A METHOD FOR REGENERATION OF AN OVERLOAD CHROMATOGRAPHY COLUMN

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. Provisional Application No. 62/857,734, filed June 5, 2019, which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention provides methods for chromatography.

BACKGROUND OF THE INVENTION

[0003] Overload (OL) cation-exchange chromatography has been shown to have equivalent impurity removal capabilities to bind and elute mode while intensifying the process to improve resin utilization and plant efficiency. The resin is loaded under strong binding conditions beyond the resin dynamic binding capacity. Impurities preferentially bind to the resin while the product breakthrough is collected. Due to the high load density (typically > 500 g/L), a higher concentration of host-cell protein remains bound to the resin. As such, the column fouls (high column pressure, >80 psi) during the sanitization phase (0.5N Sodium Hydroxide) of the purification process (**FIG. 1**). What is needed is as improved process that minimizes pressure increases during the sanitization phase with no impurity carry-over between chromatography runs.

[0004] Methods of overload chromatography are described in US Patent Application Publications US 2013/0079272A1 and US 2014/0301977A1, each of which is incorporated by reference herein in its entirety.

[0005] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

BRIEF SUMMARY

[0006] In some examples, the disclosure provides methods to clean an ion exchange chromatography material (*e.g.*, a cation exchange chromatography material) for reuse wherein the chromatography material is used in overload mode in the purification of a polypeptide, the method comprising the steps of a) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises sodium acetate; b) passing of sanitization buffer through the ion exchange chromatography material, wherein the

sanitization buffer comprises about 0.5 N NaOH; and c) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH. In one aspect, the present disclosure provides a method to clean an ion exchange chromatography material for reuse wherein the chromatography material is used in overload mode in the purification of a polypeptide, the method comprising the steps of a) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises sodium acetate (NaOAc); b) passing of sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and c) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH. In some examples, the regeneration buffer comprises about 50 to about 600 mM sodium acetate (NaOAc). In some examples, the regeneration buffer comprises about 350 mM NaOAc. In some examples, the pH of the regeneration buffer is about pH 4.0 to 11. In some examples, the pH of the regeneration buffer is about pH 8.3. In some examples, about 3 to about 12 material volumes of regeneration buffer are passed through the ion exchange chromatography material. In some examples, about 10 material volumes of regeneration buffer are passed through the ion exchange chromatography material. In some examples, about 2 to about 12 material volumes of sanitization buffer are passed through the ion exchange chromatography material. In some examples, about 10 material volumes of sanitization buffer are passed passing a regeneration buffer through the ion exchange chromatography material. In some examples, the ion exchange chromatography material is stored in about 1 to 5 material volumes of storage buffer. In some examples, the ion exchange chromatography material is stored in about 4 material volumes of storage buffer.

[0007] In some aspects, the disclosure provides a method to clean an ion exchange chromatography material for reuse wherein the chromatography material is used in overload mode in the purification of a polypeptide, the method comprising the steps of a) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises sodium acetate; b) passing an acid wash through the ion exchange chromatography material, c) passing of sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH and about 1 M NaCl; and d) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH. In some examples, the regeneration buffer and the second regeneration buffer comprises about 25 to about 600 mM NaOAc. In some examples, the regeneration buffer comprises about 350 mM NaOAc. In some examples, the regeneration buffer comprises about 40 mM NaOAc. In some examples, the pH of the regeneration buffer is about pH 2.0 to 7.0. In some examples, the pH of the regeneration buffer is about pH 4.0. In some examples, the pH of the regeneration buffer is about pH 5.0. In some examples, about 3 to about 12 material volumes of regeneration buffer are passed through the ion exchange chromatography material. In some examples, about 7 material volumes of regeneration buffer are passed through the ion exchange chromatography material. In some examples, the acid wash is about 167M NaOAc and about 0.3M phosphoric acid. In some examples, about 1 to about 5 material

volumes of the acid wash are passed through the ion exchange chromatography material. In some examples, about 3 material volumes of the acid wash are passed through the ion exchange chromatography material. In some examples, about 2 to about 12 material volumes of sanitization buffer are passed through the ion exchange chromatography material. In some examples, about 4 material volumes of sanitization buffer are passed passing a regeneration buffer through the ion exchange chromatography material. In some examples, the ion exchange chromatography material is stored in about 1 to 5 material volumes of storage buffer. In some examples, the ion exchange chromatography material is stored in about 3 material volumes of storage buffer.

[0008] In some examples, the disclosure provides methods to clean a ion exchange chromatography material (*e.g.*, a cation exchange chromatography material) for reuse wherein the chromatography material is used in overload mode in the purification of a polypeptide, the method comprising the steps of a) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises Tris and sodium acetate; b) passing a sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and c) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH. In one aspect, the present disclosure provides a method to clean an ion exchange chromatography material for reuse wherein the chromatography material is used in overload mode in the purification of a polypeptide, the method comprising the steps of a) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises Tris and sodium acetate; b) passing a sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and c) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH. In some examples, the regeneration buffer comprises about 25 to about 75 mM Tris and about 50 to about 100 mM NaOAc. In some examples, the regeneration buffer comprises about 50 mM Tris and about 85 mM NaOAc. In some examples, the pH of the regeneration buffer is about pH 7 to 9. In some examples, the pH of the regeneration buffer is about pH 8.0. In some examples, about 3 to about 12 material volumes of regeneration buffer are passed through the ion exchange chromatography material. In some examples, about 10 material volumes of regeneration buffer are passed through the ion exchange chromatography material. In some examples, about 2 to about 12 material volumes of sanitization buffer are passed through the ion exchange chromatography material. In some examples, about 10 material volumes of sanitization buffer are passed passing a regeneration buffer through the ion exchange chromatography material. In some examples, the ion exchange chromatography material is stored in about 1 to 5 material volumes of storage buffer. In some examples, the ion exchange chromatography material is stored in about 4 material volumes of storage buffer.

