[0047] In yet another aspect, the present invention provides method for treating immune deficiencies, inflammatory and autoimmune diseases, and acute infections comprising the administration of an IgG composition prepared using the improved methods provided herein.

II. Methods of IVIG Manufacture

[0048] Generally, immunoglobulin preparations according to the present invention can be prepared from any suitable starting materials, for example, recovered plasma or source plasma. In a typical example, blood or plasma is collected from healthy donors. Usually, the blood is collected from the same species of animal as the subject to which the immunoglobulin preparation will be administered (typically referred to as "homologous" immunoglobulins). The immunoglobulins are isolated from the blood by suitable procedures, such as, for example, precipitation (alcohol fractionation or polyethylene glycol fractionation), chromatographic methods (ion exchange chromatography, affinity chromatography, immunoaffinity chromatography, *etc.*) ultracentrifugation, and 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~~; the disclosures of which are hereby incorporated by reference in their entireties for all purposes~~).

[0049] In many cases, immunoglobulins are prepared from gamma globulin-containing products produced by alcohol fractionation and/or ion exchange and affinity chromatography methods well known to those skilled in the art. For example, purified Cohn Fraction II is commonly used as a starting point for the isolation of immunoglobulins. The starting Cohn Fraction II paste is typically about 95 percent IgG and is comprised of the four IgG subtypes. The different subtypes are present in Fraction II in approximately the same ratio as they are found in the pooled human plasma from which they are obtained. The Fraction II is further purified before formulation into an administrable product. For example, the Fraction II paste can be dissolved in a cold purified aqueous alcohol solution and impurities removed via precipitation and filtration. Following the final filtration, the immunoglobulin suspension can be dialyzed or diafiltered (*e.g.*, using ultrafiltration membranes having a nominal molecular weight limit of less than or equal to 100,000 daltons) to remove the alcohol. The solution can be concentrated or diluted to obtain the desired protein concentration and can be further purified by techniques well known to those skilled in the art.

[0050] Furthermore, additional preparative steps can be used to enrich a particular isotype or subtype of immunoglobulin. For example, protein A, protein G or protein H sepharose chromatography can be used to enrich a mixture of immunoglobulins for IgG, or for specific IgG subtypes. *See generally* Harlow and Lane, *Using Antibodies*, Cold Spring Harbor Laboratory Press (1999); Harlow and Lane, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press (1988); and U.S. Patent No. 5,180,810~~, the disclosures of which are hereby incorporated by reference in their entireties for all purposes~~.

[0051] Unlike the methods described above, in one aspect the present invention provides methods of preparing concentrated IgG compositions that utilize a cryo-poor starting material. Generally, the methods provided herein utilize both modified Cohn-Oncley alcohol fractionation steps and ion exchange chromatography to provide superior IgG yields, while maintaining the same, if not improved, quality as found in currently available commercial IVIG preparations. For example, in certain embodiments methods are provided that yield a final bulk IgG composition containing close to 75% of the IgG content found in the raw plasma starting material. These methods represent at least a 10% to 12% increase in the overall IgG yield over existing state of the art purification methods. For example, it is estimated that the GAMMAGARD® LIQUID manufacturing process provide a final yield of between about 60% and 65% of the IgG content found in the starting material. As such, the methods provided herein provide a significant improvement over the existing IgG purification technologies.

[0052] In one embodiment, the present invention provides a purified IgG composition that contains at least 70% of the IgG content found in the raw plasma starting material. In another embodiment, a purified IgG composition is provided that contains at least 75% of the IgG content found in the raw plasma starting material. In other embodiments, a purified IgG composition provided herein will contain at least about 65% of the IgG content found in the raw plasma starting material, or at least 66%, 67%, 68%, 69%, 70%, 71%, 72%73%, 74%, 75%, or more of the IgG content found in the raw plasma starting material.

