FRACTION I-IV-1 PRECIPITATION OF IMMUNOGLOBINS FROM PLASMA

CROSS REFERNECES TO APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 61/602,488 filed February 23, 2012~~, the disclosure of which is hereby incorpated herein by reference in its entirety for all purposes~~.

BACKGROUND OF THE INVENTION

[0002] Immune globulin products from human plasma were first used in 1952 to treat immune deficiency. Initially, intramuscular or subcutaneous administration of immunoglobulin isotype G (IgG) isolated from plasma were the methods of choice. However, these methods did not allow for the administration of larger amounts of IgG necessary for effective treatment of various diseases. Accordingly, IgG products that could be administered intravenously were developed. Usually, intravenous immunoglobulin (IVIG) contains the pooled immunoglobulin G (IgG) immunoglobulins from the plasma of more than a thousand blood donors. The preparations usually contain more than 95% unmodified IgG, which has intact Fc-dependent effector functions and only trace amounts of immunoglobulin A (IgA) and immunoglobulin M (IgM). Typically, IVIGs are sterile filtered and the manufacturing process contains steps to inactivate and/or remove viruses. These purified IgG products are primarily used in treating three main categories of medical conditions: (1) immune deficiencies: X-linked agammaglobulinemia, hypogammaglobulinemia (primary immune deficiencies), and acquired compromised immunity conditions (secondary immune deficiencies), featuring low antibody levels; (2) inflammatory and autoimmune diseases; and (3) acute infections.

[0003] Specifically, many people with primary immunodeficiency disorders lack antibodies needed to resist infection. In certain cases these deficiencies can be supplemented by the infusion of purified IgG, commonly through intravenous administration (*i.e.*, IVIG therapy). Several primary immunodeficiency disorders are commonly treated in the fashion, including X-linked Agammaglobulinemia (XLA), Common Variable Immunodeficiency (CVID), Hyper-IgM Syndrome (HIM), Severe Combined Immunodeficiency (SCID), and some IgG subclass

albumin remains in the supernatant. Taken together, these advantages result in a significant increase in the overall recovery of these important commercial products.

[0018] As shown in the Examples, the use of a low pH, high alcohol precipitation step (Fraction I-IV-1 precipitation) as an initial step in the purification of IgG from cryo-poor plasma, allows for the production of pharmaceutical grade IgG compositions with unprecedented yields of 4.3-4.7 g IgG/L source plasma. These yields represent an approximate 20% to 25% increase in yield as compared to state-of-the-art manufacturing processes, such as those used to manufacture GAMMAGARD LIQUID® (Baxter International; Deerfield, IL) from the same plasma type.

[0018a] In one aspect, the present invention relates to a method for manufacturing an enriched immunoglobulin composition from a Cohn pool, the method comprising the steps of: (a) co-precipitating immunoglobulins and alpha-1-antitrypsin (A1PI) from a Cohn pool, in a first precipitation step by the addition of ethanol to the Cohn pool at a final concentration of from 20% to 30% (v/v) at a pH of from 5.0 to 6.0, to form a first precipitate and a first supernatant; (b) solubilizing immunoglobulins in the first precipitate, to form a first suspension having a soluble portion comprising immunoglobulins and an insoluble portion comprising A1PI;(c) separating the soluble portion of the first suspension from the insoluble portion of the first suspension; and (d) recovering the soluble fraction of the first suspension, thereby forming an enriched immunoglobulin composition~~.~~, wherein no alcohol precipitation is performed prior to step (a).

[0018b] In another embodiment~~aspect~~, there is provided an enriched immunoglobulin composition prepared by a method disclosed herein.

[0018c] In another embodiment~~aspect~~, there is provided the use of an enriched immunoglobulin composition as disclosed herein in the preparation of a medicament for treating an immune deficiency, an inflammatory disease, an autoimmune disease, or an acute infection in an individual in need thereof, the medicament being suitable for administration to the subject.

