pressure, an initial flow rate of 150 ml/min was measured. The flow rate quickly decayed to much lower levels as can be seen in Figure #2. The test automatically stopped after the flow rate had decayed to a user-defined Low Flow limit of 1.0 ml/min. The collected filtrate weight was 96.4 gr or 5.57 gr per sq cm of filter surface area.

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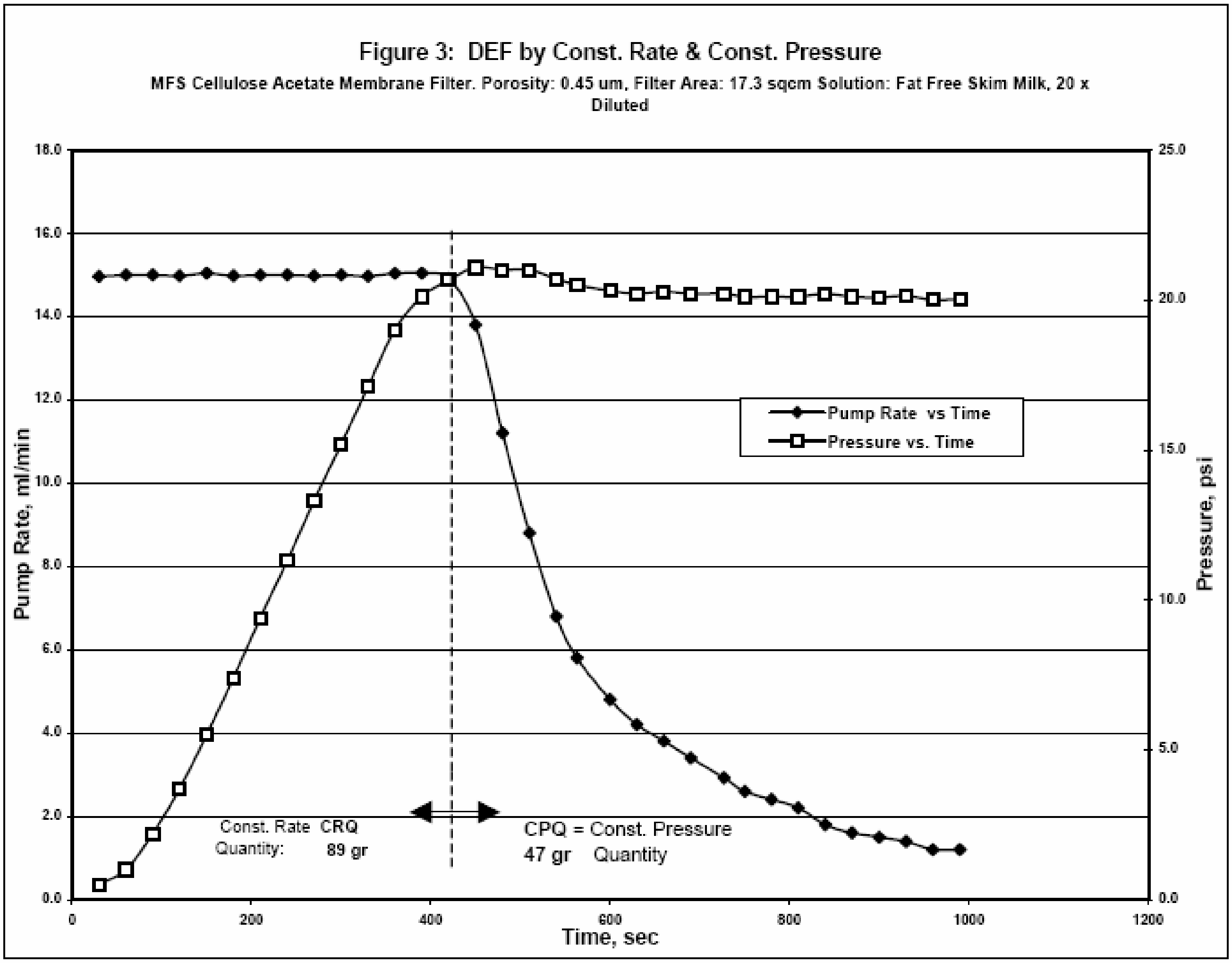
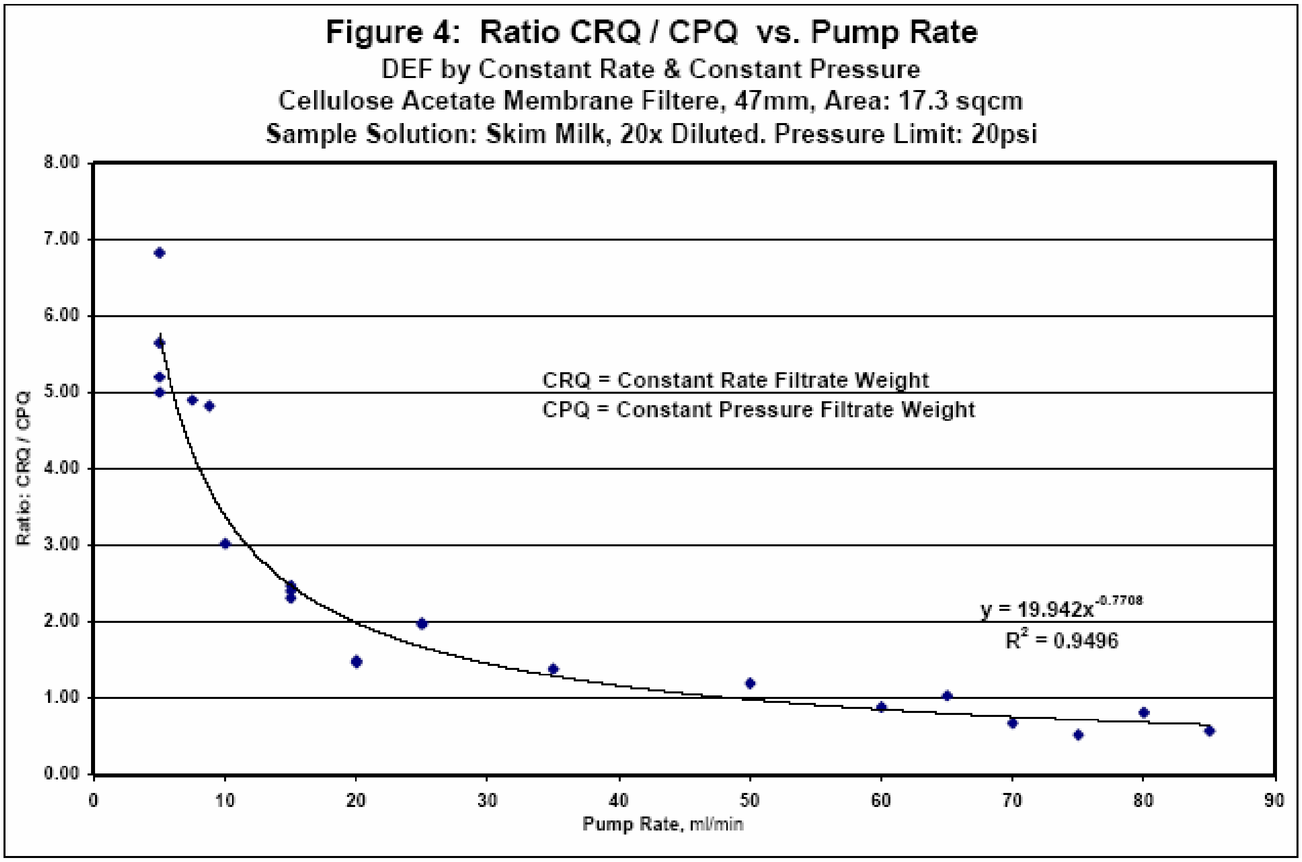
**Filtration Throughput under Constant Rate (15ml/min) and Constant Pressure (20psi) Condition:**

Utilizing the FilterTec, a constant pump rate of 15 ml/min was implemented until a backpressure of 20 psi was reached. At that point, the FilterTec switched automatically to a constant pressure delivery by modulating the pump output and thus maintaining a 20 psi pressure level. The FilterTec automatically stopped pumping after a user-defined Low Flow alarm (1.0 ml/min) had been triggered.




