[0008] The invention also provides a method of producing a purified polypeptide from a solution comprising the polypeptide and an acidic variant of the polypeptide, where the acidic variant of the polypeptide results in an inhibition of potency of the polypeptide, the method comprising: (a) contacting the polypeptide with an anion exchange (AIEX) chromatography matrix; and (b) eluting the bound polypeptide from the AIEX chromatography matrix with a high salt buffer, thereby separating said polypeptide from the acidic variant and producing a purified polypeptide.

[0009] The invention further provides a method of producing a purified polypeptide or immunoconjugate from a solution comprising the polypeptide and an acidic variant of the polypeptide, the method comprising: (a) producing the polypeptide or immunoconjugate in a bacterial cell which expresses the polypeptide or immunoconjugate; (b) isolating inclusion bodies containing the polypeptide or immunoconjugate from the bacterial cells; (c) refolding the polypeptide or immunoconjugate isolated from the inclusion bodies; (d) contacting the composition containing the polypeptide or immunoconjugate with an AIEX chromatography matrix; and (~~b~~e) eluting the bound polypeptide or immunoconjugate from the AIEX chromatography matrix with a high salt buffer, thereby purifying the polypeptide or immunoconjugte from the solution.

[0010] In certain embodiments, the acidic variant is a deamidated variant. In further embodiments, between about 75 to about 99% of the acidic or deamidated variant is removed during the purification process, in particular about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99%.

[0011] The AIEX matrix of the invention contains quaternary amine or tertiary amine ion exchange groups, a quaternary amino (Q) group, In certain embodiments, the AIEX matrix is Q sepharose.

[0012] The polypeptide or immunoconjugate of the invention is eluted with a linear or step salt gradient. In certain embodiments, the linear salt gradient is from about 150mM NaCl in Tris/HCl, pH 8.0 to about 300 mM NaCl in Tris/HCl, pH 8.0, from about 175mM NaCl in Tris/HCl, pH 8.0 to about 275 mM NaCl in Tris/HCl, pH 8.0, or from about 192mM NaCl in Tris/HCl, pH 8.0 to about 245 mM NaCl in Tris/HCl, pH 8.0.

[0013] In one embodiment, the polypeptide or immunoconjugate of the invention comprises an antibody or antigen binding fragment thereof, where the antibody or antigen binding fragment comprises a Fab, a Fab', a F(ab')2, a Fd, a single chain Fv or scFv, a

disulfide linked Fv, a V NAR domain, an IgNar, an intrabody, an IgG$\Delta$CH2, a minibody, a F(ab')3, a tetrabody, a triabody, a diabody, a single-domain antibody, DVD-Ig, Fcab, mAb2, a (scFv)2, or a scFv-Fc. In certain embodiments, the antibody or antigen binding fragment binds a cell surface receptor, such as the cell surface receptor is CD22. In further embodiments, the antibody or antigen binding fragment thereof comprises a VH and VL sequence, where the VH sequence is selected from the group consisting of SEQ ID NOs: 6-11 and the VL sequence is selected from the group consisting of SEQ ID NOs: 2, and 12-15.

**[0014]** In another embodiment, the polypeptide or immunoconjugate comprises a toxin, where the toxin is selected from the group consisting of: *Pseudomonas* exotoxin, ricin, abrin, diphtheria toxin and subunits thereof, as well as botulinum toxins A through F or variants, or derivatives thereof. In certain embodiments, the *Pseudomonas* exotoxin, or variant thereof has an amino acid sequence selected from the group consisting of SEQ ID NOs:16-22. In a particular embodiment, the immunoconjugate is the CAT-8015 immunotoxin comprising the VH-PE38 subunit of SEQ ID NO:1 and the VL subunit of SEQ ID NO:2.

**[0015]** The invention also provides a composition comprising a purified immunoconjugate having less than between about 25% and about 1% deamidated species, wherein said immunoconjugate is purified by any of the methods described above. The composition can have less than about 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1% of the deamidated species present. In certain embodiments, the composition is a pharmaceutical composition comprising a purified polypeptide or immunoconjugate and a pharmaceutically acceptable carrier.

