# Comparability of In-line and At-line Variable Pathlength Technology for Concentration Monitoring in UF/DF Processes

White Paper

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## Abstract

In contemporary biomanufacturing practices, process analytical technology (PAT) instruments have become indispensable tools for enhancing process understanding and facilitating automation. Among these, the CTech™ FlowVPX® System represents a highly effective PAT solution for real-time concentration monitoring, employing UV spectrophotometry as its core analytical method. Using Variable Pathlength Technology (VPT), concentration can be directly measured during process operation without the need for buffer correction or scatter correction, as these interferences are minimized or rendered negligible through slope-based measurement.

In this study, we evaluated the performance of both the FlowVPX and CTech™ SoloVPE® PLUS Systems in tangential flow filtration (TFF) processes, employing a variety of monoclonal antibody (mAb) and antibody-drug conjugate (ADC) materials. Tests were run primarily at the lab scale, with selected assessment at the pilot scale , including an assessment of the impact of certain method modifications.

Both systems were found to be comparable across concentration ranges spanning from 5 mg/mL to 230 mg/mL. Furthermore, the results suggest that off-line sampling methods may introduce deviations, particularly when large sampling ports are used and when there are significant concentration differences between consecutive sampling points. Real-time monitoring was shown to mitigate errors associated with manual sampling by operators and to potentially improve yield by eliminating the need for off-line sampling and measurement.

## Variable Pathlength Technology Principle

Variable Pathlength Technology (VPT) is a UV-based analytical method that determines concentration by using the slope of absorbance versus pathlength data, derived from the Beer-Lambert Law. According to this principle, the slope, ***m***, of the absorbance versus pathlength data is proportional to the extinction coefficient, ***ε***, and concentration, ***c***, as shown below:

$$10 \cdot m = \varepsilon \cdot c$$

The factor of 10 arises because slope measurements are conventionally in Abs·mm$^{-1}$, while extinction coefficients are in (mg/mL)$^{-1}$·cm$^{-1}$.

The FlowVPX System automatically identifies the pathlength corresponding to approximately 1.0 Abs and subsequently acquires 5 to 10 measurements at decreasing pathlengths to generate the slope. This slope value, when divided by the extinction coefficient, yields concentration at each time point. Concentration data are collected continuously, with measurement intervals ranging from 10 to 30 seconds depending on the method configuration, enabling real-time process monitoring.

## Materials

- CTech FlowVPX System
- CTech SoloVPE PLUS System
- CTech SoloVPE System
- TFF systems
- Monoclonal antibody samples
- Antibody-drug conjugate samples
- TFF filters
- Tubing and connectors

## Experiment Setup

The FlowVPX System was installed between the feed vessel and the main pump in the TFF system. Since most of the process material was contained in the feed vessel, placing the FlowVPX instrument immediately downstream allowed direct





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measurement of the bulk process material, thereby providing a representative concentration profile for the entire process line. Additionally, a three-way connector was incorporated into the setup to facilitate off-line sampling for independent concentration measurements using the SoloVPE PLUS System.

The FlowVPX platform was employed exclusively for real-time concentration monitoring, without automated feedback control of the TFF system.

## Experiment Design

A feasibility study was conducted with five different molecules across a wide range of concentration levels. A total of seven tangential flow filtration (TFF) processes were operated and evaluated. For each run, in-line concentration measurements using the FlowVPX System were compared against off-line measurements obtained using the SoloVPE PLUS System. Table 1 provides an overview of the experiment design employed in this evaluation.

In addition, this study evaluated the impact of scatter correction settings, specifically by comparing measurement performance with and without the application of dual-wavelength scatter correction. The scalability of the FlowVPX system was further evaluated, along with its suitability for in-line monitoring of antibody-drug conjugate (ADC) concentrations during ultrafiltration/diafiltration (UF/DF) processes.

