*Title: **Novel Application of Pourbaix Diagrams to Model Precipitation in Upstream Biomanufacturing Process Solutions***

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ABSTRACT

Commercial production of therapeutic proteins using mammalian cells requires complex process solutions, and consistency of these process solutions is critical to maintaining product titer and quality between batches. Inconsistencies between process solutions prepared at bench and commercial scale may be due to differences in mixing time, temperature, and pH which can lead to precipitation and subsequent removal via filtration of critical solution components such as trace metals. Pourbaix diagrams provide a useful tool to model the solubility of trace metals and were applied to troubleshoot the scale-up of nutrient feed preparation after inconsistencies in product titer were observed between bench- and manufacturing-scale batches. Pourbaix diagrams modeled the solubility of key metals in solution at various stages of the nutrient feed preparation and identified copper precipitation as the likely root cause of inconsistent media stability at commercial scale. Copper precipitation increased proportionally with temperature in bench-scale preparations of nutrient feed and temperature was identified as the root cause of copper precipitation at the commercial scale. Additionally, cell culture copper titration studies performed in bench-scale bioreactors linked copper-deficient mammalian cell culture to inconsistent titers at the commercial scale. Pourbaix diagrams can predict when trace metals are at risk of precipitating and can be used to mitigate risk during the scale-up of complex media preparations.

BACKGROUND

Protein therapeutics are manufactured by genetically modified mammalian cells; once the DNA which codes for the therapeutic is inserted into the cell genome, the cell machinery is used to assemble the protein of interest. Since this manufacturing process relies heavily on cell metabolism, cell growth, and

productivity, the quality attributes of the therapeutic protein are all dependent on the media environment in which the cells are cultured (Bruhlmann et al., 2015; Price, 2017).

Cell Culture Media Development

The earliest documented examples of cell culture media systems date back to the late 19th century, when a media comprised of reconstituted dried ox blood supplemented with saline solution was used for the primary culture of frog cardiac muscle tissue(Ringer, 1882). In 1959, Harry Eagle developed the first chemically-defined, animal component free media for the culture of mammalian cells in an important technological development in the industry(Eagle, 1959). Today there exists an entire industry centered around the formulation of cell culture media to suit specific cell lines(Jayme, Watanabe, & Shimada, 1997), and media development is a major topic of study under the cell culture umbrella(Ritacco, Wu, & Khetan, 2018) as it has been demonstrated that improvements in media formulation (and the subsequent optimization of the process feed strategy) can result in higher productivity and lower COGS in protein manufacturing processes(Hakkinen et al., 2018; Huang et al., 2010; Xu, Gavin, Jiang, & Chen, 2017). A prominent topic in the development of media is the availability of trace metals, such as copper, iron, and manganese, which are critical to maintain product titer and quality(Ehret, Zimmermann, Eichhorn, & Zimmer, 2019; Mitchelson, Mondia, & Hughes, 2017; Vijayasankaran et al., 2013).

Commercial cell lines are becoming increasingly productive, and the requirements for the media which supports those cells are also becoming more complex. Media optimization is a balance between planning and chemistry, since these highly productive cell lines often have high cell-specific nutrient demands and so modern cell culture media systems must deliver those nutrients while keeping solubility limitations in mind. High pH, which is required to dissolve amino acids like tyrosine for example, can cause sub-visible precipitation of metal salts among other critical media components which may only be detectable after obstructing sterile filters (Cao, Loussaert, & Wen, 2016; De Chialvo, Marchiano, & Arvía, 1984). Media recipes are generally developed on a small scale for evaluation in micro-scale or bench-scale bioreactors,

and scale-up can introduce new challenges as scale-dependent differences in temperature profile and mix time may impact the amount of precipitate formed in a preparation. Media development operations can be de-risked via the application of solution modelling tools which provide useful insight into the solution state under varying conditions.