Referring to Fig #3, the area under the Pump Rate vs. Time curve represents the total, collected filtrate weight of 136 gr. or 7.86 grams per sq cm of filter area. Figure #3 allows us to differentiate between the filtrate weight (89 gr) collected under constant pump rate (CRQ) conditions and the filtrate weight (47gr) collected under constant pressure conditions (CPQ)

It should be noted that the filtrate weight collected under constant rate condition, CRQ = 89 grams, compares reasonably well with the data collected under Constant Pressure conditions, i.e. V max. = 96.4 gr. This result is not too surprising, since the two quantities, namely, CRQ and Vmax are equivalent according to the D'Arcy equation (see reference 1, page 224)



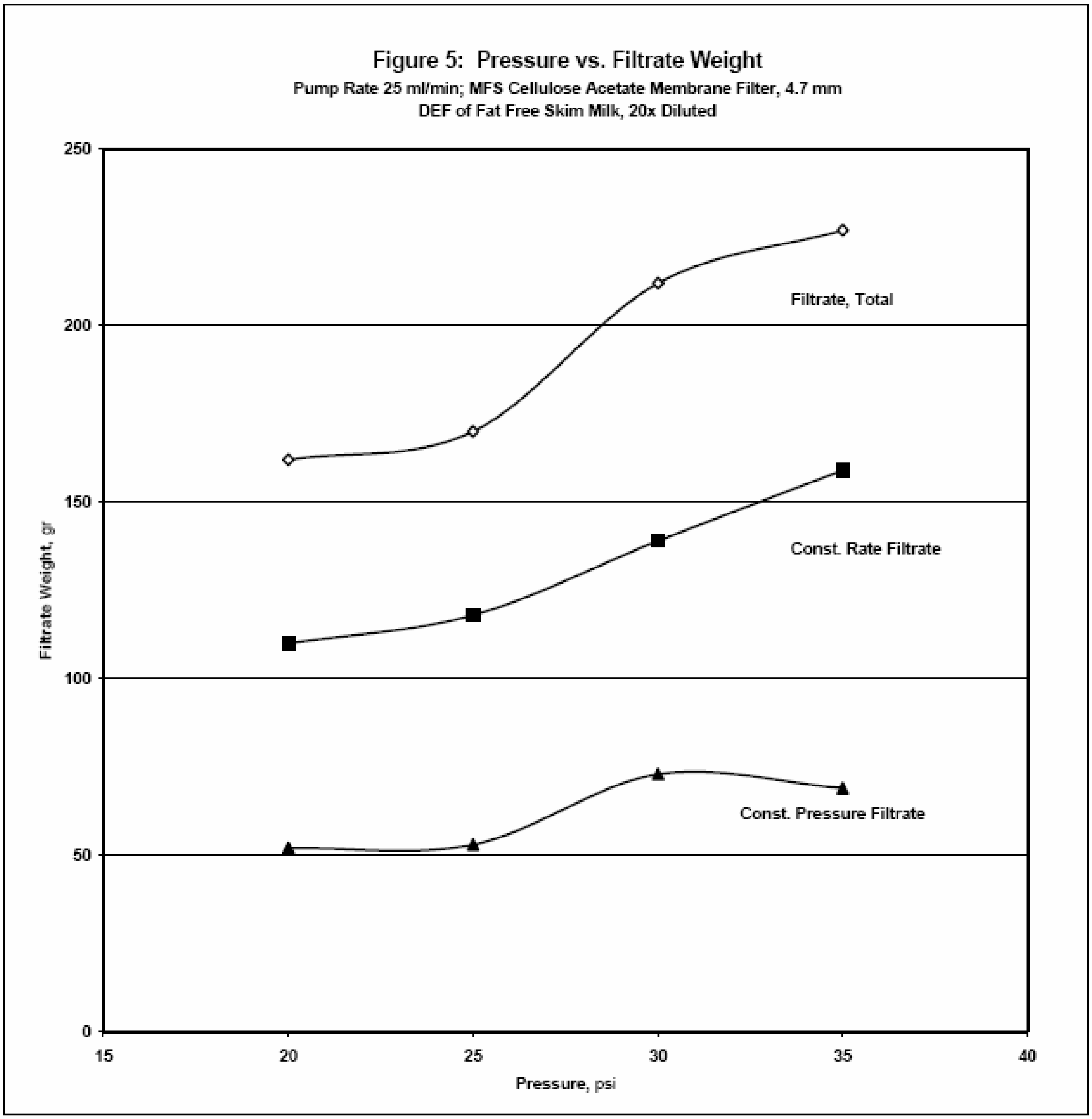

However, the actual, collected filtrate weight, CRQ + CPQ = 136 gr is considerably larger than can be reasonably explained from Vmax or Constant Rate data. Figure #4 sheds some light on this filtrate weight discrepancy. The graph of ratio CRQ / CPQ vs Pump Rate follows a power function. At the high pump rate end, the ratio CRQ / CPQ = 0.5 whereas at the low pump rate end the ratio CRQ / CPQ > 5.0.

A possible model, that is consistent with the data, postulates two filter membrane domains that are utilized during DEF. A high-flux membrane domain that is available during constant, low flow filtration and a low-flux membrane domain that is utilized primarily during high flow rate, high pressure conditions. The high-flux membrane requires a relatively low pressure differential for passing the filtrate whereas the low-flux domain requires a relatively high pressure differential. The two domains are postulated to roughly coincide with CRQ (high flux) and CPQ (low flux).

At high pump rates, the high-flux areas are quickly plugged and become rapidly unavailable for further filtrate flux. The experimental data supports this conjecture. In the 50 – 100 ml/min pump rate range CRQ is quite small in comparison to CPQ. Under Constant Pressure conditions, an initial flow rate of 150 ml/min has been measured. Thus under these conditions the high-flux filter areas are plugged, almost instantaneously, and make only a small contribution to the overall filtrate throughput.