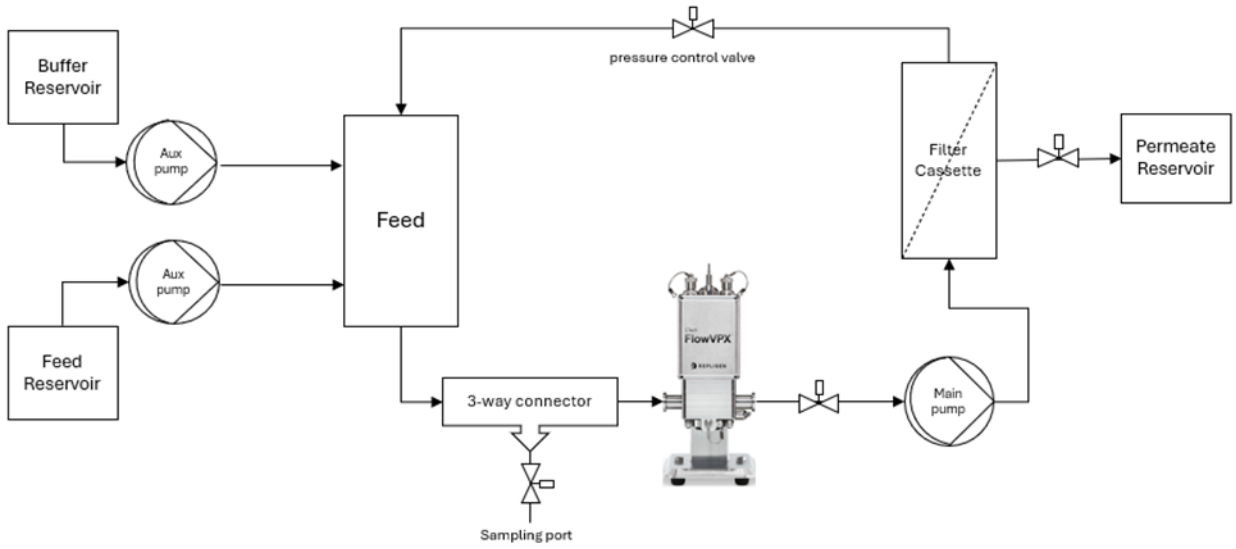



**Figure 1.** FlowVPX installation in TFF system

**Table 1.** Summary of experiment design

| Process Run | #1 | #2 | #3 | #4 | #5 | #6 | #7 |
|---|---|---|---|---|---|---|---|
| Product | mAb-1 | mAb-2 | mAb-2 | mAb-3 | mAb-4 | ADC | mAb-1 |
| Process | UF/DF | UF/DF | UF | UF | UF | UF | UF/DF |
| Scale / Flow Cell Inner Diameter | Lab scale; 3 mm | Lab scale; 3 mm | Lab scale; 3 mm | Lab scale; 3 mm | Lab scale; 3 mm | Lab scale; 3 mm | Pilot scale; 10 mm |
| FlowVPX Method | 5 data points; 280 nm; Dual WL scatter correction | 5 data points; 280 nm; Dual WL scatter correction | 5 data points; 280 nm; No scatter correction | 5 data points; 280 nm; Dual WL scatter correction | 5 data points; 280 nm; Dual WL scatter correction | 5 data points; 280 nm, *WL2*;* Dual WL scatter correction | 5 data points; 280 nm; Dual WL scatter correction |
| SoloVPE PLUS Method | 10 data points; 280 nm; Dual WL scatter correction | 10 data points; 280 nm; Dual WL scatter correction | 10 data points; 280 nm; Dual WL scatter correction | 10 data points; 280 nm; Dual WL scatter correction | 10 data points; 280 nm; Dual WL scatter correction | 10 data points; 280 nm, *WL2**; No scatter correction | 10 data points; 280 nm; Dual WL scatter correction |

**WL2 represents the secondary wavelength used in the ADC method.*

## Sampling Methods and Data Collection

An important metric of data quality in variable pathlength system is the $R^2$ value, which indicates the linearity of the absorbance vs. pathlength data collected during each concentration measurement. A higher $R^2$ value means the data complies well with the Beer-Lambert law. As shown in Figure 2, a decrease in $R^2$ values occurs during the constant feed concentration (CFC) and diafiltration (DF) phases because low-concentration materials, such as UF/DF load material or buffer, are introduced into the feed vessel, resulting in an uneven distribution of sample material and creating temporary concentration gradients. These conditions naturally yield slightly lower $R^2$ values during these process stages.

To mitigate this process-related variability and obtain more accurate concentration measurements, the system was periodically switched to recirculation mode for off-line sampling. Recirculation mode entails closing both the permeate valve and the auxiliary pump and circulating the process material within the TFF system for 3 to 5 minutes in a closed loop. Sampling was performed only after concentration was stable, ensuring that the collected samples accurately represented the process stream. The periods corresponding to this stabilized state are indicated by the orange areas in top of Figure 2, highlighting the importance of this controlled sampling approach throughout the UF1 (CFC) and DF phases.

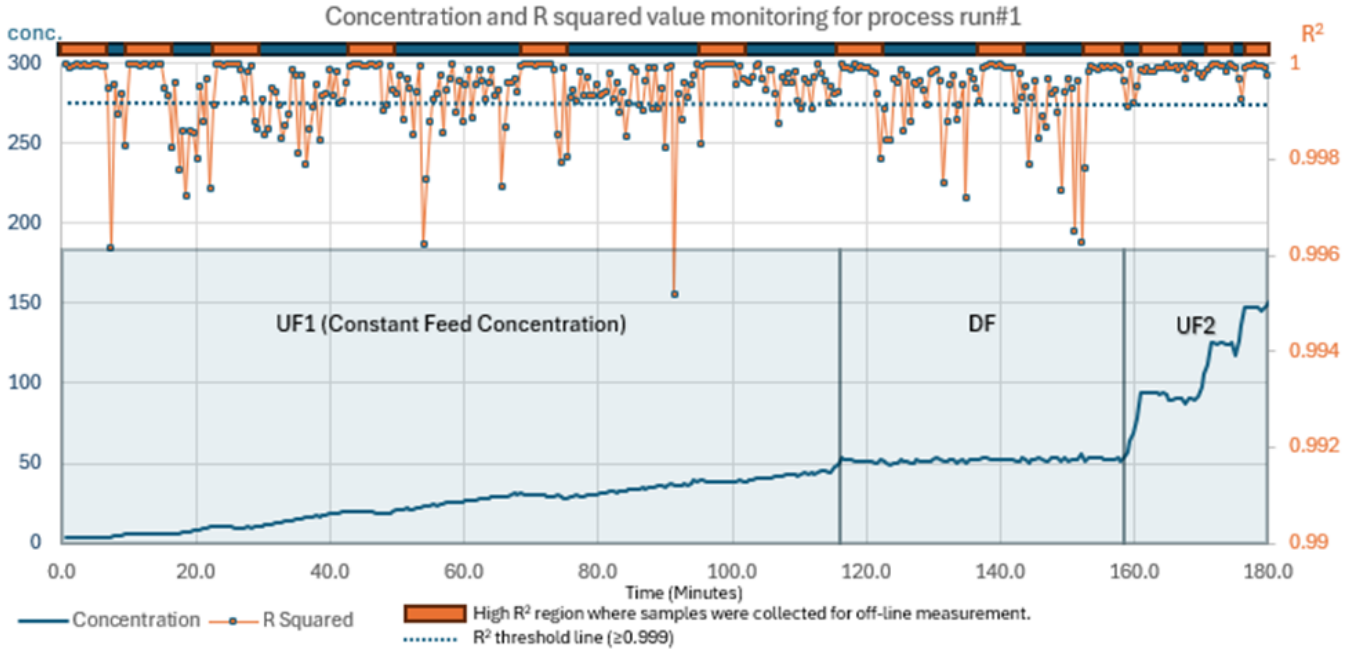



**Figure 2.** $R^2$ value and concentration during UF/DF process

## Evaluation Method

Comparability between the FlowVPX and SoloVPE PLUS Systems was evaluated based on the percent difference relative to the SoloVPE PLUS measurements, as the performance of the SoloVPE PLUS System has been previously validated.

In this evaluation, previously validated SoloVPE method configurations were applied to the SoloVPE PLUS System without modification, except for an adjustment to the number of data points used for the FlowVPX measurements. The percent difference was calculated using the following equation:

$$\%Difference = \frac{(FlowVPX\ Conc. - SoloVPE\ PLUS\ Conc.)}{SoloVPE\ PLUS\ Conc.} \times 100$$

A total of four distinct mAb products were assessed under lab -scale tangential flow filtration (TFF) conditions. To ensure diversity in molecular structure and subclass, the evaluation included two IgG1 antibodies, one IgG2 antibody, and one Fc-fusion protein. This experimental design aimed to confirm the system's robustness and reliability in accurately measuring concentration across various IgG types and molecular configurations.