[0009] In some examples, the disclosure provides methods for purifying a polypeptide from a composition comprising the polypeptide and an impurity using a ion exchange chromatography material (*e.g.*, a cation exchange chromatography material) wherein the ion exchange

chromatography material is suitable for reuse, the method comprising the steps of a) equilibrating an ion exchange chromatography material with an equilibration buffer; b) loading the composition on the ion exchange chromatography material, wherein the load density is ≤ about 1000 g/L; c) collecting fractions comprising the polypeptide; d) washing the ion exchange chromatography material with equilibration buffer; e) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises sodium acetate; f) passing of sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and g) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH. In one aspect, the present disclosure provides a method for purifying a polypeptide from a composition comprising the polypeptide and an impurity using an ion exchange chromatography material wherein the ion exchange chromatography material is suitable for reuse, the method comprising the steps of a) equilibrating an ion exchange chromatography material with an equilibration buffer; b) loading the composition on the ion exchange chromatography material, wherein the load density is ≤ about 1000 g/L; c) collecting fractions comprising the polypeptide; d) washing the ion exchange chromatography material with the equilibration buffer; e) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises sodium acetate; f) passing of sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and g) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH. In another aspect, the present disclosure provides a method for purifying a polypeptide from a composition comprising the polypeptide and an impurity using an ion exchange chromatography material wherein the ion exchange chromatography material is suitable for reuse, the method comprising the steps of a) equilibrating an ion exchange chromatography material with an equilibration buffer; b) loading the composition on the ion exchange chromatography material, wherein the load density is ≥20 g/L; c) collecting fractions comprising the polypeptide; d) washing the ion exchange chromatography material with the equilibration buffer; e) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises sodium acetate; f) passing of sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and g) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH. In yet another aspect, the present disclosure provides a method for purifying a polypeptide from a composition comprising the polypeptide and an impurity using an ion

exchange chromatography material wherein the ion exchange chromatography material is suitable for reuse, the method comprising the steps of a) equilibrating an ion exchange chromatography material with an equilibration buffer; b) loading the composition on the ion exchange chromatography material, wherein the load density exceeds the dynamic binding capacity of the ion exchange chromatography for the polypeptide; c) collecting fractions comprising the polypeptide; d) washing the ion exchange chromatography material with the equilibration buffer; e) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises sodium acetate; f) passing of sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and g) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH. In some examples, the equilibration buffer comprises about 20 mM to about 60 mM sodium acetate. In some examples, the equilibration buffer comprises about 40 mM sodium acetate. In some examples, the pH of the equilibration buffer is about pH 5.0 to about pH 6.0. In some examples, the pH of the equilibration buffer is about pH 5.5. In some examples, the ion exchange chromatography material is washed with about 3-7 material volumes of equilibration buffer. In some examples, the ion exchange chromatography material is washed with about 5 material volumes of equilibration buffer. In some examples, the regeneration buffer comprises about 50 to about 600 mM NaOAc. In some examples, the regeneration buffer comprises about 350 mM NaOAc. In some examples, the pH of the regeneration buffer is about pH 4.0 to 11. In some examples, the pH of the regeneration buffer is about pH 8.3. In some examples, about 3 to about 12 material volumes of regeneration buffer are passed through the ion exchange chromatography material. In some examples, about 10 material volumes of regeneration buffer are passed through the ion exchange chromatography material. In some examples, about 2 to about 12 material volumes of sanitization buffer are passed through the ion exchange chromatography material. In some examples, about 10 material volumes of sanitization buffer are passed passing a regeneration buffer through the ion exchange chromatography material. In some examples, the ion exchange chromatography material is stored in about 1 to 5 material volumes of storage buffer. In some examples, the ion exchange chromatography material is stored in about 4 material volumes of storage buffer.

[0010] In some aspects, the disclosure provides a method for purifying a polypeptide from a composition comprising the polypeptide and an impurity using an ion exchange chromatography material wherein the ion exchange chromatography material is suitable for reuse, the method comprising the steps of a) equilibrating an ion exchange chromatography material with an equilibration buffer; b) loading the composition on the ion exchange chromatography material,

wherein the load density exceeds the dynamic binding capacity of the ion exchange chromatography for the polypeptide; c) collecting fractions comprising the polypeptide; d) washing the ion exchange chromatography material with the equilibration buffer; e) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises sodium acetate; f) passing an acid wash through the ion exchange chromatography material, g) passing a sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH and about 1 M NaCl; and h) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH. In some examples, the load density is ≤ about 1000 g/L. In some examples, the load density is ≥ about 20 g/L. In some examples, the load density is ≥ about 70 g/L. In some examples, the load density is at least about 2× the dynamic binding capacity of the ion exchange chromatography material for the polypeptide. In some examples, the load density is about 2× to about 100× the dynamic binding capacity of the ion exchange chromatography material for the polypeptide. In some examples, the load density is about 10× the dynamic binding capacity of the ion exchange chromatography material for the polypeptide. In some examples, the equilibration buffer comprises about 50 mM to about 100 mM NaOAc and about 25 mM to about 75 mM Tris. In some examples, the equilibration buffer comprises about 85 mM sodium acetate and about 50 mM Tris. In some examples, the pH of the equilibration buffer is about pH 6.0 to about pH 10.0. In some examples, the pH of the equilibration buffer is about pH 8.0. In some examples, the ion exchange chromatography material is washed with about 5 to about 15 material volumes of equilibration buffer. In some examples, the ion exchange chromatography material is washed with about 10 material volumes of equilibration buffer. In some examples, the regeneration buffer comprises about 25 to about 600 mM NaOAc. In some examples, the regeneration buffer comprises about 350 mM NaOAc. In some examples, the regeneration buffer comprises about 40 mM NaOAc. In some examples, the pH of the regeneration buffer is about pH 2.0 to 7.0. In some examples, the pH of the regeneration buffer is about pH 4.0. In some examples, the pH of the regeneration buffer is about pH 5.0. In some examples, about 3 to about 12 material volumes of the regeneration buffer are passed through the ion exchange chromatography material. In some examples, about 7 material volumes of the regeneration buffer are passed through the ion exchange chromatography material. In some examples, the acid wash is about 167M NaOAc and about 0.3M phosphoric acid. In some examples, about 1 to about 5 material volumes of the acid wash are passed through the ion exchange chromatography material. In some examples,