A possible mechanism for the observed filter plugging behavior can be seen in figure #3. The slope of Pressure vs. Time has an extended linear segment, which becomes increasing pronounced at higher pump rates and higher pressures. The linear relationship between pressure and time is characteristic of the formation of a filter "cake" (reference 1, page 225). Under constant flow rate conditions, the formation of a filter "cake" follows the relationship $P_1 – P_0 = c\ T$, where $P_1$ represents the final pressure and $P_0$ the initial pressure condition, T is time and "c" is a constant. Thus a plot of Pressure vs. Time should be linear, the slope being equal to the constant C.

Filtration throughput data were collected at different pump rates and constant pressure limits (20 psi) as well as at constant pump rate (25 ml/min) and at different pressure limits. Figure #5 shows the relationship between Total Filtrate collected and Pressure. In the pressure range from 20 to 35 psi, the total collected filtrate varied from 162 gr (20 psi) and 227 gr (35 psi) with the largest contribution to Total Filtrate is being made by the CRQ, the Constant Rate (Filtrate) Quantity. The CPQ, on the other hand, remains almost constant in pressure limit range from 20 psi to 35 psi.



**Scale-up of Filtration Throughput under Constant Rate and Constant Pressure Conditions:**

The scalability question was addressed using a 300 sq cm 'Sartobran 300" Cellulose Acetate Membrane Filters. The porosity of the Sartorius filter consists of a 0.45 µm pre filter element followed by a 0.20 µm final filter. Since the observed filter plugging occurred with a 0.45 µm Cellulose Acetate Filter Membrane, the use of the Sartorius combination filter was considered to be reasonably safe, unless it could be shown that smaller components in the surrogate sample solution could actually plug up the 0.20 µm membrane filter. However, subsequent testing showed that the 0.20 µm membrane component did not affect the filtration outcome in any significant way.