[0019] Accordingly, among other aspects, the present invention provides a new plasma fractionation process which separates plasma or cryo-poor plasma in an initial step into a Fraction I-IV-1 precipitate and a Fraction I-IV-1 supernatant. The Fraction I-IV-1 precipitate contains nearly all immunoglobulins (*e.g.*, IgG, IgA, and IgM) and alpha 1 elastase inhibitor (A1PI), while the supernatant contains mainly albumin.

[0091] The Fraction I-IV-1 precipitate will also contain at least 70%, preferably at least 80%, more preferably at least 90%, most preferably at least 95% of the Factor H content of the starting plasma. Accordingly, this precipitate can be used as a starting material for the manufacture of a pharmaceutical Factor H composition.

[0092] The Fraction I-IV-1 precipitate will also contain at least 70%, preferably at least 80%, more preferably at least 90%, most preferably at least 95% of the inter-alpha-inhibitor (IaIp) content of the starting plasma. Accordingly, this precipitate can be used as a starting material for the manufacture of a pharmaceutical IaIp composition.

[0093] In one aspect, the present invention provides a method for separating immunoglobulins from A1PI found in the Fraction I-IV-1 precipitate. In one embodiment, the method comprises solubilizing immunoglobulins in a suspension of the Fraction I-IV-1 precipitate, while retaining A1PI in the insoluble portion of the suspension and then separating the soluble and insoluble portions, for example by filtration or centrifugation. In one embodiment, the separation is aided by treating the Fraction I-IV-1 suspension with finely divided silicon dioxide prior to separating the soluble and insoluble portions. Without being bound by theory, the silicon dioxide may bind A1PI that is co-solubilized with the immunoglobulins, enhancing the amount of A1PI partitioned into the insoluble portion of the suspension.

[0094] Furthermore, it is known that Factor H and IaIp bind particulate silicon dioxide under certain conditions (*see*, WO/2011/011753, WO/2012/012773~~PCT/US2011/45099~~, and WO/2011/150284~~PCT/US2011/038247~~; the disclosures of which are hereby expressly incorporated by reference in their entireties for all purposes). Accordingly, in one embodiment, the present invention provides a method for separating immunoglobulins from A1PI, Factor H, and IaIp found in a Fraction I-IV-1 precipitate, the method comprising suspending a Fraction I-IV-1 precipitate in water or a low conductivity buffer sufficient to solubilize immunoglobulins; treating the suspension with silicon dioxide; and separating the soluble portion of the suspension from the insoluble portion, wherein soluble portion contains immunoglobulins and the insoluble portion contains A1PI, Factor H, and IaIp.

[0095] In one embodiment of the methods provided above, the separated soluble portion of the Fraction I-IV-1 suspension contains at least 80% of the IgG content of the starting plasma

nanofiltration), ultracentrifugation, electrophoretic preparation, and the like. (*See, e.g.*, Cohn *et al.*, J. Am. Chem. Soc. 68:459-75 (1946); Oncley *et al.*, J. Am. Chem. Soc. 71:541-50 (1949); Barundern *et al.*, Vox Sang. 7:157-74 (1962); Koblet *et al.*, Vox Sang. 13:93-102 (1967); U.S. Patent Nos. 5,122,373 and 5,177,194; WO/2011/149472~~PCT/US2010/036470~~; and WO/2011/150284~~PCT/US2011/038247~~; the disclosures of which are hereby incorporated by reference in their entireties for all purposes).

A. Upstream Processing

[00109] Among other aspects, the present invention provides methods for fractionating proteins present in pooled plasma by performing an initial precipitation step that precipitates a majority of the immunoglobulin and alpha-1-antitrypsin content of the starting plasma. To further increase the purity of individual plasma proteins (*e.g.*, IgG, A1PI, Factor H, IaIp, *etc.*) found in the precipitate and supernatant of the initial precipitation step, these compositions can be further enriched by fractionation (*e.g.*, by alcohol precipitation, PEG precipitation, salting out, *etc.*), chromatography, filtration, or other method. Although use of these various techniques for the enrichment of a plasma-derived protein may be performed in any order, in a particular embodiment, the first precipitate and/or supernatant is first fractionated (*e.g.*, by ethanol precipitation) and then enriched using chromatographic and/or filtration techniques. In the context of the present invention, the initial precipitation reaction and subsequent fractionation are aggregately referred to as "upstream processing" steps, while the chromatographic and filtration steps are aggregately referred to as "downstream processing" steps.