about 3 material volumes of the acid wash are passed through the ion exchange chromatography material. In some examples, about 2 to about 12 material volumes of sanitization buffer are passed through the ion exchange chromatography material. In some examples, about 4 material volumes of sanitization buffer are passed passing a regeneration buffer through the ion exchange chromatography material. In some examples, the ion exchange chromatography material is stored in about 1 to 5 material volumes of storage buffer. In some examples, the ion exchange chromatography material is stored in about 3 material volumes of storage buffer.

[0011] In some examples, the disclosure provides methods for purifying a polypeptide from a composition comprising the polypeptide and an impurity using an ion exchange chromatography material wherein the ion exchange chromatography material is suitable for reuse, the method comprising the steps of a) equilibrating the ion exchange chromatography material with an equilibration buffer; b) loading the composition on the ion exchange chromatography material, wherein the load density is ≤ about 1000 g/L; c) collecting fractions comprising the polypeptide; d) washing the ion exchange chromatography material with equilibration buffer; e) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises Tris and sodium acetate; f) passing a sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and g) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH. In one aspect, the present disclosure provides a method for purifying a polypeptide from a composition comprising the polypeptide and an impurity using an ion exchange chromatography material wherein the ion exchange chromatography material is suitable for reuse, the method comprising the steps of a) equilibrating the ion exchange chromatography material with an equilibration buffer; b) loading the composition on the ion exchange chromatography material, wherein the load density is ≤ about 1000 g/L; c) collecting fractions comprising the polypeptide; d) washing the ion exchange chromatography material with the equilibration buffer; e) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises Tris and sodium acetate; f) passing a sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and g) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH.

[0012] In some examples, the disclosure provides methods for purifying a polypeptide from a composition comprising the polypeptide and an impurity using an ion

exchange chromatography material wherein the ion exchange chromatography material is suitable for reuse, the method comprising the steps of a) equilibrating an ion exchange chromatography material with an equilibration buffer; b) loading the composition on the ion exchange chromatography material, wherein the load density is ≥20 g/L; c) collecting fractions comprising the polypeptide; d) washing the ion exchange chromatography material with equilibration buffer; e) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises sodium acetate; f) passing of sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and g) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH. In one aspect, the present disclosure provides a method for purifying a polypeptide from a composition comprising the polypeptide and an impurity using an ion exchange chromatography material wherein the ion exchange chromatography material is suitable for reuse, the method comprising the steps of a) equilibrating the ion exchange chromatography material with an equilibration buffer; b) loading the composition on the ion exchange chromatography material, wherein the load density is ≥20 g/L; c) collecting fractions comprising the polypeptide; d) washing the ion exchange chromatography material with the equilibration buffer; e) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises Tris and sodium acetate; f) passing a sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and g) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH.

[0013] In some examples, the load density is ≥70 g/L. In some examples, the disclosure provides methods for purifying a polypeptide from a composition comprising the polypeptide and an impurity using an ion exchange chromatography material wherein the ion exchange

chromatography material is suitable for reuse, the method comprising the steps of a) equilibrating the ion exchange chromatography material with an equilibration buffer; b) loading the composition on the ion exchange chromatography material, wherein the load density is ≥20 g/L; c) collecting fractions comprising the polypeptide; d) washing the ion exchange chromatography material with equilibration buffer; e) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises Tris and sodium acetate; f) passing a sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and g) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH.

[0014] In some examples, the disclosure provides methods for purifying a polypeptide from a composition comprising the polypeptide and an impurity using an ion exchange chromatography material wherein the ion exchange chromatography material is suitable for reuse, the method comprising the steps of a) equilibrating an ion exchange chromatography material with an equilibration buffer; b) loading the composition on the ion exchange chromatography material, wherein the load density exceeds the dynamic binding capacity of the ion exchange chromatography for the polypeptide; c) collecting fractions comprising the polypeptide; d) washing the ion exchange chromatography material with equilibration buffer; e) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises sodium acetate; f) passing of sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and g) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH. In one aspect, the present disclosure provides a method for purifying a polypeptide from a composition comprising the polypeptide and an impurity using an ion exchange chromatography material wherein the ion exchange chromatography material is suitable for reuse, the method comprising the steps of a) equilibrating the ion exchange chromatography material with an equilibration buffer; b) loading the composition on the ion exchange chromatography material, wherein the load density exceeds the dynamic binding capacity of the ion exchange chromatography for the polypeptide; c) collecting fractions comprising the polypeptide; d) washing the ion exchange chromatography material with the equilibration buffer; e) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises Tris and sodium acetate; f) passing a sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and g) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH. In some examples, the load density is at least about 2× the dynamic binding capacity of the ion exchange chromatography material for the polypeptide. In some examples, the load density is about 2× to about 100× the dynamic binding capacity of the ion exchange chromatography material for the polypeptide. In some examples, the load density is about 10× the dynamic binding capacity of the ion exchange chromatography material for the polypeptide.