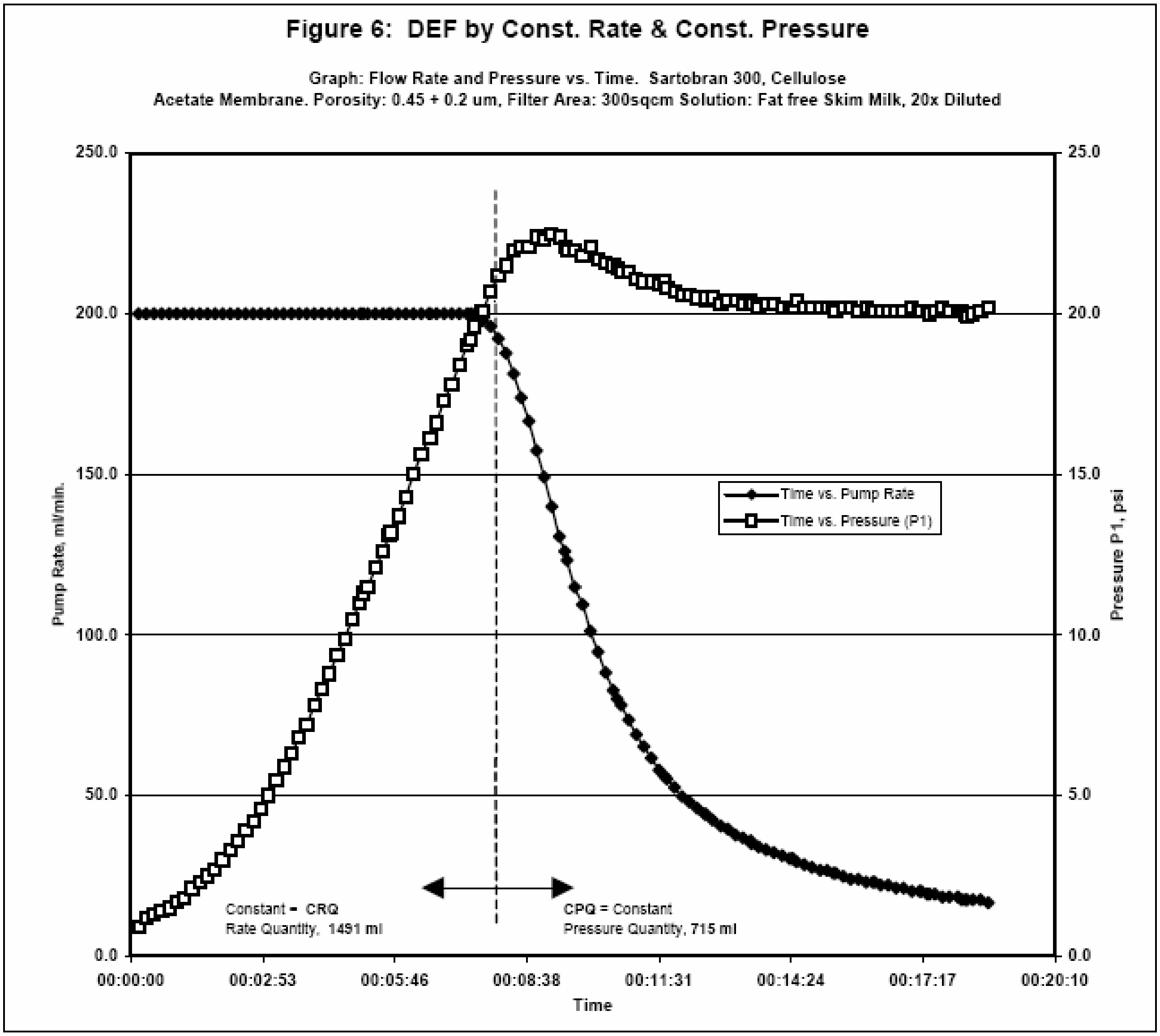



Figure 6 summarizes the DEF Sartobran filtration throughput under Constant Rate & Constant Pressure conditions. The Total Filtrate weight was 2206 gr; the Constant Rate Quantity (CRQ) contributed 1491 gr whereas the Constant Pressure Quantity (CPQ) added 715 gr to the Total Filtrate weight.

