

CLAIMS

What is claimed is:

1. A method for determining the breakthrough percentage of a chromatography column having an inlet and an outlet comprising:
 - (a) determining an initial slope (m_0) by flowing a harvested cell culture fluid through the chromatography column for enough time to establish a signal that is not changing wherein the initial slope is determined by slope spectroscopy;
 - (b) determining a first slope (m_1) by slope spectroscopy with a first sensor positioned at the inlet to the column;
 - (c) determining a second slope (m_2) by slope spectroscopy with a second sensor positioned at the outlet to the column; and
 - (d) determining the breakthrough percentage by calculating $\%BT = (m_2 - m_0) / (m_1 - m_0) * 100$.
2. The method of claim 1, wherein the method is used to determine the breakthrough percentage of the chromatography column in a batch process.
3. The method of claim 1, wherein the method is used to determine the breakthrough percentage of the chromatography column in a continuous process.
4. The method of claim 3, further comprising eluting the chromatography column.
5. The method of claim 1, further comprising calculating a real-time mass of a protein loaded onto the chromatography column (mg) as $\text{Titer} * \text{flow rate} * \text{time}$.
6. A method for determining the real-time mass of a protein loaded onto a second chromatography column in a chromatography process comprising a first and a second chromatography column comprising determining the percentage breakthrough of the first chromatography column according to claim 1 and calculating the real-time mass of a protein loaded on the second chromatography column (mg) as $\%BT * \text{titer} * \text{flow rate} * \text{time}$.
7. The method of claim 6, further comprising eluting the second chromatography column.
8. The method of claim 7, further comprising comparing a first elution peak of the chromatography column to a second elution peak of the second chromatography column.
9. The method of claim 8, further comprising determining that a resin capacity of at least one of the chromatography column or the second chromatography column has decreased over time.
10. The method of claim 9, further comprising determining that at least one of the chromatography column or the second chromatography column should be replaced based on the decrease of the resin capacity over time.
11. The method of claim 1, wherein determining the breakthrough percentage is based on a real-time mass of loading of at least one target material.

12. The method of claim 1, wherein determining the breakthrough percentage is based on a real-time mass of loading of at least one impurity.
13. The method of claim 1 or 6, further comprising determining if the chromatography column is fully loaded based on the breakthrough percentage.
14. The method of claim 1 or 6, further comprising determining a load capacity of the chromatography column based on the breakthrough percentage.
15. The method of claim 1 or 6, further comprising determining an optimization of at least one of a pH level, flow rate, conductivity, size of resin, configuration of resin, type of resin, or temperature associated with the chromatography column based on the breakthrough percentage.
16. The method of claim 6, further comprising performing at least one spectral measurement during the elution.
17. The method of claim 1, wherein determining the first slope with the first sensor, determining the second slope with the second sensor, or both, comprises determining a continuous quantification of a concentration of at least one species of interest in the harvested cell culture over time.
18. The method of claim 1 or 6, further comprising determining a final concentration of a drug substance downstream of the outlet of the column.
19. The method of claim 1, wherein m_0 , m_1 , m_2 , or any combination thereof are determined based on measurement of a single wavelength.
20. The method of claim 1, wherein m_0 , m_1 , m_2 , or any combination thereof are determined based on measurement of multiple wavelengths.
21. The method of claim 1, wherein m_0 , m_1 , m_2 , or any combination thereof are determined based on measurement of an off-peak wavelength.
22. The method of claim 1 or 6, further comprising determining the titer of the chromatography column by calculating $\text{Titer} = (m_1 - m_0)/EC$ wherein EC is the extinction coefficient of the protein in units of mL/mg*cm.
23. A method for determining the protein titer of a chromatography column having an inlet and an outlet comprising:
- determining the initial slope (m_0) by flowing a harvested cell culture fluid through the chromatography column for enough time to establish a signal that is not changing wherein the initial slope is determined by slope spectroscopy;
- determining the first slope (m_1) by positioning a sensor at the inlet to the column and measuring the slope by slope spectroscopy;
- determining the titer of the chromatography column by calculating $\text{Titer} = (m_1 - m_0)/EC$ wherein EC is the extinction coefficient of the protein in units of mL/mg*cm.

24. A method for determining the real-time mass of a protein loaded on a chromatography column comprising determining the protein titer of the chromatography column according to claim 23 and calculating the real-time mass of a protein loaded on a chromatography by calculating mass column 1 (mg) = Titer*flow rate*time.
25. The method of claim 24, wherein the method is used to determine the breakthrough percentage of the chromatography column in a batch process.
26. The method of claim 24, wherein the method is used to determine the breakthrough percentage of the chromatography column in a continuous process.
27. The method of claim 23 or 24, further comprising calculating a breakthrough percentage by calculating $\%BT = (m2-m0)/(m1-m0)*100$.
28. The method of claim 26, further comprising eluting the chromatography column.
29. A method for determining the real-time mass of a protein loaded onto a second chromatography column in a chromatography process comprising a first and a second chromatography column comprising determining the titer of the first chromatography column according to claim 23 and calculating the real-time mass of a protein loaded on the second chromatography column (mg) as $\%BT*titer *flow rate * time$.
30. The method of claim 29, further comprising eluting the second chromatography column.
31. The method of claim 30, further comprising comparing a first elution peak of the chromatography column to a second elution peak of the second chromatography column.
32. The method of claim 31, further comprising determining that a resin capacity of at least one of the chromatography column or the second chromatography column has decreased over time.
33. The method of claim 32, further comprising determining that at least one of the chromatography column or the second chromatography column should be replaced based on the decrease of the resin capacity over time.
34. The method of any of claims 23, 24 and 29, wherein the protein comprises a target material.
35. The method of claim 23 or 24, wherein determining the protein comprises an impurity.
36. The method of any of claims 23, 24 and 29, further comprising determining a load capacity of the chromatography column based on the titer.
37. The method of any of claims 23, 24 and 29, further comprising determining an optimization of at least one of a pH level, flow rate, conductivity, size of resin, configuration of resin, type of resin, or temperature associated with the chromatography column based on the titer.
38. The method of any of claims 23, 24 and 29, further comprising performing at least one spectral measurement during the elution.

39. The method of claim 23, wherein determining the first slope with the first sensor, determining the second slope with the second sensor, or both, comprises determining a continuous quantification of a concentration of at least one species of interest in the harvested cell culture over time.

40. The method of any of claims 23, 24 and 29, further comprising determining a final concentration of a drug substance downstream of the outlet of the column.

41. The method of claim 23, wherein m_0 , m_1 , m_2 , or any combination thereof are determined based on measurement of a single wavelength.

42. The method of claim 23, wherein m_0 , m_1 , m_2 , or any combination thereof are determined based on measurement of multiple wavelengths.

43. The method of claim 23, wherein m_0 , m_1 , m_2 , or any combination thereof are determined based on measurement of an off-peak wavelength.