[0015] In some examples, the disclosure provides methods for purifying a polypeptide from a composition comprising the polypeptide and an impurity using an ion exchange chromatography material wherein the ion exchange chromatography material is suitable for reuse, the method comprising the steps of a) equilibrating the ion exchange chromatography material with an equilibration buffer; b) loading the composition on the ion exchange chromatography material, wherein the load density exceeds the dynamic binding capacity of the ion exchange

chromatography for the polypeptide; c) collecting fractions comprising the polypeptide; d) washing the ion exchange chromatography material with equilibration buffer; e) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises Tris and sodium acetate; f) passing a sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and g) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH.

[0016] In some examples of the above examples, the equilibration buffer comprises about 20 mM to about 60 mM sodium acetate. In some examples, the equilibration buffer comprises about 40 mM sodium acetate. In some examples, the pH of the equilibration buffer is about pH 5.0 to about pH 6.0. In some examples, the pH of the equilibration buffer is about pH 5.5. In some examples, the ion exchange chromatography material is washed with about 3-7 material volumes of equilibration buffer. In some examples, the ion exchange chromatography material is washed with about 5 material volumes of equilibration buffer. In some examples, the regeneration buffer comprises about 25 to about 75 mM Tris and about 50 to about 100 mM NaOAc. In some examples, the regeneration buffer comprises about 50 mM Tris and about 85 mM NaOAc. In some examples, the pH of the regeneration buffer is about pH 7 to 9. In some examples, the pH of the regeneration buffer is about pH 8.0. In some examples, about 3 to about 12 material volumes of regeneration buffer are passed through the ion exchange chromatography material. In some examples, about 10 material volumes of regeneration buffer are passed through the ion exchange chromatography material. In some examples, about 2 to about 12 material volumes of sanitization buffer are passed through the ion exchange chromatography material. In some examples, about 10 material volumes of sanitization buffer are passed passing a regeneration buffer through the ion exchange chromatography material. In some examples, the ion exchange chromatography material is stored in about 1 to 5 material volumes of storage buffer. In some examples, the ion exchange chromatography material is stored in about 4 material volumes of storage buffer.

[0017] In some examples of the disclosure, the ion exchange chromatography material is in a chromatography column. In some examples, the ion exchange chromatography material is a cation exchange chromatography material. In some examples, the ion exchange chromatography material comprises a sulfopropyl moiety linked to a support matrix. In some examples, the support matrix comprises cross-linked poly(styrenedivinylbenzene). In some examples, the ion exchange chromatography material is a POROS™ HS50 material. In some examples, the ion exchange chromatography material is a mixed mode cation

exchange material. In some examples, the mixed mode cation exchange material is Capto MMC™, Capto MMC™ ImpRes, Nuvia™ cPrime™, or Toyopearl MX Trp-650M.

[0018] In some examples of the disclosure, the ion exchange chromatography material is an anion exchange chromatography material. In some examples, the ion exchange chromatography material comprises a primary amine, a secondary amine, a tertiary amine or a quaternary ammonium ion functional group, a polyamine functional group, or a diethylaminoethyl functional group linked to a support matrix. In some examples, the ion exchange chromatography material is a mixed mode anion exchange material. In some examples, the mixed mode anion exchange material is Capto Adhere™ or Capto Adhere™ ImpRes.

[0019] In some examples of the disclosure, the ion exchange chromatography material is used for large-scale production of the polypeptide. In some examples, the polypeptide is an antibody, an immunoadhesin, an Fc-containing protein, or an immunoconjugate. In some examples, the polypeptide is an antibody. In some examples, the antibody is a monoclonal antibody. In some examples, the monoclonal antibody is a chimeric antibody, humanized antibody, or human antibody. In some examples, the monoclonal antibody is an IgG monoclonal antibody. In some examples, the antibody is an antigen binding fragment. In some examples, the antigen binding fragment is a Fab fragment, a Fab' fragment, a F(ab')2 fragment, a scFv, a di-scFv, a bi-scFv, a tandem (di, tri)-scFv, a Fv, a sdAb, a tri-functional antibody, a BiTE, a diabody or a triabody. In some examples, the antibody is a bispecific antibody. In some examples, the antibody is selected from the group consisting of an anti-CD20 antibody, an anti-CD40 antibody, an anti-HER2 antibody, an anti-IL6 antibody, an anti-IgE antibody, an anti-IL13 antibody, an anti-Flu A antibody, an anti-TIGIT antibody, an anti-PD-L1 antibody, an anti-VEGF-A antibody, an anti-VEGF-A/ANG2 antibody, an anti-CD79b antibody, an anti-ST2 antibody, an anti-factor D antibody, an anti-factor IX antibody, an anti-factor X antibody, an anti-abeta antibody, an anti-tau antibody, an anti-CEA antibody, an anti-CEA/CD3 antibody, an anti-CD20/CD3 antibody, an anti-FcRH5/CD3 antibody, an anti-Her2/CD3 antibody, an anti-FGFR1/KLB antibody, a FAP-4-1 BBL fusion protein, and a FAP-IL2v fusion protein. In some examples, the antibody is selected from the group consisting of ocrelizumab, pertuzumab, trastuzumab, tocilizumab, faricimab, polatuzumab, gantenerumab, cibisatamab, crenezumab, mosunetuzumab, tiragolumab, bevacizumab, rituximab, atezolizumab, obinutuzumab, lampalizumab, lebrikizumab, omalizumab ranibizumab, emicizumab, selicrelumab, prasinezumab, RO6874281 and RO7122290.