## Results and Discussion

Since calculated percent difference values may include both positive and negative numbers, directly calculating the average could lead to misinterpretation. Therefore, the average of the absolute value of each percent difference was used to quantify accuracy across different IgG types:

$$Avg.|\%Diff.| = \frac{|\%Diff_1| + |\%Diff_2| + \cdots + |\%Diff_n|}{n}$$

As summarized in Table 2, the average absolute difference across all four products ranged from 0.5% to 0.8%, and the maximum observed difference was 3.0%. Detailed datasets are presented in Appendices A, B, C, and D. The percent difference observed in this evaluation is well within the acceptance limits for a typical UF/DF process, which is commonly ±5%. Additionally, no significant differences in accuracy were observed among the IgG types.

**Table 2.** Comparability study summary for process runs #1, 2, 4, and 5

| Process Run | #1 | #2 | #4 | #5 |
|---|---|---|---|---|
| Product | mAb-1 | mAb-2 | mAb-3 | mAb-4 |
| IgG Type | IgG1 | IgG1 | IgG2 | Fc-fusion |
| Process | UF/DF | UF/DF | UF | UF |
| Concentration Range | 5 – 230 mg/mL | 5 – 150 mg/mL | 5 – 160 mg/mL | 5 – 90 mg/mL |
| Max \|%Diff.\| | 1.9% | 3.0% | 1.3% | 0.9% |
| **Average \|%Diff.\|** | **0.8%** | **0.7%** | **0.5%** | **0.7%** |

### Buffer Exchange Impact Assessment

Possible slope contribution from the buffer was previously evaluated during method development and validation for SoloVPE methods. In this study, the potential impact of buffer exchange on in-line measurement using the FlowVPX System was assessed by comparing the results to the off-line measurement obtained using SoloVPE PLUS System. To evaluate this, process run #1 and #2 were conducted via the following sequence:

1. Ultrafiltration 1 (UF1) in Constant Feed Concentration (CFC) mode: the antibody was concentrated while maintaining the same buffer composition.
2. Diafiltration (DF): the buffer composition was changed to the final formulation, while the antibody concentration remained constant.
3. Ultrafiltration 2 (UF2): the antibody concentration was increased to the target concentration while maintaining the final formulation buffer.

During the DF process, since the antibody concentration remains constant, the effect of buffer exchange on measurement accuracy can be directly assessed without interference from concentration differences. Table 3 summarizes the percent difference values between FlowVPX and SoloVPE PLUS measurements at select intervals during the DF phase, measured in diavolumes (DV). No significant accuracy differences were observed during the buffer exchange process.

Table 4 compares measurement accuracy between the UF1 and UF2 phases. Despite the combined effects of concentration and buffer changes, no significant differences in accuracy were observed between these process stages.

**Table 3.** Comparison of measurements during diafiltration phase

| Process Run #1 | | | | Process Run #2 | | | |
|---|---|---|---|---|---|---|---|
| Sampling Point | FlowVPX (mg/mL) | SoloVPE PLUS (mg/mL) | %Difference | Sampling Point | FlowVPX (mg/mL) | SoloVPE PLUS (mg/mL) | %Difference |
| 0 DV | 50.677 | 50.355 | 0.6% | 0 DV | 51.238 | 50.951 | 0.6% |
| 2 DV | 52.118 | 52.157 | -0.1% | 2 DV | 51.283 | 51.284 | 0.0% |
| 4 DV | 52.940 | 52.548 | 0.7% | 4 DV | 51.510 | 51.311 | 0.4% |
| **Average \|%Diff.\|** | | | **0.5%** | **Average \|%Diff.\|** | | | **0.3%** |

**Table 4.** Comparison of measurements during ultrafiltration phases

| Process Run #1 | | | | Process Run #2 | | | |
|---|---|---|---|---|---|---|---|
| Conc. Point (mg/mL) | FlowVPX (mg/mL) | SoloVPE PLUS (mg/mL) | %Difference | Conc. Point (mg/mL) | FlowVPX (mg/mL) | SoloVPE PLUS (mg/mL) | %Difference |
| Initial | 3.431 | 3.457 | -0.8% | Initial | 9.840 | 9.882 | -0.4% |
| 5 | 5.819 | 5.765 | 0.9% | 25 | 24.612 | 24.467 | 0.6% |
| 10 | 9.959 | 9.832 | 1.3% | 35 | 34.502 | 34.541 | -0.1% |
| 20 | 19.420 | 19.288 | 0.7% | 45 | 43.719 | 43.676 | 0.1% |
| 30 | 29.790 | 29.576 | 0.7% | **UF1 Average \|%Diff.\|** | | | **0.3%** |
| 40 | 38.331 | 38.475 | -0.4% | 70 | 72.363 | 74.589 | -3.0% |
| **UF1 Average \|%Diff.\|** | | | **0.8%** | 90 | 90.616 | 90.87 | -0.3% |
| 90 | 90.124 | 91.061 | -1.0% | 110 | 111.901 | 111.995 | -0.1% |
| 120 | 125.160 | 124.488 | 0.5% | 130 | 131.595 | 132.106 | -0.4% |
| 150 | 147.067 | 148.556 | -1.0% | 150 | 151.221 | 154.895 | -2.4% |
| 170 | 163.431 | 165.442 | -1.2% | **UF2 Average \|%Diff.\|** | | | **1.2%** |
| 190 | 190.335 | 190.502 | -0.1% | | | | |
| 210 | 211.739 | 210.087 | 0.8% | | | | |
| 230 | 225.218 | 229.672 | -1.9% | | | | |
| **UF2 Average \|%Diff.\|** | | | **0.2%** | | | | |

### Scalability Study

To assess the scalability of the FlowVPX System, a pilot-scale run was performed using the FlowVPX 10 mm Flow Cell to compare against the lab-scale 3 mm Flow Cell. The TFF process used the mAb-1 product with a concentration range from 5 mg/mL to 135 mg/mL. The majority of measurements in the lab-scale process were within 2.0% (average: 0.8%), while the pilot-scale process exhibited a maximum difference of 7.3% (average: 2.0%). However, in the pilot-scale evaluation, the initial three data points—corresponding to the initial, 10 mg/mL, and 15 mg/mL concentration points—exhibited notable deviation due to sampling error. At these concentration points, insufficient flowthrough volume was discarded, leaving residual material from the previous sample in the sampling port. This diluted the subsequent samples and underestimated the concentration. From the fourth data point (20 mg/mL) onward, more than 10 mL of sample was flushed prior to collecting samples for off-line measurements, which significantly reduced measurement deviation.