Pourbaix Diagrams

Pourbaix diagrams display the chemical equilibrium of transition metals as a function of pH and electric potential, and were originally developed as a method of modelling corrosion in electrochemical systems(Pourbaix, 1974) and to characterize the formation and state of oxidized metal species(Hansen, Rossmeisl, & Norskov, 2008; Sergentu et al., 2016). Pourbaix diagrams are constructed using the Nernst equations for electrochemical reactions (horizontal lines) and Van't Hoff equations for chemical reactions (vertical lines). Each line on a Pourbaix diagram represents an equilibrium condition where each solid or ion species at the boundary condition are of equal molar concentration. Pourbaix diagrams display equilibrium functions and thus supplemental reaction kinetics equations must be applied to determine the rate of conversion between solid and ionic species as a function of time(Perry et al., 2019). However, Pourbaix diagrams are a concise method to identify the predominate chemical species of the metals in aqueous solutions at a known pH and electric potential.

Data presented in this case study demonstrate that Pourbaix diagrams can be applied to cell culture media development to predict precipitation of media components at various solution pH and electric potentials, which is a key boon for media designs that must maximize the concentrations of critical media components into a limited number of process solutions.

INTRODUCTION

This work describes a new approach to troubleshooting a media development challenge and highlights the use of Pourbaix diagrams to narrow investigative scope and drive planning and execution of bench-scale experiments. The subject of the case study is the preparation of a chemically defined nutrient feed (NF)

media for a mammalian cell culture process which contains a high concentration of multiple amino acids with optimal solubility at high pH. As a result, the final pH of the recipe is between 9.0 and 9.5.

 The NF formulation described in this case study contains multiple powdered amino acids. A copper impurity was detected in a lot of one of these amino acids, cystine, which was used to make media for several experiments during early process development. The presence of copper impurities in cystine has been documented elsewhere in literature(Keenan et al., 2018), and a different cystine supplier was used to minimize the copper impurity. However a high level of variability was observed when cystine from the new supplier was evaluated in the upstream process. The impact could be mitigated by supplementing the NF formulation with cupric sulfate pentahydrate at bench scale (5 L), but process variability still occurred at manufacturing scale (2000 L). In the development stages, NF was prepared in small batches (<50 L) for use in 5 L bench-scale bioreactor studies. However, when the process was transferred to a commercial manufacturing facility, NF was prepared in a 1000 L stirred tank and differences in process performance again indicated that the copper concentration was insufficient.

The manufacturing scale cultures were observed to have lower than expected viability and elevated lactate concentration which lead to low pH at harvest; metabolic and cell performance indicators were consistent with historical descriptions of copper deficiency(Boulet et al., 2018; Qian et al., 2011; Soma et al., 2018; Yuk et al., 2015). NF copper concentrations were measured via inductively-coupled plasma mass spectrometry (ICP-MS) and the data indicated that the large-scale NF preparations contained a lower concentration of copper than was previously measured in the bench-scale NF even though the two preparations used the same formulation. During the initial troubleshooting stages, the copper concentration prior to filtration was also evaluated via ICP-MS and was found to be consistent with the expected concentration, which suggested that copper was being removed during the filtration step. Previous studies have demonstrated that soluble copper ions can oxidize to insoluble cuprous oxide and cuprous hydroxide at alkaline pH consistent with this NF(De Chialvo et al., 1984), and additionally cuprous oxide crystals of approximately 0.2 µm in size have been shown to form on the surface of a

copper plate when exposed to air at 300K for 120 minutes(Lee, Hsu, & Tuan, 2016). These examples suggest a potential mechanism which could explain the change in copper concentration observed after filtration, but additional work was necessary to support the hypothesis and understand the unique properties of this NF system.

Pourbaix diagrams of the NF preparation were constructed to improve our understanding of copper solubility throughout the process. The process understanding gained from the modelling techniques guided the design of bench-scale experiments to explore the underlying mechanism behind the copper removal. Once the mechanism was determined to be copper precipitation, the results of bench-scale copper titration studies were used to determine the amount of cupric sulfate pentahydrate stock solution to add to the large-scale manufacturing process to successfully recover process performance.

MATERIALS AND METHODS

Cell Culture

Bench-scale bioreactor studies were conducted in 5 L glass vessels (Applikon Biotechnology, Foster City, CA) with a 2.5 L initial working volume and a seeding density of 1.2×10^6 cells/mL. The culture process was controlled by TruBio DV controllers (Finesse Solutions, San Jose, CA). Details about the bench-scale bioreactor apparatus, control strategy, and product quality analytical methods have been described elsewhere(Gilbert, McElearney, Kshirsagar, Sinacore, & Ryll, 2013; Huang et al., 2010; Mimura et al., 2018).

