Perfusion Culture Optimization Through Accurate Metabolite Analysis

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Introduction

- Continuous countercurrent tangential chromatography (CCTC) is a continuous, column-free, and single-use system for protein purification
- CCTC integrates the single-use perfusion and the CCTC platform into a single continuous and steady-state unit operation
- RX Series Analyzer provides repeatable and accurate measurement of common Cell Culture analytes and metabolites
- RX Series Analyzer experiences low-drift of measurement over time compared to membrane-based (electrochemical) technologies

Objectives

- **End-to-end integrated continuous chromatography**
- **Optimize the perfusion process to achieve the highest cell density, and consequently, mAB productivity through accurate monitoring of feed strategies**

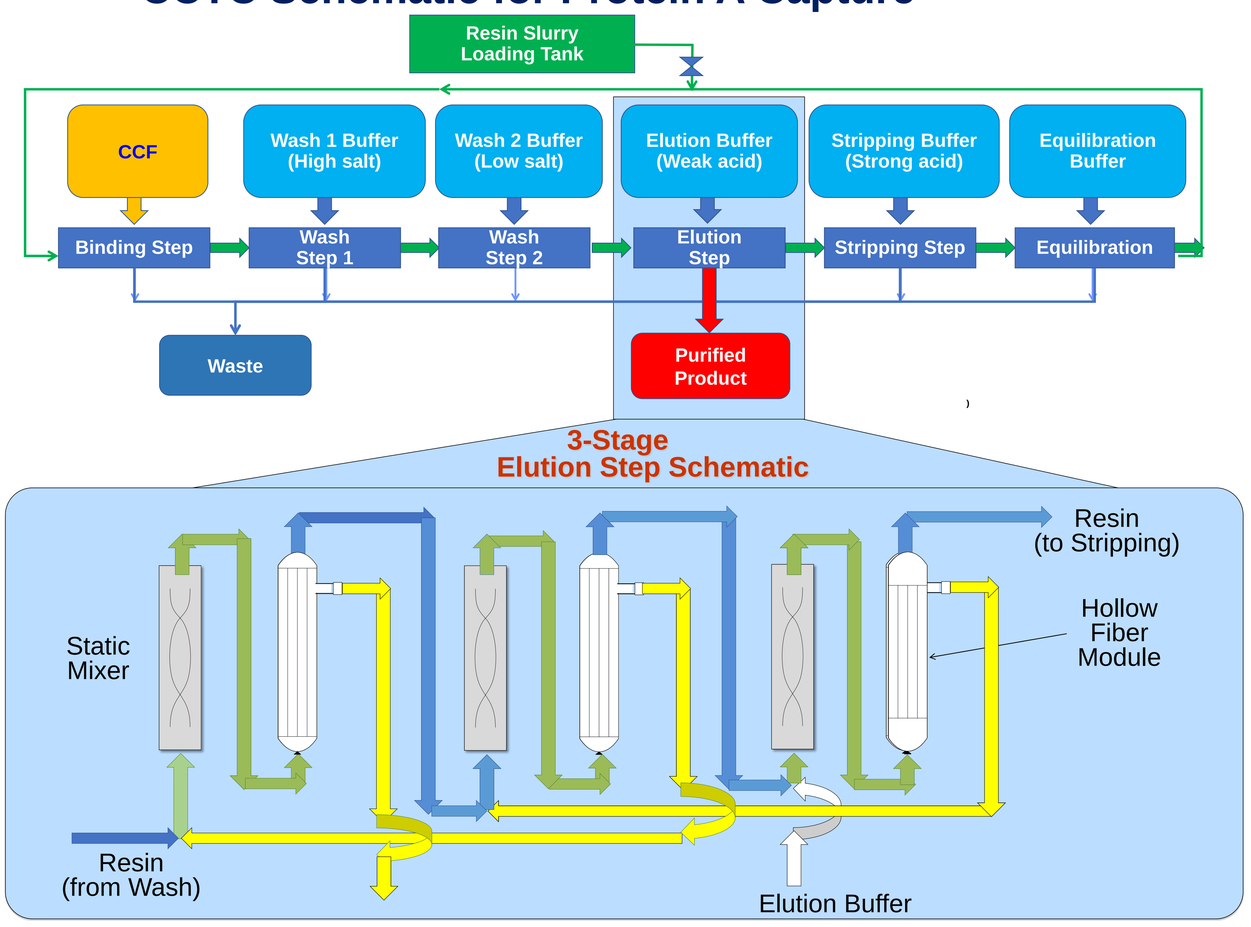
ChromaTan PD System

- Modular and saving cost of Process Development
- Capability to fill buffer directly on the skid
- Flexible, accessible, open architecture
- Fully automated
- Single-use

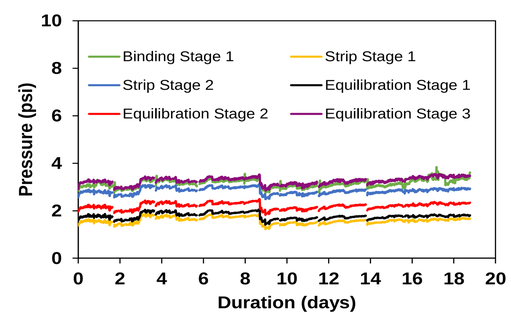