[0020] In some examples of the disclosure, the polypeptide is purified using an affinity chromatography material prior to passing through the ion exchange chromatography material, wherein the ion exchange chromatography material is a cation exchange chromatography material. In some examples, the affinity material is selected from the group consisting of a Protein A chromatography, a Protein G chromatography, a Protein A/G chromatography, a protein L chromatography, a FcXL chromatography, a protein XL chromatography, a kappa chromatography, and a kappaXL chromatography. In some examples, the Protein A affinity material is a MAbSelect material, a MAbSelect SuRe™ material or a MAbSelect SuRe™ LX material. In some examples, the buffers are passed through the ion exchange chromatography material at about 15-20 material volumes/hour. In some examples, the regeneration buffer is passed through the ion exchange chromatography material at about 10 material volumes/hour.

[0021] In some examples of the disclosure, the polypeptide is produced in a host cell. In some examples, the host cell is a Chinese hamster ovary (CHO) cell. In some examples, the impurity comprises one or more of IgG fragments, host cell proteins, or host cell nucleic acids. In some examples, the polypeptide is produced in a CHO cell and the impurity comprises CHO host cell protein (CHOP) and/or CHO nucleic acids.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] **FIG. 1** is a chromatogram of an overload chromatography run without a regeneration phase. Fouling of the resin is observed during a pressure spike at the start of the sanitization phase.

[0023] **FIG. 2** shows an example of a POROS™ 50HS chromatogram from overload chromatography of antibody mAb1.

[0024] **FIG. 3** shows a chromatogram of size exclusion chromatography (SEC) of regeneration phases from two evaluation runs. The regeneration buffer for evaluation run 1 was 50 mM Tris, 85 mM NaOAc pH 8.0. The regeneration buffer for evaluation run 2 was 350 mM NaOAc pH 8.3.

[0025] **FIG. 4** shows a chromatogram of Evaluation Run A for antibody mAb1.

[0026] **FIG. 5** shows a chromatogram of Evaluation Run B for antibody mAb1.

[0027] **FIG. 6** shows a chromatogram of Evaluation Run A for antibody mAb2.

[0028] **FIG. 7** shows a chromatogram of Evaluation Run B for antibody mAb2.

[0029] **FIGs. 8A and 8B** show SEC-HPLC chromatograms of POROS™ 50HS mock-run cleaning procedures. In **FIG. 8A** the order of regeneration buffers is 1.) 50 mM Tris, 85 mM

CLAIMS

What is claimed is:

1. A method to clean an ion exchange chromatography material for reuse wherein the chromatography material is used in overload mode in the purification of a polypeptide, the method comprising the steps of

a) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises sodium acetate (NaOAc);

b) passing of sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and

c) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH.

2. The method of claim 1, wherein the regeneration buffer comprises about 50 to about 600 mM NaOAc, wherein optionally the regeneration buffer comprises about 350 mM NaOAc, wherein optionally the pH of the regeneration buffer is about pH 4.0 to 11, wherein optionally the pH of the regeneration buffer is about pH 8.3, wherein optionally about 3 to about 12 material volumes of regeneration buffer are passed through the ion exchange chromatography material, and wherein optionally about 10 material volumes of regeneration buffer are passed through the ion exchange chromatography material.

3. The method of claim 1 or 2, wherein about 2 to about 12 material volumes of sanitization buffer are passed through the ion exchange chromatography material, wherein optionally about 10 material volumes of sanitization buffer are passed through the ion exchange chromatography material.

4. The method of any one of claims 1-3, wherein the ion exchange chromatography material is stored in about 1 to 5 material volumes of storage buffer, wherein optionally the ion exchange chromatography material is stored in about 4 material volumes of storage buffer.

5. A method to clean an ion exchange chromatography material for reuse wherein the chromatography material is used in overload mode in the purification of a polypeptide, the method comprising the steps of

a) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises sodium acetate;

b) passing an acid wash through the ion exchange chromatography material,

c) passing of sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH and about 1 M NaCl; and

d) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH.

6. The method of claim 5, wherein the regeneration buffer comprises about 25 to about 600 mM NaOAc, wherein optionally the regeneration buffer comprises about 350 mM NaOAc or about 40 mM NaOAc, wherein optionally the pH of the regeneration buffer is about pH 2.0 to 7.0, wherein optionally the pH of the regeneration buffer is about pH 4.0, wherein optionally about 3 to about 12 material volumes of regeneration buffer are passed through the ion exchange chromatography material, and wherein optionally about 7 material volumes of regeneration buffer are passed through the ion exchange chromatography material.

7. The method of claim 5 or 6, wherein the acid wash is about 167M NaOAc and about 0.3M phosphoric acid, wherein optionally about 1 to about 5 material volumes of the acid wash are passed through the ion exchange chromatography material, and wherein optionally about 3 material volumes of the acid wash are passed through the ion exchange chromatography material.

8. The method of any one of claims 5-7, wherein about 2 to about 12 material volumes of sanitization buffer are passed through the ion exchange chromatography material, wherein optionally about 4 material volumes of sanitization buffer are passed through the ion exchange chromatography material.

9. The method of any one of claims 5-8, wherein the ion exchange chromatography material is stored in about 1 to 5 material volumes of storage buffer, wherein optionally the ion exchange chromatography material is stored in about 3 material volumes of storage buffer.

10. A method to clean an ion exchange chromatography material for reuse wherein the chromatography material is used in overload mode in the purification of a polypeptide, the method comprising the steps of

a) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises Tris and sodium acetate;

b) passing a sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and

c) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH.