1. Mass Immunoglobulin Precipitation

[00110] As described herein, the inventors have discovered an improved method for fractionating human plasma in order to purify therapeutically beneficial blood proteins such as immunoglobulins, alpha-1-antitrypsin (A1PI), factor H, Inter-alpha-Inhibitor proteins (IaIp), albumin, fibrinogen, *etc.* This method incorporates an initial purification step in which the majority of the immunoglobulin, A1PI, factor H, IaIp, and fibrinogen content of the starting Cohn pool (*i.e.*, blood, plasma, and/or pre-treated plasma) is precipitated and the majority of the albumin content of the starting Cohn pool remains in the supernatant. Advantageously, the inventors have developed a method of separating immunoglobulins from A1PI, factor H, and

2. Silicon Dioxide (SiO_2) Treatment

[00159] As compared to Cohn-Oncley and Kistler-Nitschmann purifications, the initial immunoglobulin-containing precipitates formed by the methods provided herein contain substantially higher levels of non-immunoglobulin proteins, including A1PI, fibrinogen, Factor H, and IaIp. It is known that Factor H and IaIp can be bound by and subsequently eluted off of finely divided silicon dioxide (*see*, WO 2011/011753 and WO 2012/012773 ~~PCT/US2011/045099~~, the disclosures of which are both expressly incorporated by reference in their entireties for all purposes). Advantageously, it was found that inclusion of a SiO_2 treatment step after extraction of the Fraction I-IV-1 precipitate and prior to filtration of the Fraction I-IV-1 suspension aids in the separation of A1PI, fibrinogen, Factor H, and IaIp into the insoluble filter cake formed during filtration of the Fraction I-IV-1 suspension.

[00160] Accordingly, in one embodiment, finely divided silicon dioxide is admixed with the Fraction I-IV-1 suspension prior to filtration. In one embodiment, this pretreatment step comprises addition of finely divided silica dioxide particles (*e.g.*, fumed silica; Aerosil®) followed by a 40 to 80 minute incubation period during which the suspension is constantly mixed. In certain embodiments, the incubation period will be between about 50 minutes and about 70 minutes, or about 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, or more minutes. Generally, the treatment will be performed at between 0°C and 10°C, or between 2°C and 8°C. In certain embodiments, the treatment may be performed at about 0°C, 1°C, 2°C, 3°C, 4°C, 5°C, 6°C, 7°C, 8°C, 9°C, or 10°C. In a particular embodiment, the treatment is performed at between 2°C and 10°C. In a preferred embodiment, the treatment is performed at between 5°C and 10°C.

[00161] In certain embodiments, fumed silica is added at a concentration of between 20 g/kg Fraction I-IV-1 precipitate and 100 g/kg Fraction I-IV-1 precipitate. In certain embodiments, the fumed silica may be added at a concentration of about 20 g/kg Fraction I-IV-1 precipitate, or about 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 g/kg Fraction I-IV-1 precipitate. In one specific embodiment, fumed silica (*e.g.*, Aerosil 380 or equivalent) is added to the Fraction I-IV-1 suspension to a final concentration of 40±10 g/kg Fraction I-IV-1 precipitate. Mixing takes place at 2°C to 8°C for at least 50 to 70 minutes.

fractionation. ~~The disclosures of the above publications are hereby incorporated by reference in their entireties for all purposes~~

[00180] In one embodiment, the S/D treated PptG filtrate may be subjected to both cation exchange chromatography and anion exchange chromatography. For example, in one embodiment, the S/D treated PptG filtrate is contacted by a cation exchange resin under suitable conditions to binds the IgG to the resin. The S/D reagents can then be washed away from the adsorbed IgG, which is subsequently eluted off of the resin with a suitable elution buffer. In this fashion, the cation exchange chromatography step can be used to remove the S/D reagents from the preparation, concentrate the IgG containing solution, and/or remove impurities from the composition.