After excluding the initial three deviated data points in the pilot-scale process, the average difference was reduced to 1.1%, which is comparable to the results obtained from lab-scale evaluation. These results are shown in Table 5.

**Table 5.** Comparison of measurements during lab-scale and pilot-scale processes

| Process Run #1—Lab Scale (3 mm Flow Cell) | | | | Process Run #7—Pilot Scale (10 mm Flow Cell) | | | |
|---|---|---|---|---|---|---|---|
| Conc. Point (mg/mL) | FlowVPX (mg/mL) | SoloVPE PLUS (mg/mL) | %Difference | Conc. Point (mg/mL) | FlowVPX (mg/mL) | SoloVPE PLUS (mg/mL) | %Difference |
| Initial | 3.431 | 3.457 | -0.8% | Initial | 3.667 | 3.499 | **4.8%** |
| 5 | 5.819 | 5.765 | 0.9% | 10 | 10.73 | 10.433 | **2.8%** |
| 10 | 9.959 | 9.832 | 1.3% | 15 | 15.776 | 14.709 | **7.3%** |
| 20 | 19.420 | 19.288 | 0.7% | 20 | 21.446 | 21.579 | -0.6% |
| 30 | 29.790 | 29.576 | 0.7% | 25 | 25.977 | 25.701 | 1.1% |
| 40 | 38.331 | 38.475 | -0.4% | 30 | 32.291 | 31.918 | 1.2% |
| 50 (0 DV) | 50.677 | 50.355 | 0.6% | 40 | 44.108 | 43.024 | 2.5% |
| 50 (2 DV) | 52.118 | 52.157 | -0.1% | 50 (0 DV) | 52.088 | 51.742 | 0.7% |
| 50 (4 DV) | 52.940 | 52.548 | 0.7% | 50 (2 DV) | 53.503 | 53.344 | 0.3% |
| 90 | 90.124 | 91.061 | -1.0% | 50 (4 DV) | 55.145 | 54.945 | 0.4% |
| 120 | 125.160 | 124.488 | 0.5% | 90 | 88.409 | 85.924 | 2.9% |
| 150 | 147.067 | 148.556 | -1.0% | 110 | 106.293 | 105.081 | 1.2% |
| 170 | 163.431 | 165.442 | -1.2% | 120 | 119.808 | 120.993 | -1.0% |
| 190 | 190.335 | 190.502 | -0.1% | 130 | 134.244 | 135.575 | -1.0% |
| 210 | 211.739 | 210.087 | 0.8% | **Average \|%Diff.\|*** | | | **1.1%** |
| 230 | 225.218 | 229.672 | -1.9% | | | | |
| Recovery | 24.528 | 24.895 | -1.5% | | | | |
| **Average \|%Diff.\|** | | | **0.8%** | | | | |

**The initial three data points were excluded from the calculation of* Average |%Diff.| *in process run #7.*

### Scatter Correction Impact Study

To assess the impact of the implementation of a scatter correction algorithm, FlowVPX measurements were conducted both with and without scatter correction. The SoloVPE PLUS System was configured with dual wavelength scatter correction and used as the reference data for comparison.

As summarized in Table 6, the scatter correction method was found to have no significant impact on measurement performance during the UF/DF process. Data acquired with scatter correction showed a maximum difference of 3.0% and an average of 0.7%, while data without scatter correction showed a maximum difference of 3.1% and an average of 1.0%. Both methods provided comparable results, indicating that the absence of scatter correction did not adversely affect measurement accuracy.

Notably, without scatter correction, the system consistently achieved higher slope linearity ($R^2 > 0.999$) and faster data acquisition (one slope measurement every 0.2 minutes) than with the scatter correction method.

**Table 6.** Comparison of TFF process data with and without scatter correction

| Process Run #2—Dual Wavelength Scatter Correction Enabled | | | | Process Run #3—No Scatter Correction | | | |
|---|---|---|---|---|---|---|---|
| Conc. Point (mg/mL) | FlowVPX (mg/mL) | SoloVPE PLUS (mg/mL) | %Difference | Conc. Point (mg/mL) | FlowVPX (mg/mL) | SoloVPE PLUS (mg/mL) | %Difference |
| Initial | 9.840 | 9.882 | -0.4% | 30 | 31.700 | 31.916 | -0.7% |
| 25 | 24.612 | 24.467 | 0.6% | 50 | 50.674 | 50.146 | 1.1% |
| 35 | 34.502 | 34.541 | -0.1% | 70 | 72.359 | 72.827 | -0.6% |
| 45 | 43.719 | 43.676 | 0.1% | 90 | 94.662 | 91.827 | 3.1% |
| 50 (0 DV) | 51.238 | 50.951 | 0.6% | 110 | 112.074 | 111.890 | 0.2% |
| 50 (2 DV) | 51.283 | 51.284 | 0.0% | 130 | 136.025 | 135.566 | 0.3% |
| 50 (4 DV) | 51.510 | 51.311 | 0.4% | 150 | 161.279 | 160.107 | 0.7% |
| 70 | 72.363 | 74.589 | -3.0% | **Average \|%Diff.\|** | | | **1.0%** |
| 90 | 90.616 | 90.870 | -0.3% | | | | |
| 110 | 111.901 | 111.995 | -0.1% | | | | |
| 130 | 131.595 | 132.106 | -0.4% | | | | |
| 150 | 151.221 | 154.895 | -2.4% | | | | |
| **Average \|%Diff.\|** | | | **0.7%** | | | | |

### Capability of real-time antibody-drug conjugate (ADC) concentration monitoring

In the case of Antibody-Drug Conjugates (ADCs), payloads usually exhibit significant absorbance at 280 nm, which interferes with conventional concentration measurement methods typically used for monoclonal antibodies. This spectral overlap restricts accurate determination of ADC concentration using standard single-wavelength UV measurements. Therefore, the concentration of ADC is determined using a multi-wavelength method that mathematically corrects for the additional absorbance introduced by the payload. The method requires the extinction coefficients of both the antibody and the payload to be known.

In this evaluation, slope data were collected at two wavelengths: 280 nm and a secondary wavelength, WL2, corresponding to the payload. The ViPER software performed real-time ADC concentration analysis. The software automatically calculated the ADC concentration based on the collected slope data, applying the following equation:

$$ADC\ Conc. = \frac{\boldsymbol{m}(280nm) - \boldsymbol{m}(WL2) \times \frac{\boldsymbol{\varepsilon}(payload, 280nm)}{\boldsymbol{\varepsilon}(payload, WL2)}}{\boldsymbol{\varepsilon}(antibody, 280nm)}$$

The results summarized in Table 7 indicate that the measurement accuracy for ADCs remained within an acceptable range, demonstrating the applicability of the FlowVPX System for real-time monitoring during the ADC UF/DF process.