11. The method of claim 10, wherein the regeneration buffer comprises about 25 to about 75 mM Tris and about 50 to about 100 mM NaOAc, wherein optionally the regeneration buffer comprises about 50 mM Tris and about 85 mM NaOAc, wherein optionally the pH of the regeneration buffer is about pH 7 to 9, wherein optionally the pH of the regeneration buffer is about pH 8.0, wherein optionally about 3 to about 12 material volumes of regeneration buffer are passed through the ion exchange chromatography material, and wherein optionally about 10 material volumes of regeneration buffer are passed through the ion exchange chromatography material.

12. The method of claim 10 or 11, wherein about 2 to about 12 material volumes of sanitization buffer are passed through the ion exchange chromatography material, wherein optionally about 10 material volumes of sanitization buffer are passed through the ion exchange chromatography material.

13. The method of any one of claims 10-12, wherein the ion exchange chromatography material is stored in about 1 to 5 material volumes of storage buffer, wherein optionally the ion exchange chromatography material is stored in about 4 material volumes of storage buffer.

14. A method for purifying a polypeptide from a composition comprising the polypeptide and an impurity using an ion exchange chromatography material wherein the ion exchange chromatography material is suitable for reuse, the method comprising the steps of

a) equilibrating an ion exchange chromatography material with an equilibration buffer;

b) loading the composition on the ion exchange chromatography material, wherein the load density is ≤ about 1000 g/L;

c) collecting fractions comprising the polypeptide;

d) washing the ion exchange chromatography material with the equilibration buffer;

e) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises sodium acetate;

f) passing of sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and

g) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH.

15. A method for purifying a polypeptide from a composition comprising the polypeptide and an impurity using an ion exchange chromatography material wherein the ion exchange chromatography material is suitable for reuse, the method comprising the steps of

a) equilibrating an ion exchange chromatography material with an equilibration buffer;

b) loading the composition on the ion exchange chromatography material, wherein the load density is ≥20 g/L;

c) collecting fractions comprising the polypeptide;

d) washing the ion exchange chromatography material with the equilibration buffer;

e) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises sodium acetate;

f) passing of sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and

g) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH.

16. The method of claim 15, wherein the load density is ≥70 g/L.

17. A method for purifying a polypeptide from a composition comprising the polypeptide and an impurity using an ion exchange chromatography material wherein the ion exchange chromatography material is suitable for reuse, the method comprising the steps of

a) equilibrating an ion exchange chromatography material with an equilibration buffer;

b) loading the composition on the ion exchange chromatography material, wherein the load density exceeds the dynamic binding capacity of the ion exchange chromatography for the polypeptide;

c) collecting fractions comprising the polypeptide;

d) washing the ion exchange chromatography material with the equilibration buffer;

e) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises sodium acetate;

f) passing of sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and

g) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH.

18. The method of claim 17, wherein the load density is at least about 2× the dynamic binding capacity of the ion exchange chromatography material for the polypeptide, wherein optionally the load density is about 2× to about 100× the dynamic binding capacity of the ion exchange chromatography material for the polypeptide or about 10× the dynamic binding capacity of the ion exchange chromatography material for the polypeptide.

19. The method of any one of claims 14-18, wherein the equilibration buffer comprises about 20 mM to about 60 mM sodium acetate, wherein optionally the equilibration buffer comprises about 40 mM sodium acetate, wherein optionally the pH of the equilibration buffer is about pH 5.0 to about pH 6.0, and wherein optionally the pH of the equilibration buffer is about pH 5.5.

20. The method of any one of claims 14-19, wherein the ion exchange chromatography material is washed with about 3-7 material volumes of equilibration buffer, wherein optionally the ion exchange chromatography material is washed with about 5 material volumes of equilibration buffer.

21. The method of any one of claims 14-20, wherein the regeneration buffer comprises about 50 to about 600 mM NaOAc, wherein optionally the regeneration buffer comprises about 350 mM NaOAc, wherein optionally the pH of the regeneration buffer is about pH 4.0 to 11, wherein optionally the pH of the regeneration buffer is about pH 8.3, wherein optionally about 3 to about 12 material volumes of regeneration buffer are passed through the ion exchange chromatography material, and wherein optionally about 10 material volumes of regeneration buffer are passed through the ion exchange chromatography material.

22. The method of any one of claims 14-21, wherein about 2 to about 12 material volumes of sanitization buffer are passed through the ion exchange chromatography material, wherein optionally about 10 material volumes of sanitization buffer are passed through the ion exchange chromatography material.

23. The method of any one of claims 14-22, wherein the ion exchange chromatography material is stored in about 1 to 5 material volumes of storage buffer, wherein optionally the ion exchange chromatography material is stored in about 4 material volumes of storage buffer.

24. A method for purifying a polypeptide from a composition comprising the polypeptide and an impurity using an ion exchange chromatography material wherein the ion exchange chromatography material is suitable for reuse, the method comprising the steps of

a) equilibrating an ion exchange chromatography material with an equilibration buffer;

b) loading the composition on the ion exchange chromatography material, wherein the load density exceeds the dynamic binding capacity of the ion exchange chromatography for the polypeptide;

c) collecting fractions comprising the polypeptide;

d) washing the ion exchange chromatography material with the equilibration buffer;

e) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises sodium acetate;

f) passing an acid wash through the ion exchange chromatography material,

g) passing a sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH and about 1 M NaCl; and

h) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH.

25. The method of claim 24, wherein the load density is ≤ about 1000 g/L or ≥ about 20 g/L, wherein optionally the load density is ≥ about 70 g/L.