[00181] In one embodiment, IgG is eluted from the cation exchange resin using an elution buffer having a pH between about 8.0 and 9.0. In certain embodiments, the pH elution buffer may have a pH between about 8.2 and about 8.8, or between about 8.4 and about 8.6, or a pH of about 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, or 9.0. In a preferred embodiment, the pH of the elution buffer is about 8.5 ± 0.1.

[00182] Likewise, anion exchange chromatography can be used to reduce impurities in the IgG composition. In one embodiment, anion exchange chromatography is performed under solution conditions in which one or more impurities bind to the anion exchange resin but IgG does not. In a specific embodiment, anion exchange chromatography is performed at slightly acidic conditions, *i.e.*, at a pH between 5.0 and 7.0, preferably between 5.5 and 6.5, at a low ionic strength suitable for binding of contaminants but not IgG to the anion exchange resin.

[00183] In a specific embodiment, the eluate from the cation exchange column may be adjusted to a lower pH, for example between about 5.5 and about 6.5, and diluted with an appropriate buffer such that the conductivity of the solution is reduced. In certain embodiments, the pH of the cation exchange eluate may be adjusted to a pH between about 5.7 and about 6.7, or between about 5.9 and about 6.5, or a pH of about 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, or 6.7. In a preferred embodiment, the pH of the eluate is adjusted to a pH of about 6.4 ± 0.1. The eluate is then loaded onto an anion exchange column, which binds several contaminants found in the preparation. The column flow through, containing the IgG fraction, is collected during

[00255] In one embodiment, A1PI extracted from the insoluble portion of the first suspension is further enriched according to well known methods in the art, including without limitation: chromatography (*e.g.*, anion exchange chromatography, cation exchange chromatography, hydrophobic interaction chromatography (HIC), hydroxyapatite chromatography (HAP), Protein A affinity chromatography, immuno-affinity chromatography, size exclusion chromatography, *etc.*); filtration (*e.g.*, ultrafiltration and/or diafiltration); and one or more viral reduction steps (*e.g.*, nanofiltration, solvent and detergent treatment, UV irradiation, heat treatment, low pH incubation, *etc.*).

[00256] In one embodiment, A1PI extracted from the insoluble portion of the first suspension is further enriched by fractionation. Generally, any method of fractionation (*e.g.*, alcohol or polymer precipitation, salting out, *etc.*) may be used. In a preferred embodiment, the composition is further enriched by precipitating A1PI from the clarified extracted solution using a divalent cation, preferably zinc. In one embodiment, zinc chloride is added to the solution to a final concentration of from 1 mM to 15 mM. In a specific embodiment, zinc chloride is added to the solution to a final concentration of from 1 mM to 4 mM. In a more specific embodiment, zinc chloride is added to the solution to a final concentration of from 2.0 mM to 3.0 mM.

[00257] In certain embodiments, A1PI is further enriched according to purification methods known in the art, for example, those described in WO 1995/35306, WO 1998/00154, and U.S. Patent Nos. 5,981,715; 7,807,435; and 7,879,800~~, the disclosures of which are hereby expressly incorporated by reference in their entireties for all purposes~~.

VI. Pharmaceutical Compositions

[00258] In one aspect, the present invention provides compositions of plasma-derived proteins which are prepared according to any of the methods described herein. In certain embodiments, these compositions will be formulated for pharmaceutical administration (*i.e.*, pharmaceutical compositions).

[00259] In one embodiment, the present invention provides a plasma-derived protein composition prepared by a method comprising the steps of: co-precipitating immunoglobulins and alpha-1-antitrypsin (A1PI) from a Cohn pool, in a first precipitation step, to form a first

precipitate and a first supernatant; suspending the first precipitate to form a first suspension; solubilizing immunoglobulins from the first suspension to form a soluble portion of the suspension comprising immunoglobulins and an insoluble portion of the suspension comprising A1PI, fibrinogen, Factor H, and IaIp; and separating the soluble portion of the suspension from the insoluble portion of the suspension. In certain embodiments, the composition comprises a plasma-derived protein is selected from an immunoglobulin (Ig), albumin, alpha-1-antitrypsin (A1PI), butyrylcholinesterase, Factor H, a protein of the complement system, and an inter-alpha-trypsin inhibitor (IαI) protein.