**Table 7.** ADC ultrafiltration process results

| Concentration Point (mg/mL) | FlowVPX (mg/mL) | SoloVPE PLUS (mg/mL) | %Difference |
|---|---|---|---|
| 5 | 4.722 | 4.871 | -3.1% |
| 10 | 10.459 | 10.732 | -2.5% |
| 15 | 13.989 | 14.377 | -2.7% |
| 20 | 19.184 | 19.474 | -1.5% |
| 25 | 24.636 | 25.018 | -1.5% |
| 30 | 32.987 | 33.744 | -2.2% |
| 40 | 39.868 | 39.843 | 0.1% |
| **Average \|%Difference\|** | | | **1.9%** |

**Comparability between SoloVPE and SoloVPE PLUS Systems**

A comparison study was conducted to evaluate the analytical performance of the SoloVPE and SoloVPE PLUS Systems, with a particular focus on the impact of the minimum pathlength step size on slope accuracy. The primary objective was to assess whether the finer pathlength resolution of SoloVPE PLUS offers improved linearity and consistency, particularly for high concentration mAb sample.

As summarized in Table 8, the SoloVPE PLUS instrument features a pathlength step size of 0.002 mm, compared to 0.005 mm for the predecessor SoloVPE System. This allows the SoloVPE PLUS System to operate within a lower and narrower pathlength range, even when configured with a higher number of data points. For instance, with 10 data points, the SoloVPE PLUS instrument can achieve a concentration measurement utilizing a pathlength range of 0.002–0.020 mm, which is lower than the 0.005–0.025 mm range required by the SoloVPE instrument to obtain just 5 data points.

Typically, VPT systems achieve the best results when the absorbance is less than 1.0, as this falls within the optimal dynamic range of the detector. Due to the limitations in the minimum achievable pathlength, the SoloVPE System requires a reduced number of data points when analyzing high concentration samples (>100 mg/mL) to prevent saturation of the detector and thus underestimation of the concentration. In contrast, the SoloVPE PLUS System maintains absorbance values below 1.0 even when configured with 10 data points up to 230 mg/mL (the highest concentration tested in this study), thereby enabling the use of more data points within the linear absorbance range. This results in improved measurement consistency and overall method robustness.

For the mAb-1 product, limitations of the SoloVPE System necessitated a reduction in data points when the sample concentration exceeded 150 mg/mL. The SoloVPE PLUS System demonstrated better linearity under these conditions, supporting its suitability for future applications involving high concentration processes. The findings from this study indicate that the SoloVPE PLUS System provides enhanced analytical performance through lower pathlength step size, especially for high concentration samples, compared to the SoloVPE System.

**Table 8.** Pathlength capabilities comparison between SoloVPE and SoloVPE PLUS Systems

| Instrument | SoloVPE | SoloVPE PLUS |
|---|---|---|
| Pathlength step size | 0.005 mm | 0.002 mm |
| Lowest pathlength range* (10 data points) | 0.005 mm – 0.050 mm | 0.002 mm – 0.020 mm |
| Lowest pathlength range* (5 data points) | 0.005 mm – 0.025 mm | N/A** |

**The lowest pathlength range is used to measure the highest possible concentration.*
***The SoloVPE PLUS System did not require a reduced number of data points to measure any samples in this study.*

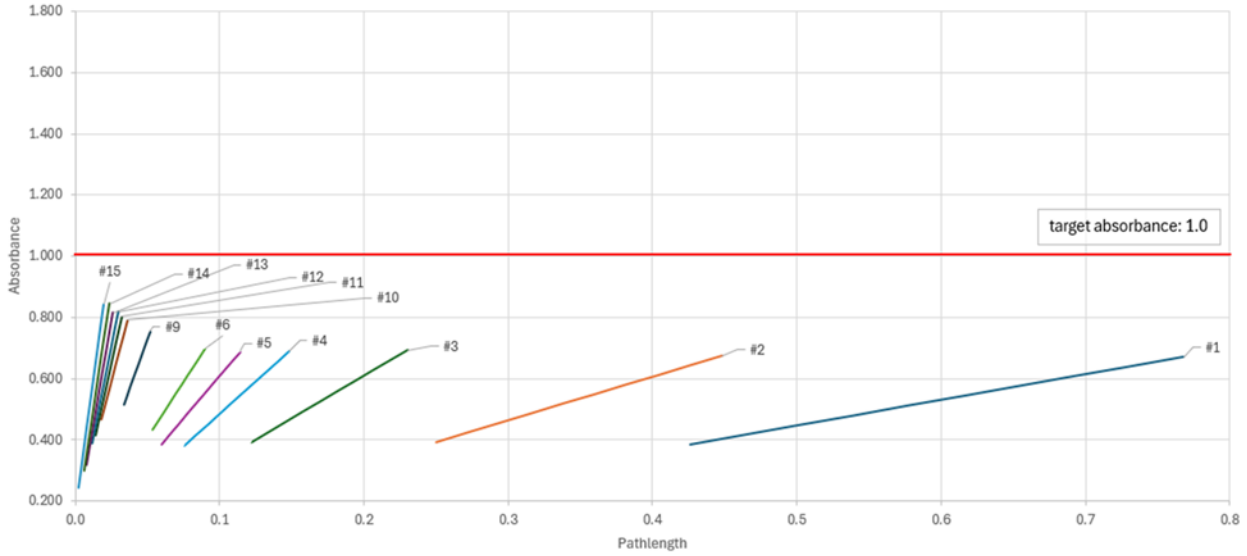



**Figure 3.** SoloVPE PLUS slope graphs for mAb-1 sample. Each line represents the absorbance vs. pathlength data of a single concentration measurement. The SoloVPE PLUS System was able to keep absorbance readings in the optimal range below 1.0 Abs, even for the highest concentrations.

