2014:487101) and is currently the standard method for determining mAb titer for batch-based production processes. However, there are difficulties in using HPLC for measurement of titer in a continuous process, as running HPLC requires that a sample be taken from the process.

One method is to manually take samples from an aseptic sampling manifold, and then manually run the sample on an HPLC. This approach is labor intensive, as it requires an operator that can take frequent samples and run the HPLC.

Another option is to use an automated sampling device with an HPLC designed for in-line operation, such as the Waters Patrol. However, this requires that the HPLC instrument be placed in the GMP suite close to the sampling point, which is difficult from a compliance perspective. Additionally, HPLCs are relatively complex instruments with many moving parts, and instrument maintenance becomes more difficult if the instrument is placed in a GMP space.

More recently, spectroscopic methods have gained favor as a method for determination of protein concentration. International Publication No. WO2010151214A1 describes a spectroscopic method in which UV sensors were placed in the feed and effluent streams of a Protein A capture chromatography system.

There remains a need for methods of determining protein concentration and informing target load volumes in capture chromatography where the feedstream protein concentration varies over time.

SUMMARY

The subject technology is illustrated, for example, according to various aspects and examples listed below.

A method for optimizing continuous chromatography (e.g., continuous protein A chromatography) in a system of continuous production of a biologic product (e.g. a perfusion bioreactor) is disclosed, comprising (i) taking an optical density (OD) measurement of the eluate from the chromatography column; (ii) applying a mathematical formula to the OD measurement, wherein the mathematical formula is Equation 1; and (iii) adjusting the continuous load of the chromatography column to achieve the optimization.

Equation 1:

$$Titer = \frac{k_{col}}{2\varepsilon_m V_l}\left(\sum_{i=0}^{n} Q_i D_i \; + \sum_{i=0}^{n} Q_{i+1} D_{i+1}\right)$$

In one aspect, the present disclosure provides a method for optimizing continuous chromatography in a system of continuous production of a biologic product, comprising (i) taking an optical density (OD) measurement of an eluate from a chromatography column; (ii) applying a mathematical formula to the OD measurement, wherein the mathematical formula is **Equation 1:**

$$Titer = \frac{k_{col}}{2\varepsilon_m V_l}\left(\sum_{i=0}^{n} Q_i D_i \; + \sum_{i=0}^{n} Q_{i+1} D_{i+1}\right)$$

and (iii) adjusting the continuous load volume of the chromatography column to achieve the optimization.

A method for optimizing continuous chromatography (e.g., continuous protein A chromatography), where the method is optionally computer implemented, in a system of continuous production of a biologic product (e.g. a perfusion bioreactor) is disclosed, comprising (i) taking an optical density (OD) measurement of the eluate from the chromatography column; (ii) applying a mathematical formula to the OD measurement, wherein the mathematical formula is Equation 2; and (iii) adjusting the continuous load of the chromatography column to achieve the optimization.

Equation 2:

$$m = \frac{n\sum x_i y_i - (\sum x_i)(\sum y_i)}{n\sum {x_i}^2 - (\sum x_i)^2}$$

$$b = \frac{\sum y_i - m\sum x_i}{n}$$

In another aspect, the present disclosure provides a method for optimizing continuous chromatography in a system of continuous production of a biologic product, comprising (i) taking an optical density (OD) measurement of an eluate from a chromatography column; (ii) applying a mathematical formula to the OD measurement, wherein the mathematical formula is **Equation 2:**

$$m = \frac{n\sum x_i y_i - (\sum x_i)(\sum y_i)}{n\sum {x_i}^2 - (\sum x_i)^2}$$

$$b = \frac{\sum y_i - m\sum x_i}{n}$$

; and (iii) adjusting the continuous load of the chromatography column to achieve the optimization.

In certain examples, the methods disclosed herein permit an improvement in one or more properties selected from increased productivity, reduced facility footprint, more agile manufacturing processes and reduced overall specific cost-of-goods (COG).

