SUMMARY

[007a] In one aspect of the invention, there is provided a method of collecting an elution from a column, the method comprising the steps of:

a) applying a sample comprising Factor VIII to a cation exchange chromatography column, wherein the application causes the Factor VIII to be retained by the cation exchange chromatography column;

b) applying a mobile phase to the cation exchange chromatography column, the mobile phase comprising a buffered solution, wherein application of the mobile phase establishes a conductivity gradient of from about 8 mS/cm to about 90 mS/cm;

c) starting a single fraction eluate collection, wherein the single fraction eluate collection includes a single start conductivity value and single stop conductivity value, wherein the single start conductivity value starts when (i) an inlet conductivity value measured for the mobile phase entering the cation exchange chromatography column is about 26.0 mS/cm or (ii) when an outlet conductivity value measured for the mobile phase exiting the cation exchange chromatography column is about 20.0 mS/cm; and

d) stopping the single fraction eluate collection when the single stop conductivity value measured for the mobile phase (i) at the inlet entering the cation exchange chromatography column is about 42.0 mS/cm or (ii) the outlet conductivity value measured for the mobile phase exiting the cation exchange chromatography column is about 35.5 mS/cm;

wherein the single fraction eluate collection comprises at least 83% of the Factor VIII retained by the cation exchange chromatography column in step (a).

[007b] In another aspect, there is provided a method of collecting an elution from a column, the method comprising the steps of:

a) applying a sample comprising Factor VIII to a anion exchange chromatography column, wherein the application causes the Factor VIII to be retained by the anion exchange chromatography column;

b) applying a mobile phase to the anion exchange chromatography column, the mobile phase comprising a buffered solution, wherein application of the mobile phase establishes a conductivity gradient of from about 4 mS/cm to about 80 mS/cm;

c) starting a single fraction eluate collection, wherein the single fraction eluate collection includes a single start conductivity value and single stop conductivity value, wherein the single start conductivity value starts when (i) an inlet conductivity value measured for the mobile phase entering the anion exchange chromatography column is about 28.8 mS/cm or (ii) an outlet conductivity value measured for the mobile phase exiting the anion exchange chromatography column is about 22.0 mS/cm; and

d) stopping the single fraction eluate collection when (i) the inlet conductivity value measured for the mobile phase entering the anion exchange chromatography column is about 52.0 mS/cm or (ii) the outlet conductivity value measured for the mobile phase exiting the anion exchange chromatography column is about 46.5 mS/cm;

wherein the single fraction eluate collection comprises at least 83% of the Factor VIII retained by the anion exchange chromatography column in step (a).

[008] ~~Thus, aspects~~Embodiments of the present specification disclose a method of collecting an elution from a column, the method comprising the steps of: a) applying a sample comprising a protein to a cation exchange chromatography column, wherein the application causes the protein to be retained by the column; b) applying a mobile phase to the cation exchange chromatography column, the mobile phase comprising a buffered solution, wherein application of the mobile phase establishes a conductivity gradient of from about 8 mS/cm to about 90 mS/cm; c) starting an eluate collection when an inlet conductivity value measured for the mobile phase entering the cation exchange chromatography column is from about 25.0 mS/cm to about 27.0 mS/cm; and d) stopping the eluate collection when the inlet conductivity value measured for the mobile phase entering the cation exchange chromatography column is from about 41.0 mS/cm to about 43.0 mS/cm; wherein the eluate collection comprises at least 25% of the protein retained by the cation exchange chromatography column in step (a). The buffered solution disclosed herein can further comprise an electrolyte and/or a surfactant. The electrolyte disclosed herein may comprise sodium ions, potassium ions, calcium ions, magnesium ions, chloride ions, hydrogen phosphate ions, hydrogen carbonate ions, or any combination thereof. The surfactant disclosed herein can be an ionic surfactant like an anion surfactant or cationic surfactant, a zwitterionic (amphoteric) surfactant, or a non-ionic surfactant. The conductivity gradient disclosed herein can be defined by a linear function, a sigmoidal function, a logarithmic function, or an exponential function. The method can be performed as a closed system.

[009] Other ~~aspects~~embodiments of the present specification disclose a method of collecting an elution from a column, the method comprising the steps of: a) applying a sample comprising a protein to a cation exchange chromatography column, wherein the application causes the protein to be retained by the column; b) applying a mobile phase to the cation exchange chromatography column, the mobile phase comprising a buffered solution, wherein application of the mobile phase establishes a conductivity gradient of from about 8 mS/cm to about 90 mS/cm; c) starting an eluate collection when an outlet conductivity value measured for the mobile phase exiting the cation exchange chromatography column is from about 19.0 mS/cm about 21.0 mS/cm; and d) stopping the eluate collection when the outlet conductivity value measured for the mobile phase exiting the cation exchange chromatography column is from about 34.5 mS/cm to about 36.5 mS/cm; wherein the eluate collection comprises at least 25% of the protein retained by the cation exchange chromatography column in step (a). The method can be performed as a closed system.

[010] Yet other ~~aspects~~embodiments of the present specification disclose a method of collecting an elution from a column, the method comprising the steps of: a) applying a sample comprising a protein to an anion exchange chromatography column, wherein the application causes the protein to be retained by the column; b) applying a mobile phase to the anion exchange chromatography column, the mobile phase comprising a buffered solution, wherein application of the mobile phase establishes a conductivity

gradient of from about 4 mS/cm to about 80 mS/cm; c) starting an eluate collection when an inlet conductivity value measured for the mobile phase entering the anion exchange chromatography column is from about 27.8 mS/cm to about 29.8 mS/cm; and d) stopping the eluate collection when the inlet conductivity value measured for the mobile phase entering the anion exchange chromatography column is from about 51.0 mS/cm to about 53.0 mS/cm; wherein the eluate collection comprises at least 25% of the protein retained by the anion exchange chromatography column in step (a). The method can be performed as a closed system.