A. Modified Alcohol Precipitation/Ion Exchange Chromatography Fractionation Methods

[0053] In one aspect, the present invention provides improved methods for the manufacture of IgG compositions suitable for use in IVIG therapy. Generally, these methods provide IgG

[0123] In one embodiment, a process improvement is realized by adding the S/D reagents (*e.g.*, Triton X-100, Tween-20, and TNBP) by spraying rather than by fluent addition. In other embodiments, the detergent reagents may be added as solids to the clarified PptG filtrate, which is being mixed to ensure rapid distribution of the S/D components. In certain embodiments, it is preferable to add solid reagents by sprinkling the solids over a delocalized surface area of the filtrate such that local overconcentration does not occur, such as in fluent addition.

10. Ion Exchange Chromatography

[0124] In order to further purify and concentrate IgG from the S/D treated PptG filtrate, cation exchange and/or anion exchange chromatography can be employed. Methods for purifying and concentrating IgG using ion exchange chromatography are well known in the art. For example, U.S. Patent No. 5,886,154 describes a method in which a Fraction II+III precipitate is extracted at low pH (between about 3.8 and 4.5), followed by precipitation of IgG using caprylic acid, and finally implementation of two anion exchange chromatography steps. U.S. Patent No. 6,069,236 describes a chromatographic IgG purification scheme that does not rely on alcohol precipitation at all. PCT Publication No. WO 2005/073252 describes an IgG purification method involving the extraction of a Fraction II+III precipitate, caprylic acid treatment, PEG treatment, and a single anion exchange chromatography step. U.S. Patent No. 7,186,410 describes an IgG purification method involving the extraction of either a Fraction I+II+III or a Fraction II precipitate followed by a single anion exchange step performed at an alkaline pH. U.S. Patent No. 7,553,938 describes a method involving the extraction of either a Fraction I+II+III or a Fraction II+III precipitate, caprylate treatment, and either one or two anion exchange chromatography steps. U.S. Patent No. 6,093,324 describes a purification method comprising the use of a macroporous anion exchange resin operated at a pH between about 6.0 and about 6.6. U.S. Patent No. 6,835,379 describes a purification method that relies on cation exchange chromatography in the absence of alcohol fractionation. ~~The disclosures of the above publications are hereby incorporated by reference in their entireties for all purposes~~.

[0125] In one embodiment of the methods of the present invention, 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 passed through a cation exchange column, which binds the IgG in the solution. The S/D reagents can then be washed away from the absorbed IgG, which is subsequently eluted off of the

preferably less than about 0.5 mg/L fibrinogen, most preferably less than about 0.25 mg/L fibrinogen.

[0158] In yet another embodiment, IgG compositions are provided that consist of primarily IgG monomers/dimmers. In one embodiment, an IgG composition is provided in which at least 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% of the IgG is monomeric or dimeric. In a preferred embodiment, an IgG composition is provided in which at least 97% of the IgG is monomeric or dimeric. In a more preferred embodiment, at least 99% of the IgG is monomeric or dimeric. In a more preferred embodiment, at least 99.5% of the IgG is monomeric or dimeric. In a more preferred embodiment, at least 99.7% of the IgG is monomeric or dimeric.

2. Pharmaceutical Compositions

[0159] In another aspect, the present invention provides pharmaceutical compositions and formulations comprising purified IgG prepared by the methods provided herein. Generally, the IgG pharmaceutical compositions and formulations prepared by the novel methods described herein will have high IgG content and purity. For example, IgG pharmaceutical compositions and formulations provided herein may have a protein concentration of at least about 7% (w/v) and an IgG content of greater than about 95% purity. These high purity IgG pharmaceutical compositions and formulations are suitable for therapeutic administration, *e.g.*, for IVIG therapy. In a preferred embodiment, a pharmaceutical IgG composition is formulated for intravenous administration (*e.g.*, IVIG therapy).

[0160] In one embodiment, the pharmaceutical compositions provided herein are prepared by formulating an aqueous IgG 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 Fibrinolysis* 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).