A method of manufacturing a biologic product of interest is provided, the method comprising the steps of:

(I) cultivating a eukaryotic cell expressing the biologic product of interest in cell culture;

(II) harvesting the biologic product of interest from the cell culture in the form of a fluid feed comprising biologic product of interest and one or more impurities or buffer components;

(III) capturing or purifying the biologic product of interest comprising continuous chromatography of the fluid feed comprising biologic product of interest and one or more impurities or buffer components; and

(IV) optionally formulating the biologic product of interest into a pharmaceutically acceptable formulation suitable for administration; and

wherein the method further comprises:

(i) taking an optical density (OD) measurement of an eluate from a chromatography column; (ii) applying a mathematical formula to the OD measurement, wherein the mathematical formula is Equation 1 or Equation 2; and (iii) adjusting the continuous load of the chromatography column to achieve the optimization.

In another aspect, the present disclosure provides a method of manufacturing a biologic product of interest comprising the steps of:

(I) cultivating a eukaryotic cell expressing the biologic product of interest in cell culture;

(II) harvesting the biologic product of interest from the cell culture in the form of a fluid feed comprising biologic product of interest and one or more impurities or buffer components;

(III) capturing or purifying the biologic product of interest, wherein said capturing or purifying comprises continuous chromatography of the fluid feed comprising biologic product of interest and one or more impurities or buffer components; and

(IV) optionally formulating the biologic product of interest into a pharmaceutically acceptable formulation suitable for administration; and

wherein the method further comprises:

(i) taking an optical density (OD) measurement of an eluate from a chromatography column; (ii) applying a mathematical formula to the OD measurement, wherein the mathematical formula is **Equation 1:**

$$Titer = \frac{k_{col}}{2\varepsilon_m V_l}\left(\sum_{i=0}^{n} Q_i D_i + \sum_{i=0}^{n} Q_{i+1} D_{i+1}\right)$$

; and (iii) adjusting the continuous load of the chromatography column to achieve the optimization.

In another aspect, the present disclosure provides a method of manufacturing a biologic product of interest comprising the steps of:

(I) cultivating a eukaryotic cell expressing the biologic product of interest in cell culture;

(II) harvesting the biologic product of interest from the cell culture in the form of a fluid feed comprising biologic product of interest and one or more impurities or buffer components;

(III) capturing or purifying the biologic product of interest, wherein said capturing or purifying comprises continuous chromatography of the fluid feed comprising biologic product of interest and one or more impurities or buffer components; and

(IV) optionally formulating the biologic product of interest into a pharmaceutically acceptable formulation suitable for administration; and

wherein the method further comprises:

(i) taking an optical density (OD) measurement of an eluate from a chromatography column; (ii) applying a mathematical formula to the OD measurement, wherein the mathematical formula is **Equation 2:**

$$m = \frac{n\sum x_i y_i - (\sum x_i)(\sum y_i)}{n\sum {x_i}^2 - (\sum x_i)^2}$$

$$b = \frac{\sum y_i - m\sum x_i}{n}$$

; and (iii) adjusting the continuous load of the chromatography column to achieve the optimization.

The term "adjusting" as used herein, refers to changing or updating the titer value used in DeltaV to determine the load volume requirement,

Additional advantages of the subject technology will become readily apparent to those skilled in this art from the following drawings and the detailed description. The drawings and description are to be regarded as illustrative in nature, and not as restrictive.

BRIEF DESCRIPTION OF FIGURES

Figure 1 depicts a continuous protein manufacturing system, for example, an iSkid system, as an exemplary embodiment of a continuous production system for which the disclosed method can be utilized.

Figure 2A and 2B show chromatograms from a representative Protein A chromatography cycle. The shaded area indicates the area under the curve for the elution peak, which is used to calculate the mass of product of product eluted from the column. Volume = 0 is pegged to the start of collection of the eluate. Figure 2A shows a chromatogram for a full chromatography cycle. Figure 2B shows a zoomed view of the elution peak. The elution collection volume was 3L as indicated by the arrows.

Figure 3 shows predicted titer using the "Feed Minus Effluent" Difference Method. Absorbance was measured at 300 nm. Volume was normalized. Eight separate continuous batches across three different sites have been included. Inlet UV data was not available for all cycles, so some cycles have been excluded from this dataset.

Figure 4 shows predicted vs. actual titer using the elution UV titer prediction method. Titer predictions were compared against the actual bioreactor permeate protein concentration at the time of elution.