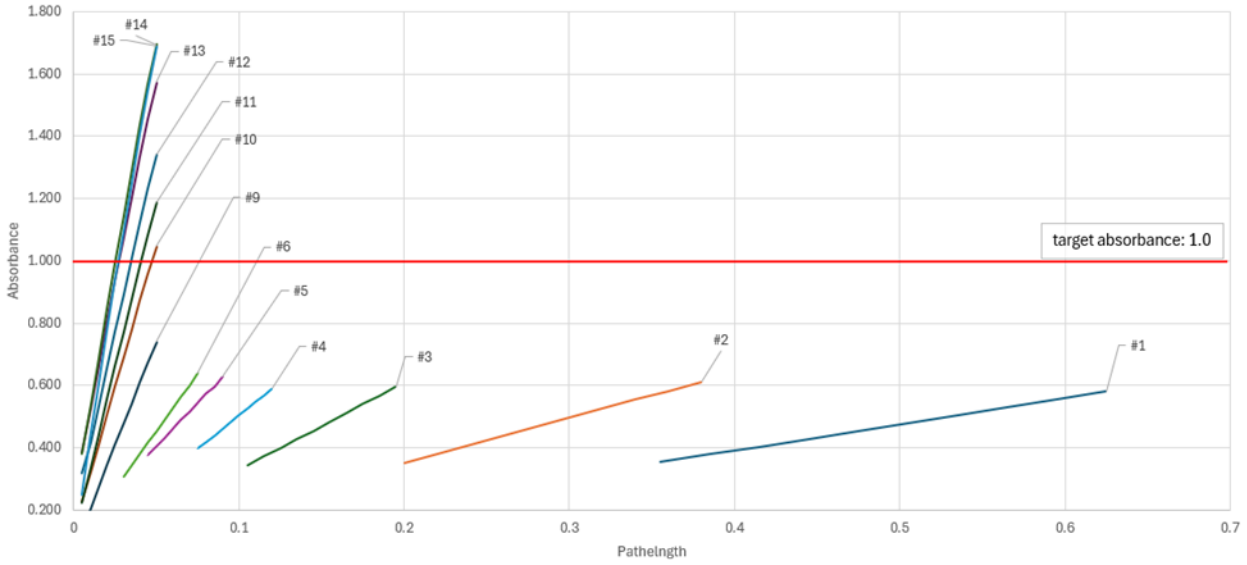



**Figure 4.** SoloVPE slope graphs for mAb-1 sample. Each line represents the absorbance vs. pathlength data of a single concentration measurement. Samples with very high concentrations result in absorbance measurements above 1.0 Abs, which can potentially lead to saturation of the detector.

**Table 9.** Comparability between SoloVPE and SoloVPE PLUS Systems

| Sample Number | SoloVPE PLUS (10 data points) | SoloVPE (10 data points) | SoloVPE (5 data points) | %Difference (10 data points) | %Difference (5 data points) |
|---|---|---|---|---|---|
| #1 | 5.765 | 5.783 | N/A | -0.3% | N/A |
| #2 | 9.832 | 9.898 | | -0.7% | |
| #3 | 19.288 | 19.255 | | 0.2% | |
| #4 | 29.576 | 29.483 | | 0.3% | |
| #5 | 38.475 | 38.147 | | 0.9% | |
| #6 (0 DV) | 50.355 | 50.751 | | -0.8% | |
| #7 (2 DV) | 52.157 | 52.981 | | -1.6% | |
| #8 (4 DV) | 52.548 | 52.815 | | -0.5% | |
| #9 | 91.061 | 92.566 | | -1.7% | |
| #10 | 124.488 | 126.070 | | -1.3% | |
| #11 | 148.556 | 147.437 | | 0.8% | |
| #12 | 165.442 | 158.798 | 163.611* | 4.0% | 1.1% |
| #13 | 190.502 | 182.979 | 190.532 | 3.9% | 0.0% |
| #14 | 210.087 | 203.003 | 211.015 | 3.4% | -0.4% |
| #15 | 229.672 | 214.984 | 224.015* | 6.4% | 2.5% |

**For samples #12 and #15, a pathlength range of 0.010–0.030 mm was used due to low linearity ($R^2$) observed at 0.005 mm pathlength.*

## Conclusion

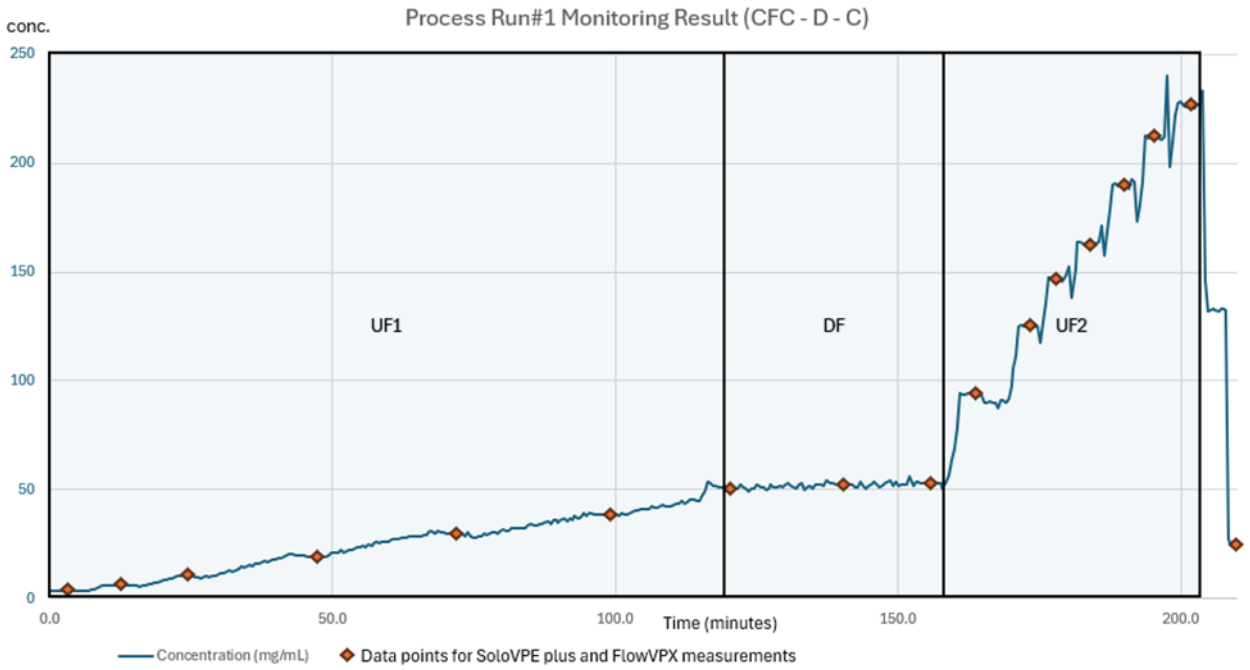
This study comprehensively evaluated the performance of the FlowVPX and SoloVPE PLUS Systems in TFF processes, demonstrating that both systems provide comparable measurement accuracy over a wide range of concentration levels. Real-time in-line monitoring using the FlowVPX system was shown to effectively mitigate errors associated with manual sampling and off-line measurements, thereby improving process efficiency and reducing material loss.