**[0016]** The invention also provides a formulation comprising 1 mg/mL CAT-8015 in 25 mM sodium phosphate, 4% sucrose, 8% glycine, 0.02% polysorbate 80 (PS80), pH 7.4. In further embodiments, the formulation is lyophilized.

**[0017]** The invention also provides a method of modifying the bioactivity of a polypeptide solution comprising a polypeptide and a deamidated variant, the method comprising separating the polypeptide from the deamidated variant by linear elution AIEX chromatography; and combining the purified polypeptide and deamidated variant in fixed quantities to obtain the desired bioactivity of the polypeptide solution.

myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-betaine (*e.g.*, lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleoyl-taurate; and the MONAQUAT™ series (Mona Industries, Inc., Paterson, N.J.), Useful detergents is a polysorbate, such as polysorbate 20 (TWEEN 20®) or polysorbate 80 (TWEEN 80®).

**[0055]** A "buffer" used in the present invention is a solution that resists changes in pH by the addition of acid or base by the action of its acid-base conjugates components. Various buffers can be employed in a method of the present invention depending on the desired pH of the buffer and the particular step in the purification process [*see* Buffers. A Guide for the Preparation and Use of Buffers in Biological Systems, Gueffroy, D., ed. Calbiochem Corporation (1975)]. Non-limiting examples of buffer components that can be used to control the pH range desirable for a method of the invention include acetate, citrate, histidine, phosphate, ammonium buffers such as ammonium acetate, succinate, MES, CHAPS, MOPS, MOPSO, HEPES, Tris, and the like, as well as combinations of these TRIS-malic acid-NaOH, maleate, chloroacetate, formate, benzoate, propionate, pyridine, piperazine, ADA, PIPES, ACES, BES, TES, tricine, bicine, TAPS, ethanolamine, CHES, CAPS, methylamine, piperidine, O-boric acid, carbonic acid, lactic acid, butaneandioic acid, diethylmalonic acid, glycylglycine, HEPPS, HEPPSO, imidazole, phenol, POPSO, succinate, TAPS, amine-based, benzylamine, trimethyl or dimethyl or ethyl or phenyl amine, ethylenediamine, or morpholine Additional components (additives) can be present in a buffer as needed, *e.g.*, salts can be used to adjust buffer ionic strength, such as sodium chloride, sodium sulfate and potassium chloride; and other additives such as amino acids (such as glycine and histidine), chaotropes (such as urea), alcohols (such as ethanol, mannitol, glycerol, and benzyl alcohol), detergents (see supra.), and sugars (such as sucrose, mannitol, maltose, trehalose, glucose, and fructose). The buffer components and additives, and the concentrations used, can vary according to the type of chromatography practiced in the invention.

**[0056]** The "loading buffer" is that which is used to load the composition comprising the polypeptide molecule of interest and one or more impurities onto the ion exchange resin. The loading buffer has a conductivity and/or pH such that the polypeptide molecule of interest (and generally one or more impurities) is/are bound to the ion exchange resin or

**[00120]** Before the sample is applied to the column, the column can be equilibrated in the buffer or salt that will be used to chromatograph the protein. As discussed below, chromatography (and loading of the protein to be purified) can occur in a variety of buffers or salts including sodium, potassium, ammonium, magnesium, calcium, chloride, fluoride, acetate, phosphate, and/or citrate salts and/or Tris buffer. Citrate buffers and salts are preferred by those skilled in the art for their ease of disposal. Such buffers or salts can have a pH of at least about 5.5. In some embodiments, equilibration may take place in a solution comprising a Tris or a sodium phosphate buffer. In some embodiments, equilibration takes place at a pH of at least about 5.5. Equilibration may take place at pHs between about 6.0 and about 8.6, preferably at pHs between about 6.5 and 7.5. Most preferably, the solution comprises a sodium phosphate buffer at a concentration of about 25 millimolar and at a pH of about 6.8.