[0161] In certain embodiments, pharmaceutical formulations are provided having an IgG content of between about 80 g/L IgG and about 120 g/L IgG. Generally, these IVIG formulations are prepared by isolating an IgG composition from plasma using a method described herein, concentrating the composition, and formulating the concentrated composition in a solution suitable for intravenous administration. The IgG compositions may be concentrated using any suitable method known to one of skill in the art. In one embodiment, the composition is concentrated by ultrafiltration/diafiltration. In some embodiments, the ultrafiltration device used to concentrate the composition will employ an ultrafiltration membrane having a nominal molecular weight cut off (NMWCO) of less than about 100 kDa or less than about 90, 80, 70, 60, 50, 40, 30, or fewer kDa. In a preferred embodiment, the ultrafiltration membrane has a NMWCO of no more than 50 kDa. Buffer exchange may be achieved using any suitable technique known to one of skill in the art. In a specific embodiment, buffer exchange is achieved by diafiltration.

[0162] In one specific embodiment, a pharmaceutical composition of IgG is provided, wherein the IgG composition was purified from plasma using a method comprising the steps of (a) precipitating a cryo-poor plasma fraction, in a first precipitation step, with between about 6% and about 10% alcohol at a pH of between about 6.7 and about 7.3 to obtain a supernatant enriched in IgG, (b) precipitating IgG from the supernatant with between about 20% and about 30% alcohol at a pH of between about 6.7 and about 7.3 to form a first precipitate, (c) re-suspending the first precipitate formed in step (b) to form a suspension, (d) treating the suspension formed in step (c) with a detergent, (e) precipitating IgG from the suspension with between about 20% and about 30% alcohol at a pH of between about 6.7 and about 7.3 to form a second precipitate, (f) re-suspending the second precipitate formed in step (e) to form a suspension, (g) treating the suspension formed in step (f) with a solvent and/or detergent, (h) performing at least one ion exchange chromatography fractionation; (i) performing a solvent detergent treatment; and (j) subjecting the composition to nanofiltration, thereby preparing a composition of IgG.

[0163] In a specific embodiment, a pharmaceutical composition of IgG is provided, wherein the IgG composition was purified from plasma using a method comprising the steps of (a) adjusting the pH of a cryo-poor plasma fraction to about 7.0, (b) adjusting the ethanol concentration of the cryo-poor plasma fraction of step (a) to about 25% (v/v) at a temperature between about -5°C and about -9°C, thereby forming a mixture, (c) separating liquid and

[0178] The FDA has approved the use of IVIG to treat various indications, including allogeneic bone marrow transplant, chronic lymphocytic leukemia, idiopathic thrombocytopenic purpura (ITP), pediatric HIV, primary immunodeficiencies, Kawasaki disease, chronic inflammatory demyelinating polyneuropathy (CIDP), and kidney transplant with a high antibody recipient or with an ABO incompatible donor. In certain embodiments, the IVIG compositions provided herein are useful for the treatment or management of these diseases and conditions.

[0179] Furthermore, off-label uses for IVIG are commonly provided to patients for the treatment or management of various indications, for example, chronic fatigue syndrome, clostridium difficile colitis, dermatomyositis and polymyositis, Graves' ophthalmopathy, Guillain-Barré syndrome, muscular dystrophy, inclusion body myositis, Lambert-Eaton syndrome, Lupus erythematosus, multifocal motor neuropathy, multiple sclerosis (MS), myasthenia gravis, neonatal alloimmune thrombocytopenia, Parvovirus B19 infection, pemphigus, post-transfusion purpura, renal transplant rejection, spontaneous Abortion/Miscarriage, stiff person syndrome, opsoclonus Myoclonus, severe sepsis and septic shock in critically ill adults, toxic epidermal necrolysis, chronic lymphocytic leukemia, multiple myeloma, X-linked agammaglobulinemia, and hypogammaglobulinemia. In certain embodiments, the IVIG compositions provided herein are useful for the treatment or management of these diseases and conditions.