[011] Yet other ~~aspects~~embodiments of the present specification disclose a method of collecting an elution from a column, the method comprising the steps of: a) applying a sample comprising a protein to an anion exchange chromatography column, wherein the application causes the protein to be retained by the column; b) applying a mobile phase to the anion exchange chromatography column, the mobile phase comprising a buffered solution, wherein application of the mobile phase establishes a conductivity gradient of from about 4 mS/cm to about 80 mS/cm; c) starting an eluate collection when an outlet conductivity value measured for the mobile phase exiting the column is from about 21.0 mS/cm to about 23.0 mS/cm; and d) stopping the eluate collection when the outlet conductivity value measured for the mobile phase exiting the column is from about 45.5 mS/cm to about 47.5 mS/cm; wherein single eluate collection comprises at least 25% of the protein retained by the anion exchange chromatography column in step (a). The method can be performed as a closed system.

[012] Still other ~~aspects~~embodiments of the present specification disclose a method of collecting an elution from a column, the method comprising the steps of: a) applying a sample comprising a protein to a cation exchange chromatography column, wherein the application causes the protein to be retained by the column; b) applying a mobile phase to the cation exchange chromatography column, the mobile phase comprising a buffered solution, wherein application of the mobile phase establishes a conductivity gradient of from about 8 mS/cm to about 90 mS/cm; c) starting an eluate collection when an inlet conductivity value measured for the mobile phase entering the cation exchange chromatography column is from about 25.0 mS/cm to about 27.0 mS/cm or an outlet conductivity value measured for the mobile phase exiting the cation exchange chromatography column is from about 19.0 mS/cm about 21.0 mS/cm; and d) stopping the eluate collection when the inlet conductivity value measured for the mobile phase entering the cation exchange chromatography column is from about 41.0 mS/cm to about 43.0 mS/cm or the outlet conductivity value measured for the mobile phase exiting the cation exchange chromatography column is from about 34.5 mS/cm to about 36.5 mS/cm; wherein the eluate collection comprises at least 25% of the protein retained by the cation exchange chromatography column in step (a). The method can be performed as a closed system.

[013] Still other ~~aspects~~embodiments of the present specification disclose a method of collecting an elution from a column, the method comprising the steps of: a) applying a sample comprising a protein to an anion exchange chromatography column, wherein the application causes the protein to be retained by the column; b) applying a mobile phase to the anion exchange chromatography column, the mobile phase

comprising a buffered solution, wherein application of the mobile phase establishes a conductivity gradient of from about 4 mS/cm to about 80 mS/cm; c) starting an eluate collection when an inlet conductivity value measured for the mobile phase entering the anion exchange chromatography column is from about 27.8 mS/cm to about 29.8 mS/cm or an outlet conductivity value measured for the mobile phase exiting the anion exchange chromatography column is from about 21.0 mS/cm to about 23.0 mS/cm; and d) stopping the eluate collection when the inlet conductivity value measured for the mobile phase entering the anion exchange chromatography column is from about 51.0 mS/cm to about 53.0 mS/cm or the outlet conductivity value measured for the mobile phase exiting the anion exchange chromatography column is from about 45.5 mS/cm to about 47.5 mS/cm; wherein single eluate collection comprises at least 25% of the protein retained by the column in step (a). The method can be performed as a closed system.

[014] Further ~~aspects~~embodiments of the present specification disclose a method of collecting an elution from a column, the method comprising the steps of: a) applying a sample comprising a protein to a column, wherein the application causes the protein to be retained by the column; b) applying a mobile phase to the column, the mobile phase comprising a buffered solution, wherein application of the mobile phase establishes a conductivity gradient of from about 4 mS/cm to about 90 mS/cm; c) starting an eluate collection when an conductivity value measured for the mobile phase is from about 19.0 mS/cm to about 30.0 mS/cm; and d) stopping the eluate collection when the conductivity value measured for the mobile phase is from about 34.0 mS/cm to about 53.0 mS/cm; wherein the eluate collection comprises at least 25% of the protein retained by the column in step (a). The method can be performed as a closed system.

DESCRIPTION

[015] Ion exchange chromatography is a chromatographical method that is widely used for chemical analysis and separation of charged molecules. Ion exchange is a reversible chemical reaction wherein an ion from a compound contained in a sample is exchanged for a similarly charged ion attached to an immobile ion-exchange resin of the stationary phase. An important area of the application is extraction and purification of biologically produced substances such as, *e.g.*, amino acids, proteins, and polynucleotide molecule like DNA and RNA.

[016] Ion exchange columns include a stationary phase, which typically comprises an ion-exchange resin fixed in place for the chromatography procedure, and a mobile phase, which comprises a buffered solution that moves in a definite direction during the chromatography procedure.

[017] A stationary phase comprises an ion-exchange resin. Ion exchange resin is a an insoluble matrix (or support structure) normally in the form of small beads of about 1 mm to about 2 mm in diameter. The resin has highly developed structure of surface pores which serve as ion exchange sites that easily trapped and released ions. The trapping of ions takes place only with simultaneous releasing of other ions; thus the process is called ion-exchange. Ion exchange resins are classified as cation exchangers