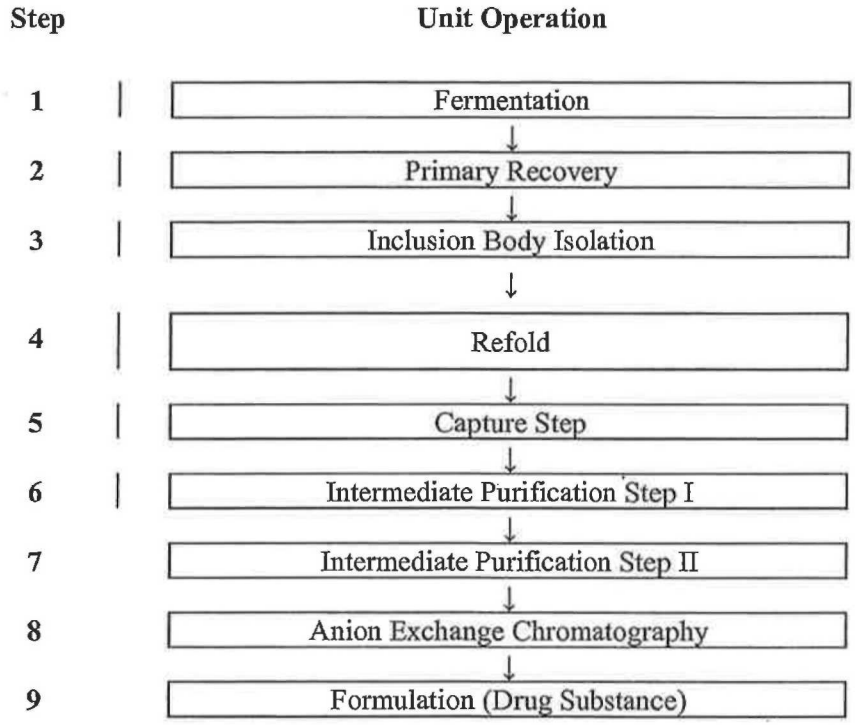
**[00121]** The protein purification process of the present invention is applicable to removal of an acidic variant from any protein. Some proteins specifically contemplated for use with the invention include antibodies or fragments thereof. Other proteins include, but are not limited to recombinant fusion proteins comprising one or more constant antibody immunoglobulin domains, optionally an Fc portion of an antibody, and a protein ~~of~~of interest.

Formulations

**[00122]** Formulations of the purified polypeptides or immunoconjugates are prepared for storage and use by combining a purified polypeptide or immunoconjugate of the present invention with a pharmaceutically acceptable vehicle (*e.g.*, carrier, excipient) (Remington, The Science and Practice of Pharmacy 20th Edition Mack Publishing, 2000). Suitable pharmaceutically acceptable vehicles include, but are not limited to, nontoxic buffers such as phosphate, citrate, and other organic acids; salts such as sodium chloride; antioxidants including ascorbic acid and methionine; preservatives (*e.g.*, octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens, such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight polypeptides (*e.g.*, less than about 10 amino acid residues); proteins

[00137] CAT-8015 was renatured from isolated inclusion bodies and subsequently purified by a 4-column process. Table 1 provides an overview of the renaturation and purification unit operations.

**Table 1 - CAT-8015 production flowchart**




[00138] Figure 1 shows the analytical ion exchange chromeataography (IEC) profile of a sample of a CAT-8015 reference standard. As shown in the profile, a pre-peak emerges prior to the elution of the main peak. The individual fractions eluted from the IEC are assayed for CAT-8015 biological activity relative to a reference standard using an apoptosis bioassay that measures the ability of the test sample to induce dose dependent apoptotic death of the CD22 receptor-expressing Daudi cell line. Once bound to CD22 and internalized, CAT-8015 induces Daudi cells to undergo apoptosis via Caspase 3/7 activation that can be measured by Caspase-GloTM 3/7 Assay System. The potency of the test sample is determined by dividing the 50% effective concentration (EC50) of the Reference Standard by the EC50 of the test sample. The results of the apoptosis bioassay

WHAT IS CLAIMED IS:

1. A method of purifying an active immunoconjugate, wherein said immunoconjugate is deamidated at one or more residues, and wherein said deamidation results in an inhibition of potency of said immunoconjugate, the method comprising: (a) contacting the immunoconjugate with an anion exchange AIEX chromatography matrix; and (b) eluting the bound immunoconjugate from the AIEX chromatography matrix with a high salt buffer, thereby separating the active immunoconjugate from the deamidated variant.