[0180] Finally, experimental use of IVIG for the treatment or management of diseases including primary immune deficiency, RRMS, Alzheimer's disease, and Parkinson's disease has been proposed (U.S. Patent Application Publication No. U.S. 2009/0148463~~, which is herein incorporated by reference in its entirety for all purposes~~). In certain embodiments, the IVIG compositions provided herein are useful for the treatment or management of primary immune deficiency, RRMS, Alzheimer's disease, or Parkinson's disease. In certain embodiments comprising daily administration, an effective amount to be administered to the subject can be determined by a physician with consideration of individual differences in age, weight, disease severity, route of administration (*e.g.*, intravenous v. subcutaneous) and response to the therapy. In certain embodiments, an immunoglobulin preparation of this invention can be administered to a subject at about 5 mg/kilogram to about 2000 mg/kilogram each day. In additional embodiments, the immunoglobulin preparation can be administered in amounts of at least about 10 mg/kilogram, at last 15 mg/kilogram, at least 20 mg/kilogram, at least 25 mg/kilogram, at least 30 mg/kilogram, or at least 50 mg/kilogram. In additional embodiments, the immunoglobulin preparation can be administered to a subject at doses up to

Amidolytic activity, C3, and fibrinogen, as well as to determine the percentage of IgG monomers/dimers in the final composition. As can be seen in Table 19 below, the improve methods manufacture provided herein result in final bulk compositions with increased IgG recovery, 73.6% to 78.5% of the starting material as compared to 60% to 70% for currently employed methods, while maintaining purity profiles that are as good, if not better, than current IgG manufacting standards.

Table 19. Levels of impurities and IgG monomer/dimmer composition in IgG composition prepared according to the improved manufacturing methods provided herein.

Option	**1**	**3**	**6**
Experiment # (P0..10NG2)	19	20	21
Protein yield in ANX flow-through [g/L plasma]	**4.35**	**4.35**	**4.38**
IgG recovery: Cohn starting material to bulk [%]	73.6	78.5	78.5
Characterization of final bulk			
IgA [μg/mL @ 10% protein]	29	26	pend.
IgM [μg/mL @ 10% protein]	1.1	1.1	pend.
Amidolytic activity [PL-1 nmol/mL min]	pend.	<10	<10
MSD monomers/dimers [%]	99.7	99.9	99.7
C3 [μg/mL @ 10% protein]	pend.	pend.	pend.
Fibrinogen [μg/mL @ 10% protein]	<0.2	pend.	pend.

[0001] 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.~~

CLAIMS

1. A method for preparing an enriched IgG composition from plasma, the method comprising the steps of:

(a) precipitating a cryo-poor plasma fraction, in a first precipitation step, with between 6% and 10% alcohol at a pH of between 7.0 and 7.5 to obtain a first precipitate and a first supernatant;

(b) precipitating IgG from the first supernatant, in a second precipitation step, with between 20% and 25% alcohol at a pH of between 6.7 and 7.3 to form a second precipitate;

(c) re-suspending the second precipitate to form a suspension;

(d) precipitating IgG from the suspension formed in step (c), in a third precipitation step, with between 22% and 28% alcohol at a pH of between 6.7 and 7.3 to form a third precipitate;

(e) re-suspending the third precipitate to form a suspension; and

(f) separating the soluble fraction from the suspension formed in step (e), thereby forming an enriched IgG composition,

wherein at least one of the first precipitation step, second precipitation step, or third precipitation step comprises spray addition of the alcohol.

2. The method of claim 1, wherein all of the first precipitation step, second precipitation step, and third precipitation step comprise spray addition of the alcohol.

3. The method of claim 1 or 2, wherein the pH of at least one of the first precipitation step, second precipitation step, or third precipitation step is achieved by addition of a pH modifying solution after addition of the alcohol.