The ability of the FlowVPX System to deliver continuous, real-time concentration data presents significant advantages for biomanufacturing applications, particularly in enhancing process control and minimizing variability. The scalability assessment confirmed that the system maintained high measurement accuracy even under pilot-scale conditions. Although some deviations were initially observed, these were attributed to at-line sampling errors, which were effectively addressed by implementing appropriate sampling protocols, including the use of recirculation mode to stabilize concentrations before measurement.

The evaluation of scatter correction methods indicated that their impact on measurement accuracy was minimal under the tested conditions, suggesting that simpler analytical approaches without scatter correction may be sufficient, particularly when process conditions do not induce significant light scattering. Furthermore, the study demonstrated the applicability of the FlowVPX System for real-time ADC concentration monitoring during UF/DF processes. Despite the inherently more complex nature of ADC analysis, the system achieved acceptable accuracy, highlighting its potential as a valuable PAT tool for advanced biomanufacturing environments.

Future studies should further explore the long-term implementation and integration of the FlowVPX System within full-scale manufacturing processes to comprehensively validate its benefits for real-time monitoring and automated process control.

## Appendix A: Process Run #1 Concentration Data




| Conc. Point (mg/mL) | FlowVPX (mg/mL) | SoloVPE PLUS (mg/mL) | %Difference |
|---|---|---|---|
| Initial | 3.431 | 3.457 | -0.8% |
| 5 | 5.819 | 5.765 | 0.9% |
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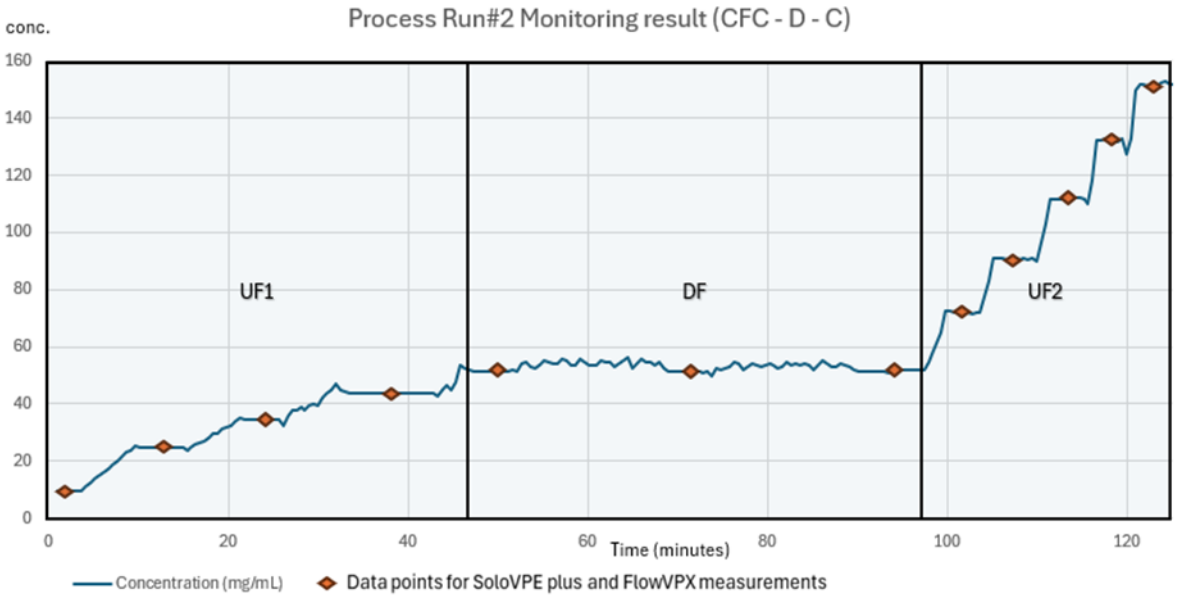
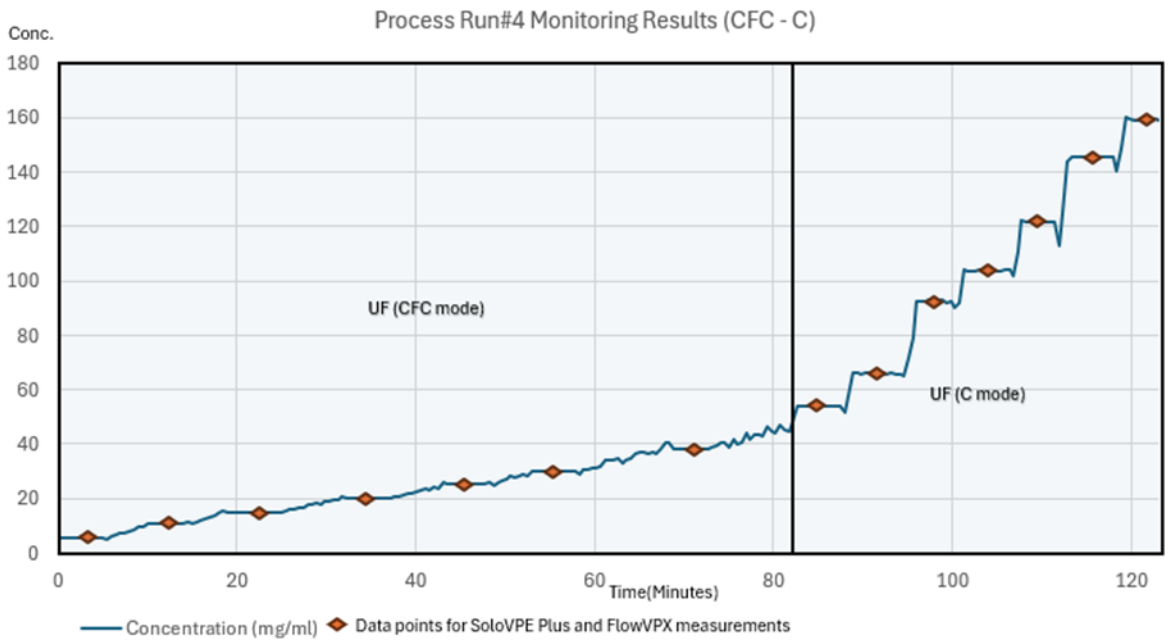
| 40 | 38.331 | 38.475 | -0.4% |
| 50 (0 DV) | 50.677 | 50.355 | 0.6% |
| 50 (2 DV) | 52.118 | 52.157 | -0.1% |
| 50 (4 DV) | 52.940 | 52.548 | 0.7% |
| 90 | 90.124 | 91.061 | -1.0% |
| 120 | 125.160 | 124.488 | 0.5% |
| 150 | 147.067 | 148.556 | -1.0% |
| 170 | 163.431 | 165.442 | -1.2% |
| 190 | 190.335 | 190.502 | -0.1% |
| 210 | 211.739 | 210.087 | 0.8% |
| 230 | 225.218 | 229.672 | -1.9% |
| Recovery | 24.528 | 24.895 | -1.5% |