2. A method of producing a purified polypeptide from a solution comprising the polypeptide and an acidic variant of the polypeptide, wherein said acidic variant of the polypeptide results in an inhibition of potency of said polypeptide, the method comprising: (a) contacting the polypeptide with an anion exchange (AIEX) chromatography matrix; and (b) eluting the bound polypeptide from the AIEX chromatography matrix with a high salt buffer, thereby separating said polypeptide from the acidic variant and producing a purified polypeptide.

3. A method of producing a purified polypeptide or immunoconjugate from a solution comprising the polypeptide and an acidic variant of the polypeptide, the method comprising: (a) producing the polypeptide or immunoconjugate in a bacterial cell which expresses the polypeptide or immunoconjugate; (b) isolating inclusion bodies containing the polypeptide or immunoconjugate from the bacterial cells; (c) refolding the polypeptide or immunoconjugate isolated from the inclusion bodies; (d) contacting the composition containing the polypeptide or immunoconjugate with an AIEX chromatography matrix; and (e~~b~~) eluting the bound polypeptide or immunoconjugate from the AIEX chromatography matrix with a high salt buffer, thereby purifying the polypeptide or immunoconjugte from the solution.

~~3. The method of claim 2 or 3, wherein the acidic variant is a deamidated variant.~~

4. The method of any of claims 1-3 or 54, wherein the AIEX matrix contains quaternary amine and tertiary amine ion exchange groups.

5. The method of claim 4, wherein the AIEX matrix contains a quaternary amino (Q) group.

6. The method of claim 5, wherein the AIEX matrix is Q sepharose.

7. The method of any of claims 1-6 or 54, wherein the polypeptide is eluted with a linear or step salt gradient.

8. The method of claim 7, wherein the polypeptide is eluted with a linear salt gradient.

9. The method of claim 8, wherein the linear salt gradient is from about 150mM NaCl in Tris/HCl, pH 8.0 to about 300 mM NaCl in Tris/HCl, pH 8.0.

10. The method of claim 9, wherein the linear salt gradient is from about 175mM NaCl in Tris/HCl, pH 8.0 to about 275 mM NaCl in Tris/HCl, pH 8.0.

1 1. The method of claim 9, wherein the linear salt gradient is from about 192mM NaCl in Tris/HCl, pH 8.0 to about 245 mM NaCl in Tris/HCl, pH 8.0.

12. The method of any of claims 1-11 or 54, wherein between about 75 to about 99% of the acidic or deamidated variant is removed.

13. The method of claim 12, wherein about 80% of the acidic or deamidated variant is removed.

14. The method of claim 12, wherein about 85% of the acidic or deamidated variant is removed.

15. The method of claim 12, wherein about 90% of the acidic or deamidated variant is removed.

16. The method of claim 12, wherein about 95% of the acidic or deamidated variant is removed.

17. The method of claim 12, wherein about 96%, 97%, 98%, or 99% of the acidic or deamidated variant is removed.

18. The method of any of claims 1-17 or 54, wherein the polypeptide or immunoconjugate comprises an antibody or antigen binding fragment thereof.

19. The method of claim 18, wherein the antibody or antigen binding fragment comprises a Fab, a Fab', a F(ab')2, a Fd, a single chain Fv or scFv, a disulfide linked Fv, a V-NAR domain, an IgNar, an intrabody, an IgGACH2, a minibody, a F(ab')3 a tetrabody, a triabody, a diabody, a single-domain antibody, DVD-Ig, Fcab, $mAb^2$, a (scFv)2, or a scFv-Fc.