## Summary:

***The new DEF filtration method, namely, the "SciLog Constant Rate / Constant Pressure Procedure" can have a very significant economic impact by enhancing DEF filtration throughput by 30 to 35%.*** However, additional filterability tests, using different filter materials and conditions, are necessary to demonstrate the universal applicability of the new DEF liquid handling procedure. Fig 7 & 8 summarize the experimental results:

Figure 7:

*MFS Membrane Filters : Cellulose Acetate, Porosity: 0.45um, Area: 17.3 sqcm*

Solution: Fat Free Skim Milk, 20x Diluted

| Filtration Method: | Pressure Limit: | Pump Rate: | Collected Filtrate: | | CRQ | CPQ |
|---|---|---|---|---|---|---|
| Const. Pressure | 20 psi | | 96 gr | 71% | | |
| Const. Rate | 20 psi | 15 ml/min | 89 gr | 65% | | |
| Rate + Pressure | 20 psi | 15 ml/min | 136 gr | 100% | 89 gr<br>65% | 47 gr<br>35% |

* CRQ = Constant Rate (Filtrate) Quantity CPQ = Constant Pressure (Filtrate) Quantity

Figure 8

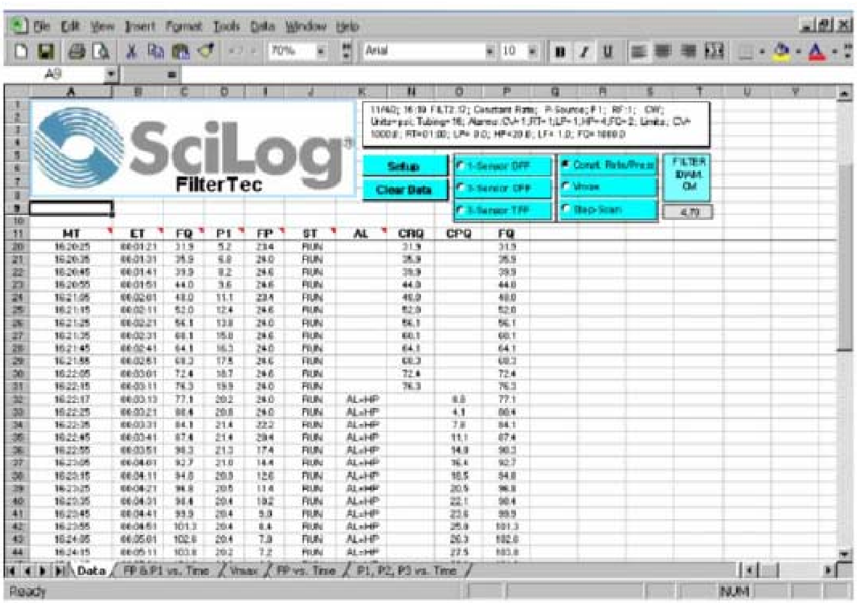
*Sartobran 300 : Cellulose Acetate, Porosity: 0.45um + 0.20um, Filter Area: 300sqcm*

Solution: Fat Free Skim Milk, 20x Diluted

| Filtration Method: | Pressure Limit: | Pump Rate: | Collected Filtrate: | | CRQ* | CPQ* |
|---|---|---|---|---|---|---|
| Const. Pressure | 20 psi | | 1591 gr | 72% | | |
| Const. Rate | 20 psi | 200 ml/min | 1491 gr | 68% | | |
| Rate + Pressure | 20 psi | 200 ml/min | 2206 gr | 100% | 1491 gr<br>68% | 715 gr<br>32% |

* CRQ = Constant Rate (Filtrate) Quantity CPQ = Constant Pressure (Filtrate) Quantity

The FilterTec is accompanied by an automated documentation system consisting of a copy of WinWedge32 and a custom Excel spreadsheet that imports the data from the FilterTec on a real time basis and automatically populates the spreadsheet and it's related graphs. This system was crucial in collecting an analyzing the data contained herein. The following is a screenshot of the FilterTec Custom Excel spreadsheet:




References: Zeman, L. J. & Zydney, A. L. Microfiltration and Ultrafiltration Principles and Applications. Marcel Dekker, Inc. NY, 1996.



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