## Appendix B: Process Run #2 Concentration Data



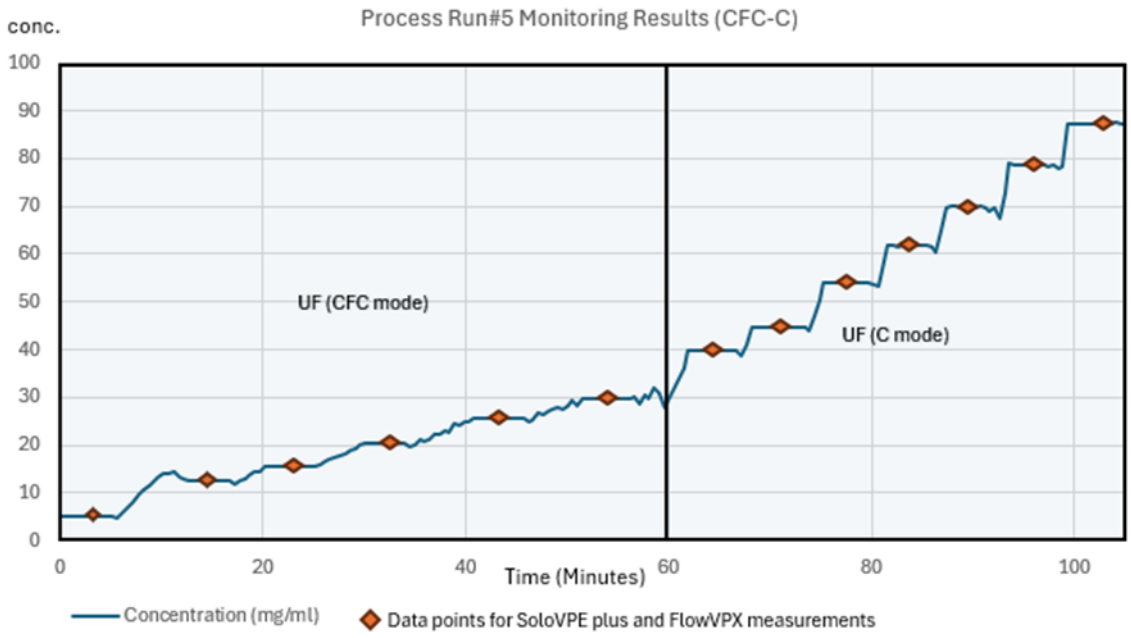

| Conc. Point (mg/mL) | FlowVPX (mg/mL) | SoloVPE Plus (mg/mL) | %Difference |
|---|---|---|---|
| Initial | 9.840 | 9.882 | -0.4% |
| 25 | 24.612 | 24.467 | 0.6% |
| 35 | 34.502 | 34.541 | -0.1% |
| 45 | 43.719 | 43.676 | 0.1% |
| 50 (0 DV) | 51.238 | 50.951 | 0.6% |
| 50 (2 DV) | 51.283 | 51.284 | 0.0% |
| 50 (4 DV) | 51.510 | 51.311 | 0.4% |
| 70 | 72.363 | 74.589 | -3.0% |
| 90 | 90.616 | 90.870 | -0.3% |
| 110 | 111.901 | 111.995 | -0.1% |
| 130 | 131.595 | 132.106 | -0.4% |
| 150 | 151.221 | 154.895 | -2.4% |

## Appendix C: Process Run #4 Concentration Data




| Conc. Point (mg/mL) | FlowVPX (mg/mL) | SoloVPE PLUS (mg/mL) | %Difference |
|---|---|---|---|
| Initial | 5.588 | 5.629 | -0.7% |
| 10 | 11.093 | 11.153 | -0.5% |
| 15 | 14.919 | 14.947 | -0.2% |
| 20 | 20.407 | 20.403 | 0.0% |
| 25 | 25.414 | 25.348 | 0.3% |
| 30 | 29.908 | 29.750 | 0.5% |
| 40 | 38.332 | 38.014 | 0.8% |
| 50 | 53.887 | 53.614 | 0.5% |
| 65 | 66.045 | 65.601 | 0.7% |
| 90 | 92.737 | 93.257 | -0.6% |
| 105 | 103.979 | 104.666 | -0.7% |
| 120 | 121.375 | 121.203 | 0.1% |
| 140 | 145.456 | 145.495 | 0.0% |
| 160 | 159.582 | 161.745 | -1.3% |

## Appendix D: Process Run #5 Concentration Data




| Conc. Point (mg/mL) | FlowVPX (mg/mL) | SoloVPE PLUS (mg/mL) | %Difference |
|---|---|---|---|
| Initial | 5.021 | 4.978 | 0.9% |
| 10 | 12.431 | 12.368 | 0.5% |
| 15 | 15.479 | 15.392 | 0.6% |
| 20 | 20.317 | 20.212 | 0.5% |
| 25 | 25.604 | 25.559 | 0.2% |
| 30 | 29.641 | 29.400 | 0.8% |
| 40 | 39.842 | 39.490 | 0.9% |
| 45 | 44.723 | 44.597 | 0.3% |
| 55 | 53.833 | 53.484 | 0.7% |
| 60 | 61.820 | 61.396 | 0.7% |
| 70 | 69.815 | 69.206 | 0.9% |
| 80 | 78.950 | 78.236 | 0.9% |
| 90 | 86.965 | 87.587 | -0.7% |

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