Nutrient feeds were added daily, starting on day 3 of the culture, and targeted a fixed percentage of the culture volume at the time of feeding. A 40% w/v glucose stock solution was fed as needed to maintain a non-limiting glucose concentration between 3-6 g/L.

The copper addition to experimental nutrient feeds was modulated using an aqueous 20 mg/L cupric sulfate pentahydrate stock solution. A titration study was performed which increased the theoretical copper concentration at harvest up to 1.7-fold, 2.6-fold, 3.7-fold, and 4.8-fold (1.7x, 2.6x, 3.7x, and 4.8x

respectively) relative to the theoretical harvest concentration without additional copper (1x). Additionally, cupric sulfate pentahydrate was supplemented to a target harvest concentration of a 770-fold increase (770x) to theoretical copper harvest concentration to evaluate the impacts of an extreme high concentration (far in excess of biological requirements) on cell productivity and product quality.

Method of Modelling Media Preparation Conditions

The NF media described in this work was prepared via the sequential addition of powdered chemical components to water at 55°C. As the preparations were not heated, the temperature decreased over time and this temperature was only monitored in manufacturing-scale batches. Once the final component was added, the solution was sterilized by passing through a 0.1 µm polyethersulfone (PES) filter (Millipore, Burlington, MA).

For bench-scale experiments evaluating the impact of different preparation temperatures on copper precipitation, NF media was prepared according to the recipe in a single 50L aliquot. Prior to the addition of copper, the incomplete media was transferred to 5L Applikon bioreactors (Applikon Biotechnology, Foster City, CA) and mixed at a power-per-volume of 27 W/m^3 to simulate the mixing of the commercial-scale media prep tanks. The mixing times after each component addition were fixed to remain consistent between the small- and large-scale preparations. Copper was added in the form of cupric sulfate pentahydrate (Thermo-Fisher, Waltham, MA) to the incomplete media targeting a final copper concentration that was demonstrated to have no negative impacts to cell culture. The final steps of the preparation titrated the pH via the addition of 10 N sodium hydroxide and 6 N hydrochloric acid (both from Thermo-Fisher, Waltham, MA) and these final 3 stages of the NF preparation and the corresponding Pourbaix diagrams have been summarized in Figure 1. The preparation temperature in each 5L vessel was controlled at either 30°C, 40°C, or 50°C to simulate the difference in rate of temperature decline between small and large preparations. Samples were taken at each step of the preparations and immediately filtered through 0.1 µm PES filters and analyzed via IC-PMS to determine the soluble copper concentration. As the composition, temperature and dissolved oxygen are changing throughout the preparation of the feed

media, a single solution potential cannot be used in the model. Instead, redox potential was measured in triplicate preparations using redox probes (Hamilton, Reno, Nevada) to determine the redox state of the NF preparation. (Pluschkell & Flickinger, 1995)

Pourbaix Diagram

Pourbaix diagrams were generated in HSC Chemistry 9 software (Outotec, Denver, CO) using 3 soluble metals (Cu, Zn, Fe) and 3 ion or polyatomic ion elements (chlorine, sulfur, carbon). The concentrations of copper, iron and zinc were determined via ICPMS measurements of feed preparations, while the chlorine concentration was measured using the Beckman MetaFlex (Beckman, Indianapolis, IN). The concentrations of sulfur and carbon used in the Pourbaix diagrams were based on the theoretical sulfate and carbonate concentration in the NF. Temperature for the model ranged from 30-50°C and the model pressure was set to 1 atmosphere.

Analytical Methods

Daily viable cell concentrations and viability was measured using the Beckman Coulter Vi-CELL XR (Beckman Coulter, Brea, CA) using trypan blue exclusion. Glucose, glutamine, lactate, ammonium, potassium, and sodium were measured using the Roche Cedex BioHT (Roche Diagnostics, Indianapolis, IN). Daily pH, pCO2, and p02 were measured using a RAPIDPoint 500 blood gas analyzer (Siemens AG, Berlin, Germany).

Harvest samples were centrifuged at 3,000 rpm for five minutes and filtered through a 0.2 µm filter. The centrate was then purified using a Protein A column and the eluate was measured for glycosylation composition. The antibody concentration and charge variant of the antibodies were determined using various methods previously described (Tsang, Wang, Yusuf-Makagiansar, & Ryll, 2014; Yang et al., 2014; Zhang et al., 2016).