[00260] Generally, any chemical means for the precipitation of immunoglobulins and A1PI may be used, including but not limited to, alcohol precipitation (*e.g.*, using ethanol or methanol), precipitation using water soluble polymers (*e.g.*, PEG or dextran), and salting out (*e.g.*, using ammonium phosphate, ammonium sulfate, sodium citrate, *etc.*). In a preferred embodiment, the precipitation is alcohol precipitation, preferably ethanol precipitation.

[00261] In one embodiment, the composition is formulated for pharmaceutical administration. In a specific embodiment, the composition is formulated for intravenous administration. In another specific embodiment, the composition is formulated for intramuscular administration. In another embodiment, the composition is formulated for subcutaneous administration. In yet another embodiment, the composition is formulated for intraocular administration.

[00262] In one embodiment, the pharmaceutical compositions provided herein are prepared by formulating a plasma-derived protein composition isolated using a method provided herein. Generally, the formulated composition will have been subjected to at least one, preferably at least two, most preferably at least three, viral inactivation or removal steps. Non-limiting examples of viral inactivation or removal steps that may be employed with the methods provided herein include, solvent detergent treatment (Horowitz et al., Blood Coagul fibrinogenolysis 1994 (5 Suppl 3):S21-S28 and Kreil et al., Transfusion 2003 (43):1023-1028~~, both of which are herein expressly incorporated by reference in their entirety for all purposes~~), nanofiltration (Hamamoto et al., Vox Sang 1989 (56)230-236 and Yuasa et al., J Gen Virol. 1991 (72 (pt 8)):2021-2024, both of which are herein expressly incorporated by reference in their entirety for all purposes), and low pH incubation at high temperatures (Kempf et al., Transfusion 1991 (31)423-427 and Louie et al., Biologicals 1994 (22):13-19). In certain embodiments, the composition comprises a

HPLC	(% monomers)	98.1
Citrate	µmol/mL	<0.05
PKA	IE/mL	<4
Visual inspection	Vials rejected (e.g., due to particles)	None

~~[00312] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.~~

WHAT IS CLAIMED IS:

1. A method for manufacturing an enriched immunoglobulin composition from a Cohn pool, the method comprising the steps of:

(a) co-precipitating immunoglobulins and alpha-1-antitrypsin (A1PI) from a Cohn pool, in a first precipitation step by the addition of ethanol to the Cohn pool at a final concentration of from 20% to 30% (v/v) at a pH of from 5.0 to 6.0, to form a first precipitate and a first supernatant;

(b) solubilizing immunoglobulins in the first precipitate, to form a first suspension having a soluble portion comprising immunoglobulins and an insoluble portion comprising A1PI;

(c) separating the soluble portion of the first suspension from the insoluble portion of the first suspension; and

(d) recovering the soluble fraction of the first suspension, thereby forming an enriched immunoglobulin composition~~.~~, wherein no alcohol precipitation is performed prior to step (a).

~~2. The method of claim 1, wherein the final concentration of ethanol is 25±4%.~~

~~3. The method of claim 1, wherein the final concentration of ethanol is 25±3%.~~

~~4.~~2. The method of claim 1, wherein the final concentration of ethanol is 25±2%.

~~5. The method of claim 1, wherein the final concentration of ethanol is 25±1%.~~

~~6.~~3. The method of claim ~~5~~1, wherein the final concentration of ethanol is 25%.

~~7. The method according to any one of claims 1 to 6, wherein the pH is 5.5±0.4.~~

~~8. The method of claim 7, wherein the pH is 5.5±0.3.~~

~~9.~~4. The method according to any one of claims 1 to 3~~of claim 7~~, wherein the pH is 5.5±0.2.

~~10. The method of claim 7, wherein the pH is 5.5±0.1.~~

~~11.~~5. The method according to any one of claims 1 to 3~~of claim 7~~, wherein the pH is 5.5.

