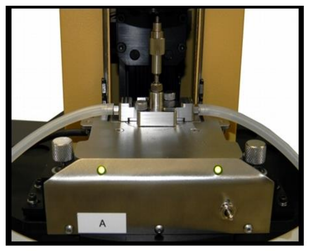
FlowVPE: Evaluation of an In-line Protein Concentration Device

By Gerald Cruz, Joseph Sexton

ABSTRACT

Typical UV spectrophotometric analysis is limited to a fixed path-length for both bench-top analytics and in-process OD meters. On the bench top side, a fixed path-length cuvette system introduces dilution errors as the sample must be diluted to within an acceptable absorbance range. On the process side, UV-flow cells are fixed at a path-length that allows for accurate linear pool cuts from ~0.1 to only ~1 OD, however this comes with the tradeoff of complete saturation of the detector and a loss of OD signal above ~5OD. The FlowVPE is a flow-cell from C Technologies designed for in-line measurement of protein concentration. This flow-cell, which is based on the bench top SoloVPE system, was subjected to multiple tests in order to evaluate proof of concept. Our team was able to demonstrate FlowVPE real time protein concentration with bench top level accuracy. By integrating real time concentration to volume, process efficiency such as real time protein mass and mass balance was also demonstrated. With the ability to produce greater linear range, the FlowVPE has shown the capability to accurately make higher pool cuts than what has been historically used. This technology can serve as a powerful analytical tool with immediate applications such as full peak characterization, novel load breakthrough detection, and enabling alternative purification schemes.

PROTOTYPE



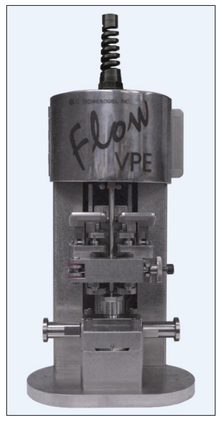
Preliminary Testing

The Prototype was able to demonstrate it's capability of reading protein concentration in-line. Although there were some design issues that were brought to light during testing (a more robust zeroing method, observed leakage from flow cell, locking screw accessibility), the device was still able to show that it can produce comparable readings to our in-house devices, show it's cleanable, can detect small changes in OD, as well as detect OD above the upper limits of our current meters. C Technologies was given the feedback which were addressed in the next generation FlowVPE.

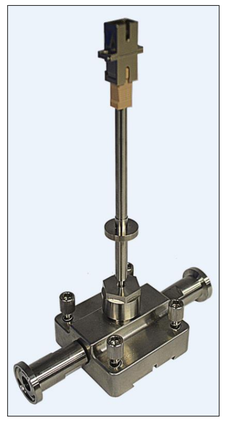
Areas further developed from Prototype -

- Was a raw prototype piggybacked onto an existing system
- Zeroing-position technique was raw
- Software development was needed to "instrumentalize"
- Prototype had a low flow rate

NEXT GENERATION FLOWVPE



FlowVPE Unit



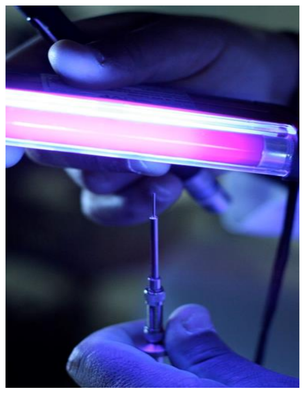
Flow-cell

Design

The FlowVPE was redesigned with a more robust zeroing mechanism than its predecessor. The flow-cell is now outfitted with tri-clamp 0.5" fittings which will enable a full flow path with existing Pilot Scale skids.

Cleaning

Initial cleaning test was performed with the fibrette first in a stationary state (2 positions to see if lowering the fibrette introduced an "unclean" portion of it) and then later with the fibrette in motion. Both tests were conducted similarly to the cleaning protocol used for the prototype: After contact with protein the flow-cell was subjected to a series of washes which included an initial PW rinse, 0.5N NaOH wash, and final PW rinse TOC samples were taken at the end of each final PW rinse and submitted to QCIP. Methods approximating current standard practices were found to have cleaned the flow-cell adequately by meeting the criteria of <1 ppm.