ICP-MS

Elemental analysis was performed using inductively coupled plasma mass spectrometry (ICP-MS). These measurements were taken using a PerkinElmer Nexion 350D quadrupole spectrometer (PerkinElmer Inc., Waltham, MA) utilizing an Elemental Scientific baffled cyclonic spray chamber and concentric nebulizer (Elemental Scientific, Omaha, NE). Plasma power was 1600 W and the nebulizer gas flow rate was 0.98 L/min. All elements were analyzed using Kinetic Energy Dispersion (KED) mode employing helium as the collision gas. Daily tuning activities were performed to minimize the impact of doubly charged and oxidized species.

The instrument was calibrated with 8 stock solutions ranging from 0.5 µg/L to 1000 µg/L for all elements of interest. These stock solutions were generated through the serial dilution of PerkinElmer Instrument Calibration Standard 2, which contains 100 µg/mL of Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sn, Sr, Ti, Tl, V, and Zn in 5% HNO_3, trace tartaric acid, and trace HF. All sample and standard dilutions were performed using a 2% solution of Fisher Trace Metal nitric acid in MilliQ ($\geq$18.2 MΩ) water. Feed samples were prepared by adding 1.0 mL of sample solution to 8.9 mL of diluent and 0.1 mL of an internal standard solution. All standard and sample results were normalized to the internal standard signal variation.

RESULTS

Media System Modelling

Trace metals essential to glucose metabolism were identified and the concentrations were used as inputs for Pourbaix diagrams generated by HSC Chemistry 9 software. Pourbaix diagrams were generated to model the state of the metals present in the NF preparation to screen for metals at risk of precipitation and thus subsequent removal via filtration leading to an impact on the cell culture process. The Pourbaix diagrams did not identify solubility issues with iron, zinc, or carbon-containing ions between the

preparation-relevant pH range 9.1-9.3, however the Pourbaix diagrams predicted copper precipitation in the NF.

To better understand the copper precipitation issue, Pourbaix diagrams were generated to model the state of copper prior to and after the addition of cupric sulfate pentahydrate (Fig. 1). Prior to the addition of cupric sulfate pentahydrate, the predominate species of copper estimated by the Pourbaix diagram (pH range 9.1 to 9.3, Eh range -0.1 to 0 V) was aqueous cupric chloride (Fig. 2).

With the addition of cupric sulfate pentahydrate and NaOH in the next preparation step, the predominate species of copper predicted by the Pourbaix diagram shifted to insoluble elemental copper (Fig. 3). While elemental copper was the predominate species predicted by the Pourbaix diagram, elevated copper concentrations (>1 M) and temperature (>200°C) have been reported to be essential to induce elemental copper precipitaton.(Regenspurg, Geigenmüller, Milsch, & Kühn, 2017; Teichert, Doert, & Ruck, 2018) Additionally, precipitation of elemental copper from cupric chloride has been shown to require a transition first to insoluble copper oxide, indicating copper oxide was the likely precipitate forming in the media solution.(Teichert et al., 2018) While copper oxide is bioavailable to the cell(Richards, Zhao, Harrell, Atwell, & Dibner, 2010), the precipitate was removed when the feed solution was filtered through a 0.1 µm filter and the total copper concentration in the media was reduced. This stage mixed for 60 minutes during NF preparation, which may have provided suffiencient time for conversion of cupric chloride to copper oxide.

The final step of the preparation was the addition of hydrochloric acid which shifted the copper species equilibrium toward aqueous cuprous chloride in the Pourbaix diagram (Fig. 4). However, insoluble copper oxide formed during the prior NF step was unlikely to convert to aqueous copper chloride as copper oxide can only be readily dissolved in strong acids such as HCl or at temperatures in excess of 200°C(Palmer, 2011).The results of the Pourbaix diagram suggested copper oxide precipitation was occurring during the final 2 steps of the NF preparation (Fig. 2 and 3) and an increase in the duration or

temperature during these preparation steps in the NF preparation process should lead to an increase in copper precipitation. Since the rate of cooling for the large-scale preparation was slower than that of the bench-scale preparations, the increased time at elevated temperature likely facilitated the oxidation reaction of copper ions to cuprous oxide ($2Cu+ + H_2O = Cu_2O(s) + 2H+$)(Lee et al., 2016).

