CLAIMS

1. A method of collecting an elution from a column, the method comprising the steps of:

a) applying a sample comprising ~~a protein~~ Factor VIII to a cation exchange chromatography column, wherein the application causes the ~~protein~~ 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~~n~~ 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 ~~at least~~ about ~~21~~ 26.0 mS/cm or (ii) when an outlet conductivity value measured for the mobile phase exiting the cation exchange chromatography column is ~~at least~~ about ~~15~~ 20.0 mS/cm; and

d) stopping the single fraction eluate collection when the single stop ~~inlet~~ conductivity value measured for the mobile phase (i) at the inlet entering the cation exchange chromatography column is ~~at most~~ about ~~47~~ 42.0 mS/cm or (ii) the outlet conductivity value measured for the mobile phase exiting the cation exchange chromatography column is ~~at most~~ about ~~41~~ 35.5 mS/cm;

wherein the single fraction eluate collection comprises at least ~~25~~83% of the ~~protein~~ Factor VIII retained by the cation exchange chromatography column in step (a).

2. The method of claim 1 wherein in step c) starting of the single fraction eluate collection begins when the inlet conductivity value measured for the mobile phase entering the cation exchange chromatography column is between about 25.0 mS/cm and about 27.0 mS/cm and in step d) stopping of the single fraction eluate collection occurs when the inlet conductivity value measured for the mobile phase entering the cation exchange chromatography column is between about 41.0 mS/cm and about 43.0 mS/cm.

3. The method of claim 1 wherein in step c) starting of the single fraction eluate collection begins when the outlet conductivity value measured for the mobile phase exiting the cation exchange chromatography column is between about 19.0 mS/cm and about 21.0 mS/cm and in step d) stopping of the single fraction eluate collection occurs when the outlet conductivity value measured for the mobile phase exiting the cation exchange chromatography column is between about 34.5 mS/cm and about 36.5 mS/cm.

4. The method of claim 1 wherein in step c) starting of the single fraction eluate collection begins when the inlet conductivity value measured for the mobile phase entering the cation exchange chromatography column is between about 25.0 mS/cm and about 27.0 mS/cm and in step d) stopping of the single fraction eluate collection occurs when the inlet conductivity value measured for the mobile phase entering the cation exchange chromatography column is between about 41.0 mS/cm and about 43.0 mS/cm or wherein in step c) starting of the single fraction eluate collection begins when the outlet conductivity value measured for the mobile phase exiting the cation exchange chromatography column is between about 19.0 mS/cm and about 21.0 mS/cm and in step d) stopping of the single fraction eluate collection occurs when the outlet conductivity value measured for the mobile phase exiting the cation exchange chromatography column is between about 34.5 mS/cm and about 36.5 mS/cm.

~~5. The method of claim 1, wherein the eluate collection comprises at least 50% of the protein retained by the cation exchange chromatography column in step (a), or at least 75% of the protein retained by the cation exchange chromatography column in step (a).~~

~~6. The method of claim 1, wherein the protein is a recombinant Factor VIII protein.~~

~~7. The method of claim 1, wherein the protein is ADAMTS-13, α1-antiplasmin, α2-antiplasmin, antithrombin, antithrombin III, cancer procoagulant, erythropoietin, Factor II, Factor V, Factor VI, Factor VII, Factor VIII, Factor IX, Factor X, Factor XI, Factor XII, Factor XIII, fibronectin, fibrinogen, heparin cofactor II, high-molecular-weight kininogen, intramuscular immunoglobulin, intravenous immunoglobulin, plasminogen, plasminogen activator inhibitor-1, plasminogen activator inhibitor-2, prekallikrein, protein C, protein S, protein Z, protein Z-related protease inhibitor, tissue factor, tissue plasminogen activator, urokinase, or Von Willebrand Factor.~~

85. The method of claim 1, wherein the buffered solution comprises 2-(*N*-morpholino) ethanesulfonic acid (MES), N-(2-acetamido)iminodiacetic acid (ADA), dimethylarsinic acid (Cacodylate), piperazine-*N*,*N*'-*bis*(2-ethanesulfonic acid) (PIPES), N-(2-Acetamido)-2-aminoethanesulfonic acid (ACES), cholamine chloride, *N*,*N*'-*bis*(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES), 3-(*N*-morpholino) propanesulfonic acid (MOPS), 2-{[tris(hydroxymethyl)methyl]amino}ethanesulfonic acid (TES), *N*-(2-hydroxyethyl) piperazine-*N*'-(2-ethanesulfonic acid) (HEPES), piperazine-*N*,*N*'-*bis*(2-hydroxypropanesulfonic acid) (POPSO), *N*-*tris*(hydroxymethyl) methylglycine (Tricine), tris(hydroxymethyl)methylamine (Tris), acetamidoglycine, N,N-bis(2-hydroxyethyl)glycine (Bicine), *N*-*tris*(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS), 3-[(1,1-dimethyl-2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid (AMPSO), 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid (CAPSO), or 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS).

96. The method of claim 1, wherein the buffered solution comprises a non-ionic surfactant selected from a polyoxyethylene glycol sorbitan alkyl ester, a poloxamer, a polyoxyethylene glycol alkyl ether, a polyoxypropylene glycol alkyl ether, a glucoside alkyl ether, a dodecoxyethanol, a polyoxyethylene glycol octylphenol ether, a polyoxyethylene glycol alkylphenol ether, a glycerol alkyl ester, a sorbitan alkyl ester, a cocamide ethanolamine, sucrose monolaurate, dodecyl dimethylamine oxide, sodium cholate, or any combination thereof.