Riboflavin Testing

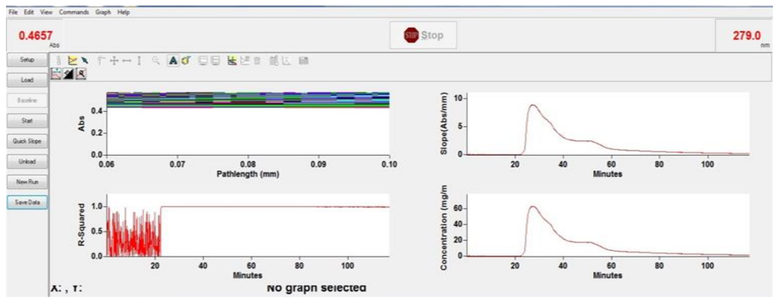
In another test, flow cell was loaded with riboflavin and fibrette cycled full range (0.005 to 8mm) with an open flow path. Flow cell was then rinsed with PW and disassembled to determine riboflavin location. Riboflavin was not visible when using a standard UV light source.

Functional Testing

The functionality of the FlowVPE as an inline protein concentration device was tested by following the Molecule B process through all 3 of its chromatography steps. It was set-up inline immediately downstream of skid OD Meters, upstream of skid flow meter. Device is connected via sanitary tubing and hose barbs. Entire process flow passes through FlowVPE unit (i.e. not split stream). Analog output box is housed within NEMA cabinet. UV Cary60 and C-Tech laptop reside on skid platform. Historian data collection was verified and the unit was "calibrated" to a known solution so that output tags matched known solution. The difference between the known and actual display was subtracted from actual display output so that the two ran true to each other. Further, an additional tag "calculator" was enabled that integrates real time concentration to volume from pool start to pool finish thereby providing real time protein mass.

Functional Testing (cont.)

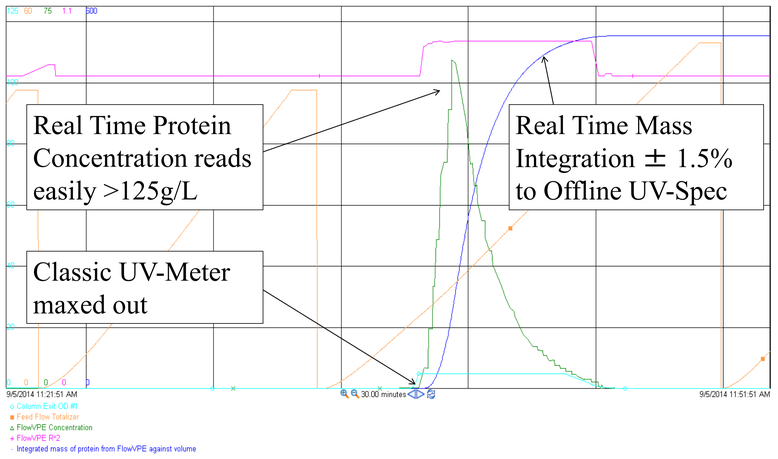
The two modes in the software, Quick Slope and Fixed Slope, performed well. Quick Slope Kinetic Mode, whereby the software self-selects the optimum path length range to scan, provided readings ~ every few seconds. Fixed slope mode, where path length range is preset, revealed shorter sampling time and adequate resolution of the peak. The unit functioned well with respect to flow, no leakage, little-to-no noticeable backpressure. All phases of chromatography operations (including column storage) were run through unit. The unit was flushed, sanitized and stored with the skid as normal.



FlowVPE Interface

The integrated mass calculator functioned exceedingly well, generating highly accurate real time protein concentration (see graph below). The integrated mass calculator trends 0.3% to 2.7% with phenomenal potential to optimize (see graph below).

Molecule B - Affinity Elution Step



SUMMARY

The latest FlowVPE device once again was able to show that it can produce comparable readings to our in-house devices, show it's cleanable, can detect small changes in OD, as well as detect OD above the upper limits of our current meters. The device can now be added to an existing Pilot Scale skid and run full flow path. Zeroing-position is robust, cleaning concerns seemingly mitigated, and software more appropriate for historian tie-in. We've found that online concentration measurement through FlowVPE increases the speed at which we acquire process data and make sound decisions. The quality of this online data is far greater than the current "clipped" UV/OD signal. This technology has immediate application, today… as well as potential for future applications and continued growth.

ACKNOWLEDGMENTS

Ramsey Shanbaky (rshanbaky@ctechnologiesinc.com)
Chris Dowd, James Patch, Paul McDonald, Neria Daniel, Chris Williams, Eileen Duenas, Bijee George, Domenic Schimizzi, Philip Chung *and the Pilot Plant Ops Tech Forum,* Debbie O'Connor, Jimmy Sugahara, Alysha de Souza… and the entire B7 Purification and Drug Product Pilot Plant.