Model Confirmation using Bench-Scale Solution Preparation Studies

As the water used in the NF preparation is charged to 55°C and allowed to cool throughout the preparation, the NF temperature at the time of cupric sulfate addition likely differed. The impact of temperature on copper oxidation was evaluated by controlling the temperature of NF preparation at 30, 40 and 50°C and collecting samples before and after filtration through a 0.1 µm PES filter. The amount of copper removed by the 0.1 µm filter was directly proportional to the preparation temperature which confirms the hypothesis that copper oxidation, and thus precipitation, increased with temperature (Fig. 5). Additionally, copper precipitation continued after the addition of hydrochloric acid which indicated that the presence of chloride ions did not prevent copper precipitation. Solubility of copper oxide has been shown to increase with temperature from ~10 nM at 25°C to ~35 nM at 50°C suggesting that the predominate soluble species was likely copper chloride.(Palmer, 2011) The 30°C condition resulted in an increase in post-filtration copper concentration after 200 minutes. This phenomenon may be the result of the copper precipitates decreasing in size to less than 0.1 µm, as it is unlikely for the insoluble copper oxide to convert to other soluble copper compounds at 30°C.(Palmer, 2011) The 50°C condition resulted in a similar decrease in soluble copper concentration in the NF as observed in the copper-deficient cultures at manufacturing-scale. While temperature is not monitored in bench-scale preparations, the manufacturing NF preparations were observed to be at 48-49°C at the time of cupric sulfate addition indicating the elevated temperature of NF preparation increased the conversion of cupric chloride to copper oxide.

Characterization of Cell Culture Impact via Copper Titration

The results of the Pourbaix diagrams and bench-scale media preparation studies demonstrate that the pH and temperature of a media solution and the concentration of components therein can lead to the precipitation of critical metals, specifically copper. The copper-deficient cultures at manufacturing scale resulted in poor cell viability and productivity. Copper titration was evaluated in a 5L bench scale model representative of the manufacturing scale process to determine the impact on cell health, metabolism, productivity, and product quality.

Cell Viability, Productivity, and Glucose Metabolism

The copper-deficient cultures at manufacturing-scale demonstrated lower cell viability which is indicative of poor cell health. Low harvest viability was also observed in replicates of the condition with the lowest copper concentration (1x), which harvested with an average viability of 60% and an average titer of 5.9 g/L (Fig. 6). Increasing the theoretical copper concentration to 1.7x improved harvest viability to >80% and harvest titer to 7.8 g/L. Minimal variation was observed in the viability and titer trends between 1.7x and 4.8x, but at the high boundary condition of 770x, a decrease in viability began on day 8 and continued until the culture harvested at approximately 80% viability without impact to harvest titer. Copper concentrations below 1.7x were detrimental to cell health and productivity for this cell line.

The culture health can be better understood when viewed in the context of lactate metabolism. An inversely proportional relationship was observed between harvest lactate concentration and theoretical total copper addition between theoretical harvest copper concentrations of 1x (the media concentration prior to supplementation) and 4.8x (Fig. 7); the high late-run lactate trend in the condition with 1x copper likely led to the viability decline. However, in conditions with high concentrations of copper, the

improvements in harvest lactate concentration and culture viability appeared to exhibit diminishing returns as the time-course trends of the 4.8x and 770x conditions were comparable.

Previous *in-vivo* and *in-vitro* studies performed on mammalian systems demonstrated that copper deficiency hinders glucose metabolism by limiting the synthesis of cytochrome-C oxidase which hinders oxidative phosphorylation(Davies, Lawrence, & Mills, 1985; Ishida, Andreux, Poitry-Yamate, Auwerx, & Hanahan, 2013; Medeiros & Jennings, 2002). This impact manifests in the process as an increase in the rate of lactate produced from glucose energy metabolism as the cell shifts to anaerobic ATP generation. In this study, we specifically examined both the impact of varying concentrations of copper on the lactate concentration in the culture and also the cell-specific glucose and lactate flux to better understand the impact of copper on energy efficiency.