26. The method of claim 24, wherein the load density is at least about 2× the dynamic binding capacity of the ion exchange chromatography material for the polypeptide or about 2× to about 100× the dynamic binding capacity of the ion exchange chromatography material for the polypeptide or about 10× the dynamic binding capacity of the ion exchange chromatography material for the polypeptide.

27. The method of any one of claims 24-26, wherein the equilibration buffer comprises about 50 mM to about 100 mM NaOAc and about 25 mM to about 75 mM Tris, wherein optionally the equilibration buffer comprises about 85 mM sodium acetate and about 50 mM Tris, wherein optionally the pH of the equilibration buffer is about pH 6.0 to about pH 10.0,

wherein optionally the pH of the equilibration buffer is about pH 8.0, wherein optionally the ion exchange chromatography material is washed with about 5 to about 15 material volumes of equilibration buffer, and wherein optionally the ion exchange chromatography material is washed with about 10 material volumes of equilibration buffer.

28. The method of any one of claims 24-27, wherein the regeneration buffer comprises about 25 to about 600 mM NaOAc, wherein optionally the regeneration buffer comprises about 350 mM NaOAc or about 40 mM NaOAc, wherein optionally the pH of the regeneration buffer is about pH 2.0 to 7.0, and wherein optionally the pH of the regeneration buffer is about pH 4.0.

29. The method of any one of claims 24-28, wherein about 3 to about 12 material volumes of the regeneration buffer are passed through the ion exchange chromatography material, wherein optionally about 7 material volumes of the regeneration buffer are passed through the ion exchange chromatography material.

30. The method of any one of claims 24-29, wherein the acid wash is about 167M NaOAc and about 0.3M phosphoric acid, wherein optionally about 1 to about 5 material volumes of the acid wash are passed through the ion exchange chromatography material, and wherein optionally about 3 material volumes of the acid wash are passed through the ion exchange chromatography material.

31. The method of any one of claims 24-30, wherein about 2 to about 12 material volumes of sanitization buffer are passed through the ion exchange chromatography material, wherein optionally about 4 material volumes of sanitization buffer are passed through the ion exchange chromatography material.

32. The method of any one of claims 24-31, wherein the ion exchange chromatography material is stored in about 1 to 5 material volumes of storage buffer, wherein optionally the ion exchange chromatography material is stored in about 3 material volumes of storage buffer.

33. A method for purifying a polypeptide from a composition comprising the polypeptide and an impurity using an ion exchange chromatography material wherein the ion exchange chromatography material is suitable for reuse, the method comprising the steps of

a) equilibrating the ion exchange chromatography material with an equilibration buffer;

b) loading the composition on the ion exchange chromatography material, wherein the load density is ≤ about 1000 g/L;

c) collecting fractions comprising the polypeptide;

d) washing the ion exchange chromatography material with the equilibration buffer;

e) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises Tris and sodium acetate;

f) passing a sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and

g) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH.

34. A method for purifying a polypeptide from a composition comprising the polypeptide and an impurity using an ion exchange chromatography material wherein the ion exchange chromatography material is suitable for reuse, the method comprising the steps of

a) equilibrating the ion exchange chromatography material with an equilibration buffer;

b) loading the composition on the ion exchange chromatography material, wherein the load density is ≥20 g/L;

c) collecting fractions comprising the polypeptide;

d) washing the ion exchange chromatography material with the equilibration buffer;

e) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises Tris and sodium acetate;

f) passing a sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and

g) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH.

35. The method of claim 34, wherein the load density is ≥70 g/L.

36. A method for purifying a polypeptide from a composition comprising the polypeptide and an impurity using an ion exchange chromatography material wherein the ion exchange chromatography material is suitable for reuse, the method comprising the steps of

a) equilibrating the ion exchange chromatography material with an equilibration buffer;

b) loading the composition on the ion exchange chromatography material, wherein the load density exceeds the dynamic binding capacity of the ion exchange chromatography for the polypeptide;

c) collecting fractions comprising the polypeptide;

d) washing the ion exchange chromatography material with the equilibration buffer;

e) passing a regeneration buffer through the ion exchange chromatography material, wherein the regeneration buffer comprises Tris and sodium acetate;

f) passing a sanitization buffer through the ion exchange chromatography material, wherein the sanitization buffer comprises about 0.5 N NaOH; and

g) storing the ion exchange chromatography material in a storage buffer, wherein the storage buffer comprises about 0.1 N NaOH.

37. The method of claim 36, wherein the load density is at least about 2× the dynamic binding capacity of the ion exchange chromatography material for the polypeptide or about 2× to about 100× the dynamic binding capacity of the ion exchange chromatography material for the polypeptide or about 10× the dynamic binding capacity of the ion exchange chromatography material for the polypeptide.

38. The method of claim 37, wherein the equilibration buffer comprises about 20 mM to about 60 mM sodium acetate.

39. The method of any one of claims 33-38, wherein the equilibration buffer comprises about 40 mM sodium acetate, wherein optionally the pH of the equilibration buffer is about pH 5.0 to about pH 6.0, wherein optionally the pH of the equilibration buffer is about pH 5.5, wherein optionally the ion exchange chromatography material is washed with about 3-7 material volumes of equilibration buffer, and wherein optionally the ion exchange chromatography material is washed with about 5 material volumes of equilibration buffer.

40. The method of any one of claims 33-39, wherein the regeneration buffer comprises about 25 to about 75 mM Tris and about 50 to about 100 mM NaOAc, wherein optionally the regeneration buffer comprises about 50 mM Tris and about 85 mM NaOAc, wherein optionally the pH of the regeneration buffer is about pH 7 to 9, wherein optionally the pH of the regeneration buffer is about pH 8.0, wherein optionally about 3 to about 12 material volumes of regeneration buffer are passed through the ion exchange chromatography material, and wherein

optionally about 10 material volumes of regeneration buffer are passed through the ion exchange chromatography material.