~~12. The method according to any one of claims 1 to 11, wherein the pH is maintained for the duration of the first precipitation step.~~

~~13. The method according to any one of claims 1 to 12, wherein the alcohol precipitation step comprises addition of the alcohol by diffuse addition.~~

~~14. The method of claim 13, wherein the diffuse addition comprises spraying.~~

~~15. The method according to any one of claims 1 to 13, wherein the alcohol precipitation step comprises addition of the alcohol at a site adjacent to an impeller.~~

~~16. The method according to any one of claims 1 to 15, wherein the first precipitation step is performed at a temperature of from -3°C to -10°C.~~

~~17.~~6. The method according to any one of claims 1 to 5~~of claim 16~~, wherein the first precipitation step is performed at a temperature of from -5°C to -9°C.

~~18. The method according to any one of claims 1 to 17, wherein the first precipitate is suspended with from 4 L to 60 L of buffer per kg precipitate.~~

~~19. The method of claim 18, wherein the first precipitate is suspended with from 8 L to 15 L of buffer per kg precipitate.~~

~~20.~~7. The method according to any one of claims 1 to 6~~19~~, wherein the first suspension has a pH of from 4.0 to 5.4.

~~21.~~8. The method according to any one of claims 1 to 6~~20~~, wherein the first suspension has a pH of from 4.7 to 5.1.

~~22.~~9. The method according to any one of claims 1 to 8~~21~~, wherein the first suspension has a conductivity of from 0 mS/cm to 4 mS/cm.

~~23.~~10. The method according to any one of claims 1 to 9~~22~~, wherein the first suspension has a conductivity of from 0.5 mS/cm to 2 mS/cm.

~~24. The method according to any one of claims 1 to 23, wherein the first precipitate is suspended in a buffer comprising acetate and/or phosphate.~~

~~25. The method according to any one of claims 1 to 24, wherein the soluble portion of the first suspension is separated from the insoluble portion of the first suspension by centrifugation or filtration.~~

~~26.~~11. The method according to any one of claims 1 to 10~~25~~, wherein the step of separating the soluble portion of the first suspension from the insoluble portion of the first suspension comprises:

(i) mixing finely divided silicon dioxide (SiO_2) with the first suspension; and

(ii) separating the SiO_2 from the suspension.

~~27. The method of claim 26~~, wherein the finely divided silicon dioxide (SiO_2) has an average surface area of from 350 m^2/g to 410 m^2/g.

~~28.~~12. The method according to any one of claims 1 to 11~~27~~, wherein the finely divided silicon dioxide (SiO_2) is added to the first suspension at a final concentration of from 15 g/kg first precipitate to 80 g/kg first precipitate.

~~29.~~13. The method according to any one of claims 1 to 12~~28~~, wherein the method comprises the steps of:

(a) precipitating immunoglobulins from a Cohn pool, in a first precipitation step, with between 24% and 26% alcohol at a pH of between 5.3 and 5.7 and temperature of between -6°C and -8°C to form a first precipitate and a first supernatant;

(b) suspending the first precipitate to form a first suspension;

(c) treating the first suspension with finely divided silicon dioxide (SiO_2);

(d) separating the soluble fraction of the first suspension from the insoluble fraction of the first suspension; and

(e) recovering the soluble fraction of the first suspension, thereby forming an enriched immunoglobulin composition.

~~30. The method according to any one of claims 1 to 29, wherein the enriched immunoglobulin composition contains at least 90% of the immunoglobulin content of at least one immunoglobulin class present in the Cohn pool used in step (a).~~

~~31. The method of claim 30, wherein the enriched immunoglobulin composition contains at least 95% of the immunoglobulin content of at least one immunoglobulin class present in the Cohn pool used in step (a).~~

~~32. The method of claim 30 or 31, wherein the immunoglobulin class is IgG.~~

~~33.~~14. The method according to any one of claims 1 to 1~~33~~1, wherein the method further comprises the steps of

(f) precipitating immunoglobulins from the soluble fraction of the first suspension, in a second precipitation step, to obtain a second precipitate and a second supernatant;

(g) suspending the second precipitate to form a second suspension; and

(h) recovering the soluble fraction of the second suspension, thereby forming a further enriched immunoglobulin composition.