CCTC Features

- 5-15x increase in productivity compared to batch column
- Ability to optimize process conditions during a single run
- Comparable yield / purification performance as column
- Low pressure (<15 psi) operation; suitable for single use

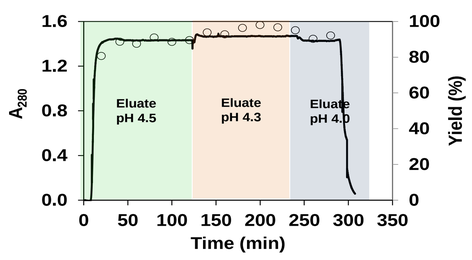
CCTC Schematic for Protein A Capture



CCTC Applications



Long term stability of the CCTC system:
Ideal for integration with perfusion bioreactor.



Polishing of mAb: Tuning of pH to optimize yield During a run

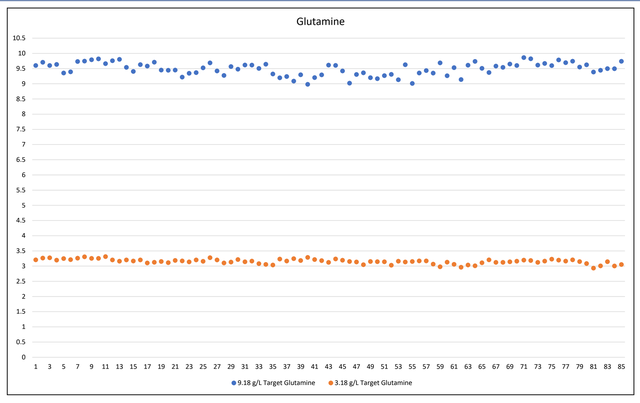
Polishing step

- ~5x increase in productivity
- 5% increase in yield

Rx Series Analyzer

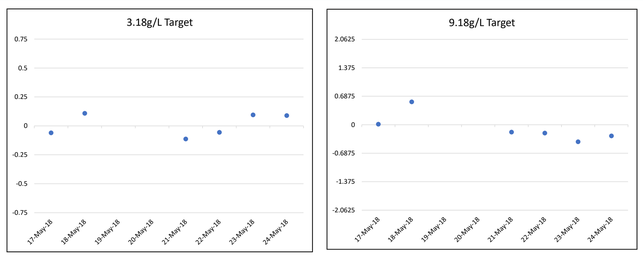
- Automated instrument to monitor cell culture metabolites: Glucose, Lactate, Ammonia, Glutamine, Glutamate, LDH, IgG, etc.)
- Photometric analyzer utilizes robotic pipetting arms to deliver sample along with a specific reagent into a measurement cuvette. Enzymatic reaction occurs, creating a color, which is correlated to an analyte concentration
- Less than 300uL required per sample
- Low maintenance and calibration intervals
- Robust and accurate performance