The glucose efficiency of the experimental conditions was estimated by normalizing the average cell-specific lactate production rate to the average cell-specific glucose uptake rate between day 5 and harvest (Fig. 8). Conditions from 1x to 2.6x theoretical copper at harvest exhibited a higher rate of cell-specific lactate production relative to cell-specific glucose uptake, but the improvement in glucose utilization efficiency had diminishing returns as copper concentration increases. These results are consistent with the time-course lactate trends shown in Figure 7 and suggest culture copper concentration directs glucose metabolism to lactate production below a certain threshold, above which glucose efficiency is no longer modulated by copper concentration.

Quality of mAb Product

While the addition of excess copper (> 4.8x) did not provide a practically significant benefit to glucose efficiency or titer at harvest, a detrimental impact was observed on two product quality attributes. Although most product quality attributes remained unchanged, the proportion of high-pI isoforms increased with the culture copper concentration, and elevated levels of both low-pI and high-pI isoforms were observed at the extreme copper concentration of 770x.

A comparison of the raw spectra from mass spectrometry between control and experimental material showed that the increase in low-pI isoforms were the result of an increase in multiple post-translational modifications, but the elevated high-pI isoforms were the result of an increase in a single modification of -58 Da relative to the main peak.

The mechanism for the increase in C-terminal amidation is likely related to the activity of the peptidyl-glycine α-amidating mono-oxygenase (PAM) enzyme, for which copper is a cofactor(Yuk et al., 2015). PAM truncates C-terminal glycine residues (via the removal of a glyoxylate molecule) and facilitates an amidation reaction on the new C-terminal amino acid(Hu et al., 2017; Yuk et al., 2015). The PAM-catalyzed removal of the glyoxylate molecule results in a loss of 58 Daltons (Da) compared to the lysine truncated molecule (Beck & Liu, 2019).

The presence of an amide group at the end of the C-terminal changes the net charge of the protein which would increase the high-pI species observed via icIEF, and an increase in this amidated protein is also consistent with the observed increase in a single peak of basic isoforms observed in the icIEF chromatograms (Fig 9). When this peak was isolated, mass spectrometry analysis revealed that the average mass of the fraction was -58 Daltons relative to the main peak, which supports the hypothesis of the involvement of the PAM enzyme.

Since harvest copper targets of less than 3.7x were found to impact process performance, and high copper concentrations impacted product quality by increasing high-pI isoforms, the cupric sulfate pentahydrate stock solution was added in such a way to target a harvest concentration of 3.7x at commercial scale. This modification was successful in restoring process performance and consistency (data not shown).

CONCLUSIONS

In this case study, Pourbaix diagrams were used to troubleshoot and improve metal precipitation in a NF preparation after variability was observed in the upstream process during scale-up operations. The approach described here led to an advanced understanding of copper solubility during NF preparation and

informed the design of bench-scale experiments, providing insight into the performance of mammalian cell culture processes when subjected to varying concentrations of copper. Bench-scale media preparation studies demonstrated copper loss post-filtration under high pH conditions (9.0-9.5) is a function of preparation temperature and time, supporting the hypothesis that copper precipitation was the mechanism of action for copper removal.

Once copper precipitation was identified, the manufacturing process was modified to prevent precipitation and copper was added as a separate stock solution to successfully recover process performance at the commercial production scale. The target harvest copper concentration was optimized using bench-scale cell culture experiments which explored the process and product impact of copper deficiency on the CHO cell culture process. The efficiency of culture glucose metabolism was improved by increasing the theoretical copper concentration in the process from 1x to 3.7x, but diminishing returns were observed for theoretical concentrations greater than 3.7x. This threshold-like effect is consistent with the biochemical system in the process since copper is used intracellularly in the structure and function of cytochrome c oxidase which is critical for oxidative phosphorylation. Detrimental impacts to product quality were observed, however, from copper concentrations above 3.7x; high-pI isoforms were observed to increase in a manner consistent with PAM-catalyzed amidation of the C-terminus. C-terminal amidation has previously been shown to impact the folding of bioactive peptides (In et al., 2005).

In this work, we applied Pourbaix diagrams as a retroactive troubleshooting tool. A similar approach could also be applied proactively to screen new process solutions prior to scale-up and identify critical trace metals which are near or beyond the limits of solubility. This information can then be used to determine an appropriate control strategy at manufacturing-scale and ensure that metal concentrations in media preparations are scalable within the process design space.

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