20. The method of claim 18 or 19, wherein the antibody or antigen binding fragment binds a cell surface receptor.

21. The method of claim 20, wherein the cell surface receptor is CD22.

22. The method of any of claims 1-21 or 54, wherein the polypeptide or immunoconjugate comprises a toxin.

23. The method of claim 22, wherein the toxin is selected from the group consisting of: Pseudomonas exotoxin, ricin, abrin, diphtheria toxin and subunits thereof, as well as botulinum toxins A through F or variants, or derivatives thereof.

24. The method of claim 22 or 23, wherein the toxin is a Pseudomonas exotoxin, or variant thereof.

25. The method of claim 24, wherein said Pseudomonas exotoxin, or variant thereof has an amino acid sequence selected from the group consisting of SEQ ID NOs: 16-22.

26. The method of claim 24, wherein said Pseudomonas exotoxin, or variant thereof has the amino acid sequence of SEQ ID NO:22.

27. The method of claim 18 or 19, wherein said antibody or antigen binding fragment thereof comprises a VH and a VL sequence.

28. The method of claim 27, wherein said VH sequence is selected from the group consisting of SEQ ID NOs: 6-11.

29. The method of claim 27, wherein said VL sequence is selected from the group consisting of SEQ ID NOs: 2, and 12-15.

30. The method of any of claims 1-29 or 54, wherein the polypeptide or immunoconjugate comprises an anti-CD22 antibody or antigen binding fragment thereof and a PE or variant thereof.

31. The method of claim 30, wherein the immunoconjugate is the CAT-8015 immunotoxin comprising the VH-PE38 subunit of SEQ ID NO: 1 and the VL subunit of SEQ ID NO:2.

32. A composition comprising a purified immunoconjugate having less than between about 25% and about 1% deamidated species, wherein said immunoconjugate is purified by the method of any of claims 1-31 or 54.

33. The composition of claim 32, wherein less than about 25% of the deamidated species is present.

34. The composition of claim 32, wherein less than about 20% of the deamidated species is present.

35. The composition of claim 32, wherein less than about 10% of the deamidated species is present.

36. The composition of claim 32, wherein less than about 5% of the deamidated species is present.

37. The composition of claim 32, wherein less than about 3% of the deamidated species is present.

46. A formulation comprising the composition of any of claims 32-43 and at least one excipient selected from the group consisting of sodium chloride, potassium dihydrogen phosphate, disodium hydrogen phosphate, and sodium hydroxide and water.

47. A formulation comprising the composition of any of claims 32-43 comprising 1 mg/mL CAT-8015 in 25 mM sodium phosphate, 4% sucrose, 8% glycine, 0.02% polysorbate 80 (PS80), pH 7.4.

48. The formulation of ~~claim~~ any one of claims 41 to 43 that is further lyophilized.

49. A method of modifying the bioactivity of a polypeptide solution comprising a polypeptide and a deamidated variant, the method comprising separating the polypeptide from the deamidated variant by linear elution AIEX chromatography; and combining the purified polypeptide and deamidated variant in fixed quantities to obtain the desired bioactivity of the polypeptide solution.

50. The method of any of claims 24-27, wherein said one or more deamidated residues are present within the Pseudomonas exotoxin or variant thereof.

51. The method of claim 47, wherein said immunoconjugate is encoded by the amino acid sequence of SEQ ID NO: 1 and is deamidated at position 358 of SEQ ID NO: 1.

52. The method of claim 3, wherein refolding the polypeptide or immunoconjugate isolated from the inclusion bodies comprises solubilization of inclusion bodies at a pH in a range of about pH 9.0 to about pH 10.5.

53. The method of claim 3 or 52, wherein refolding the polypeptide or immunoconjugate isolated from the inclusion bodies comprises solubilization of inclusion bodies at a pH of 10.5, a pH of 10.0, a pH of 9.5, or pH of 9.0.

54. The method of claim 2 or 3, wherein the acidic variant is a deamidated variant.