~~10~~7. The method of claim 1, wherein the method is performed as a closed system.

~~11~~8. A method of collecting an elution from a column, the method comprising the steps of:

a) applying a sample comprising ~~a protein~~ Factor VIII to a anion exchange chromatography column, wherein the application causes the ~~protein~~ 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~~n~~ 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 ~~at least~~ about ~~24~~ 28.8 mS/cm or (ii) an outlet conductivity value measured for the mobile phase exiting the anion exchange chromatography column is ~~at least~~ about ~~17~~ 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 ~~at most~~ about ~~57~~ 52.0 mS/cm or (ii) the outlet conductivity value measured for the mobile phase exiting the anion exchange chromatography column is ~~at most~~ about ~~52~~ 46.5 mS/cm;

wherein the single fraction eluate collection comprises at least ~~25~~83% of the ~~protein~~ Factor VIII retained by the anion exchange chromatography column in step (a).

~~12~~9. The method of claim ~~11~~8 wherein in step c) starting of the single fraction eluate collection begins when the inlet conductivity value measured for the mobile phase entering the anion exchange chromatography column is between about 27.8 mS/cm and about 29.8 mS/cm and in step d) stopping of the single fraction eluate collection occurs when the inlet conductivity value measured for the mobile phase entering the anion exchange chromatography column is between about 51.0 mS/cm and about 53.0 mS/cm.

~~13~~10. The method of claim ~~11~~8 wherein in step c) starting of the single fraction eluate collection begins when the outlet conductivity value measured for the mobile phase exiting the anion exchange chromatography column is between about 21.0 mS/cm and about 23.0 mS/cm and in step d) stopping of the single fraction eluate collection occurs when the outlet conductivity value measured for the mobile phase exiting the anion exchange chromatography column is between about 45.5 mS/cm and about 47.5 mS/cm.

~~14~~11. The method of claim ~~11~~8 wherein in step c) starting of the single fraction eluate collection begins when the inlet conductivity value measured for the mobile phase entering the anion exchange chromatography column is between about 27.8 mS/cm and about 29.8 mS/cm and in step d) stopping of the single fraction eluate collection occurs when the inlet conductivity value measured for the mobile phase entering the anion exchange chromatography column is between about 51.0 mS/cm and about 53.0 mS/cm or wherein in step c) starting of the single fraction eluate collection begins when the outlet conductivity value measured for the mobile phase exiting the anion exchange chromatography column is between about 21.0 mS/cm and about 23.0 mS/cm and in step d) stopping of the single fraction eluate collection occurs when the outlet conductivity value measured for the mobile phase exiting the anion exchange chromatography column is between about 45.5 mS/cm and about 47.5 mS/cm.

~~15. The method of claim 11, wherein the eluate collection comprises at least 50% of the protein retained by the anion exchange chromatography column in step (a), or at least 75% of the protein retained by the anion exchange chromatography column in step (a).~~

~~16. The method of claim 11, wherein the protein is a recombinant Factor VIII protein.~~

~~17. The method of claim 11, wherein the protein is ADAMTS-13, α1-antiplasmin, α2-antiplasmin, antithrombin, antithrombin III, cancer procoagulant, erythropoietin, Factor II, Factor V, Factor VI, Factor VII, Factor VIII, Factor IX, Factor X, Factor XI, Factor XII, Factor XIII, fibronectin, fibrinogen, heparin cofactor II, high-molecular-weight kininogen, intramuscular immunoglobulin, intravenous immunoglobulin, plasminogen, plasminogen activator inhibitor-1, plasminogen activator inhibitor-2, prekallikrein, protein C, protein S, protein Z, protein Z-related protease inhibitor, tissue factor, tissue plasminogen activator, urokinase, or Von Willebrand Factor.~~

~~18~~12. The method of claim ~~11~~8, wherein the buffered solution comprises 2-(*N*-morpholino) ethanesulfonic acid (MES), N-(2-acetamido)iminodiacetic acid (ADA), dimethylarsinic acid (Cacodylate), piperazine-*N*,*N'-bis*(2-ethanesulfonic acid) (PIPES), N-(2-Acetamido)-2-aminoethanesulfonic acid (ACES), cholamine chloride, *N*,*N'-bis*(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES), 3-(*N*-morpholino) propanesulfonic acid (MOPS), 2-{[tris(hydroxymethyl)methyl]amino}ethanesulfonic acid (TES), *N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid) (HEPES), piperazine-*N*,*N'-bis*(2-hydroxypropanesulfonic acid) (POPSO), *N-tris*(hydroxymethyl) methylglycine (Tricine), tris(hydroxymethyl)methylamine (Tris), acetamidoglycine, N,N-bis(2-hydroxyethyl)glycine (Bicine), *N-tris*(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS), 3-[(1,1-dimethyl-2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid (AMPSO), 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid (CAPSO), or 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS).

~~19~~13. The method of claim ~~11~~8, wherein the buffered solution comprises a non-ionic surfactant selected from a polyoxyethylene glycol sorbitan alkyl ester, a poloxamer, a polyoxyethylene glycol alkyl ether, a polyoxypropylene glycol alkyl ether, a glucoside alkyl ether, a dodecoxyethanol, a polyoxyethylene glycol octylphenol ether, a polyoxyethylene glycol alkylphenol ether, a glycerol alkyl ester, a sorbitan alkyl ester, a cocamide ethanolamine, sucrose monolaurate, dodecyl dimethylamine oxide, sodium cholate, or any combination thereof.

~~20~~14. The method of claim ~~11~~8, wherein the method is performed as a closed system.