~~34. The method of claim 33~~, wherein the second precipitation step is an alcohol precipitation step, comprising.

~~35. The method of claim 34, wherein the alcohol precipitation step comprises~~ adding ethanol to the soluble fraction of the first suspension to achieve a final concentration of between 22% and 28% ethanol at a pH between 6.5 and 7.5.

~~36. The method of claim 34 or 35, wherein the alcohol precipitation step comprises addition of the alcohol by diffuse addition.~~

~~37. The method of claim 36, wherein the diffuse addition comprises spraying.~~

~~38. The method according to any one of claims 34 to 36, wherein the alcohol precipitation step comprises addition of the alcohol at a site adjacent to an impeller.~~

~~39. The method according to any one of claims 33 to 38, wherein the second precipitation step is performed at a temperature between -3°C and -10°C.~~

~~40. The method according to any one of claims 33 to 39 wherein the enriched immunoglobulin composition contains at least 90% of the immunoglobulin G content present in the Cohn pool used in step (a).~~

~~41. The method of claim 40, wherein the enriched immunoglobulin composition contains at least 95% of the immunoglobulin G content present in the Cohn pool used in step (a).~~

~~42.~~15. The method according to any one of claims 1 to 14~~1~~, wherein the method further comprises a cation exchange chromatography enrichment step and/or~~,~~

~~43. The method according to any one of claims 1 to 42, wherein the method further comprises~~ an anion exchange chromatography enrichment step.

~~44. The method according to any one of claims 1 to 43, wherein the method further comprises a viral inactivation and/or removal step.~~

~~45.~~16. The method according to any one of claims 1 to 15~~of claim 44~~, wherein the method comprises at least one of: (i) a solvent/detergent (S/D) viral inactivation step;~~,~~

~~46. The method of claim 44 or 45, wherein the method comprises~~ (ii) a nanofiltration step; and~~,~~

~~47. The method according to any one of claims 44 to 46, wherein the method comprises~~ (iii) a step of incubating the composition at a pH from 4.0 to 5.0 and temperature from 20°C to 40°C for at least one week.

~~48. The method according to any one of claims 1 to 47, wherein the final enriched IgG composition comprises at least 98% IgG.~~

~~49. The method of claim 48, wherein the final enriched IgG composition comprises at least 99% IgG.~~

~~50.~~17. The method according to any one of claims 1 to 16~~49~~, wherein the method yields at least 4 g of IgG per L of Cohn pool used in step (a).

~~51. The method of claim 50, wherein the method yields at least 4.25 g of IgG per L of Cohn pool used in step (a).~~

~~52.~~18. The method of claim 17~~50~~, wherein the method yields at least 4.5 g of IgG per L of Cohn pool used in step (a).

~~53.~~19. The method according to any one of claims 1 to 18~~47~~, wherein alpha-1-antitrypsin (A1PI) is further purified from an insoluble fraction of the first suspension.

~~54. The method according to any one of claims 1 to 47, wherein fibrinogen is further purified from an insoluble fraction of the first suspension~~

~~55.~~20. The method according to any one of claims 1 to 18~~47~~, wherein Factor H is further purified from an insoluble fraction of the first suspension.

~~56.~~21. The method according to any one of claims 1 to 18~~47~~, wherein an Inter-alpha-Trypsin Inhibitor protein (IαIp) is further purified from an insoluble fraction of the first suspension.

~~57. The method according to any one of claims 53 to 56, wherein the insoluble fraction of the first suspension is treated with finely divided silicon dioxide (SiO_2).~~

~~58. The method according to any one of claims 1 to 47, wherein albumin is further purified from the first supernatant.~~

~~59.~~22. The method according to any one of claims 1 to 21~~57~~, wherein the Cohn pool is cryo-poor plasma.

~~60. An enriched immunoglobulin composition prepared by a method according to any one of claims 1 to 59.~~

61. Use of an enriched immunoglobulin composition of claim 60 in the preparation of a medicament for treating an immune deficiency, an inflammatory disease, an autoimmune disease, or an acute infection in an individual in need thereof, the medicament being suitable for administration to the subject.