Metabolite Measurements



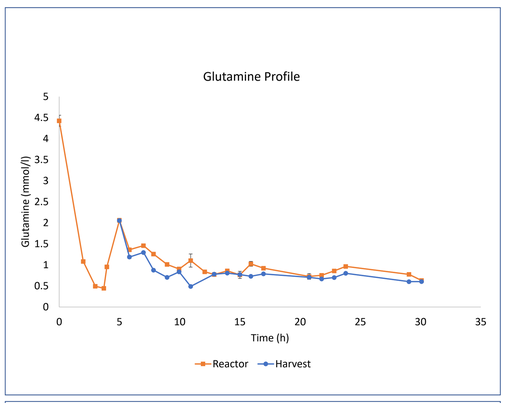
Glutamine Control Replicates

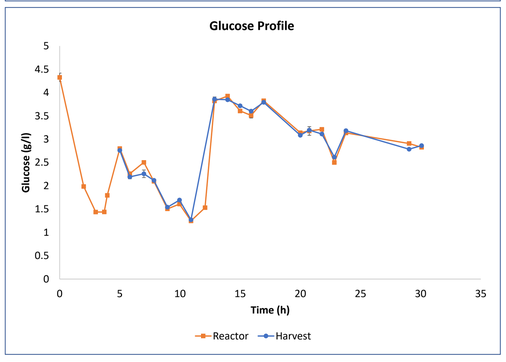
- To challenge Rx Series instrument; 85 known Glutamine samples were measured in replicates: 3.18 g/L and 9.18 g/L
- Results fell within 1 Standard Deviation



Glutamine Control Standards

- Glutamine samples ran once per day for known concentrations of 3.18g/L and 9.18g/L between May 17 and May 24, 2018
- All results fell within 1 Standard Deviation





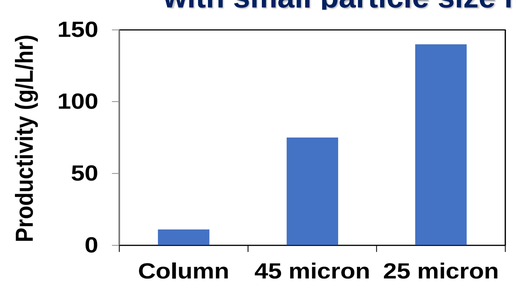
Glutamine and Glucose Monitoring

- Perfusion began on day 5
- Blue profile (●) are results from the harvest stream (cell free samples)
- Orange profile (■) are results from cell containing samples from the reactor
- Good correlation achieved: Cell free versus cell containing samples

Results and Conclusions

- Monitoring glucose and glutamine over the course of the experiment, and providing sufficient amount of nutrients, helped to achieve 60×10^6 viable cells/ml
- Analysis performed on Rx Series of both cell containing and cell free samples yielded results showing good agreement
- The Rx Series instrument was challenged with samples of known concentration (Controls) over an extended period of time for multiple metabolites showing low standard deviation in all cases
- A study of the RX Series analyzer was performed to show exceptional repeatability of Glutamine measurement, with very low drift

ProA capture productivity of 140 g of mAb /L resin /hr with small particle size resin from Purolite Corp.



Improving productivity:

- 45 micron off the shelf resin shows a 6X productivity improvement.
- Custom 25 micron particle shows 15X improvement in resin productivity for Protein A capture

Future End-to-End Continuous Integrated Bio-manufacturing Platform

Perfusion / Fed Batch Bioreactor → CCTC Capture → CCTC Intermediate → CCTC Polishing

Integration Project 1 (Funded by NIH) | Integration Project 2 (Funded by FDA)