41. The method of any one of claims 33-40, wherein about 2 to about 12 material volumes of sanitization buffer are passed through the ion exchange chromatography material, wherein optionally about 10 material volumes of sanitization buffer are passed through the ion exchange chromatography material.

42. The method of any one of claims 33-41, wherein the ion exchange chromatography material is stored in about 1 to 5 material volumes of storage buffer, wherein optionally the ion exchange chromatography material is stored in about 4 material volumes of storage buffer.

43. The method of any one of claims 1-42, wherein the ion exchange chromatography material is in a chromatography column.

44. The method of any one of claims 1-43, wherein the ion exchange chromatography material is a cation exchange chromatography material; wherein optionally the ion exchange chromatography material comprises a sulfopropyl moiety linked to a support matrix, wherein optionally the support matrix comprises cross-linked poly(styrenedivinylbenzene), wherein optionally the ion exchange chromatography material is a POROS™ HS50 material, wherein optionally the ion exchange chromatography material is a mixed mode cation exchange material, and wherein optionally the mixed mode cation exchange material is Capto MMC™, Capto MMC™ ImpRes, Nuvia™ cPrime™, or Toyopearl MX Trp-650M.

45. The method of any one of claims 1-43, wherein the ion exchange chromatography material is an anion exchange chromatography material; wherein optionally the ion exchange chromatography material comprises a primary amine, a secondary amine, a tertiary amine or a quaternary ammonium ion functional group, a polyamine functional group, or a diethylaminoethyl functional group linked to a support matrix, wherein optionally the ion exchange chromatography material is a mixed mode anion exchange material, and wherein optionally the mixed mode anion exchange material is Capto Adhere™ or Capto Adhere™ ImpRes.

46. The method of any one of claims 1-45, wherein the ion exchange chromatography material is used for large-scale production of the polypeptide, wherein optionally the

polypeptide is an antibody, an immunoadhesin, an Fc-containing protein, or an immunoconjugate.

47. The method of claim 46, wherein the polypeptide is an antibody.

48. The method of claim 47, wherein the antibody is a monoclonal antibody, wherein optionally the monoclonal antibody is a chimeric antibody, humanized antibody, or human antibody, and wherein optionally the monoclonal antibody is an IgG monoclonal antibody.

49. The method of claim 47 or 48, wherein the antibody is an antigen binding fragment, wherein optionally the antigen binding fragment is a Fab fragment, a Fab' fragment, a $F(ab')_2$ fragment, a scFv, a di-scFv, a bi-scFv, a tandem (di, tri)-scFv, a Fv, a sdAb, a tri-functional antibody, a BiTE, a diabody or a triabody.

50. The method of any one of claims 47-49, wherein the antibody is a bispecific antibody.

51. The method of claim 47 or 48, wherein the antibody is selected from the group consisting of an anti-CD20 antibody, an anti-CD40 antibody, an anti-HER2 antibody, an anti-IL6 antibody, an anti-IgE antibody, an anti-IL13 antibody, an anti-Flu A antibody, an anti-TIGIT antibody, an anti-PD-L1 antibody, an anti-VEGF-A antibody, an anti-VEGF-A/ANG2 antibody, an anti-CD79b antibody, an anti-ST2 antibody, an anti-factor D antibody, an anti-factor IX antibody, an anti-factor X antibody, an anti-abeta antibody, an anti-tau antibody, an anti-CEA antibody, an anti-CEA/CD3 antibody, an anti-CD20/CD3 antibody, an anti-FcRH5/CD3 antibody, an anti-Her2/CD3 antibody, an anti-FGFR1/KLB antibody, a FAP-4-1 BBL fusion protein, and a FAP-IL2v fusion protein or selected from the group consisting of ocrelizumab, pertuzumab, trastuzumab, tocilizumab, faricimab, polatuzumab, gantenerumab, cibisatamab, crenezumab, mosunetuzumab, tiragolumab, bevacizumab, rituximab, atezolizumab, obinutuzumab, lampalizumab, lebrikizumab, omalizumab ranibizumab, emicizumab, selicrelumab, prasinezumab, RO6874281 and RO7122290.

52. The method of any one of claims 47-51, wherein the polypeptide is purified using an affinity chromatography material prior to passing through the ion exchange chromatography material, wherein the ion exchange chromatography material is a cation exchange chromatography material; wherein optionally the affinity material is selected from the group

consisting of a Protein A chromatography, a Protein G chromatography, a Protein A/G chromatography, a protein L chromatography, a FcXL chromatography, a protein XL chromatography, a kappa chromatography, and a kappaXL chromatography, and wherein optionally the Protein A affinity material is a MAbSelect material, a MAbSelect SuRe™ material or a MAbSelect SuRe™ LX material.

53. The method of any one of claims 1-52, wherein the buffers are passed through the ion exchange chromatography material at about 15-20 material volumes/hour, wherein optionally the regeneration buffer is passed through the ion exchange chromatography material at about 10 material volumes/hour.

54. The method of any one of claims 1-53, wherein the polypeptide is produced in a host cell, wherein optionally the host cell is a Chinese hamster ovary (CHO) cell.

55. The method of any one of claims 14-54, wherein the impurity comprises one or more of IgG fragments, host cell proteins, or host cell nucleic acids, wherein optionally the polypeptide is produced in a CHO cell and the impurity comprises CHO host cell protein (CHOP) and/or CHO nucleic acids.