4. The method of any one of claims 1 to 3, wherein the pH of all of the first precipitation step, second precipitation step, and third precipitation step is achieved by addition of a pH modifying solution after addition of the alcohol.

5. The method of claim 3 or 4, wherein the addition of a pH modifying solution comprises spray addition of the pH modifying solution.

6. The method of any one of claims 3 to 5, wherein the pH of the precipitation step is modified before and after the addition of alcohol, during and after the addition of alcohol, or before, during, and after the addition of alcohol.

7. The method of any one of claims 1 to 6, wherein the pH of at least one precipitation step is maintained for the entire precipitation step by continuous adjustment of the pH.

8. The method of any one of claims 1 to 7, wherein the method further comprises an ion exchange chromatography purification step.

9. The method of claim 8, wherein the method comprises both an anion exchange chromatography purification step and a cation exchange chromatography step.

10. The method of any one of claims 1 to 9, wherein the method further comprises a nanofiltration step and/or an ultrafiltration/diafiltration step.

11. The method of any one of claims 1 to 10, wherein the enriched IgG composition obtained in step (f) contains at least 85% of the IgG content found in the cryo-poor plasma fraction used in step (a).

12. The method of claim 11, wherein the enriched IgG composition obtained in step (f) contains at least 90% of the IgG content found in the cryo-poor plasma fraction used in step (a).

13. The method of any one of claims 1 to 12, wherein the purity of γ-globulins in the final IgG composition is at least 95%.

14. The method of claim 13, wherein the purity of γ-globulins in the final IgG composition is at least 98%.

15. The method of claim 13, wherein the purity of γ-globulins in the final IgG composition is at least 99%.

16. The method of claim 1, wherein the method comprises the steps of:

(1) precipitating a cryo-poor plasma fraction, in a first precipitation step, with between 6% and 10% alcohol at a pH of between 7.0 and 7.5 to obtain a first precipitate and a first supernatant, by:

(a) cooling a mixture of pooled plasma donations to a temperature between 2°C and 10°C;

(b) separating liquid and precipitate from the mixture of step (a) by centrifugation;

(c) admixing ethanol into the liquid supernatant formed in step (b) to a final concentration of between 5% (v/v) to 10% (v/v) ethanol, thereby forming a mixture;

(d) cooling the mixture formed in step (c) to between -4°C and 0°C; and

(e) separating liquid and precipitate from the mixture of step (d) by centrifugation; and isolating the supernatant, thereby forming a first plasma fraction;

(2) precipitating IgG from the first plasma fraction, in a second precipitation step, with between 20% and 25% alcohol at a pH of between 6.7 and 7.3 to form a second precipitate by:

(f) adjusting the pH of the first plasma fraction to 7.0;

(g) adjusting the ethanol concentration of the first plasma fraction of step (f) to 25% (v/v) at a temperature between -7°C and -9°C, thereby forming a mixture;

(h) separating liquid and precipitate from the mixture of step (g);

(3) re-suspending the second precipitate to form a suspension by:

(i) re-suspending the precipitate of step (h) with a buffer containing phosphate and acetate, wherein the pH of the buffer is adjusted with between 300 mL and 700 mL of glacial acetic acid per 1000L of buffer, thereby forming a suspension;

(j) mixing finely divided silicon dioxide (SiO_2) with the suspension from step (i) for at least 30 minutes;

(k) filtering the suspension with a filter press, thereby forming a filtrate;

(l) washing the filter press with at least 3 filter press dead volumes of a buffer containing phosphate and acetate, wherein the pH of the buffer is adjusted with between 50 mL and 200 mL of glacial acetic acid per 1000L of buffer, thereby forming a wash solution;

(m) combining the filtrate of step (k) with the wash solution of step (g), thereby forming a solution, and treating the solution with a detergent;

(4) precipitating IgG from the solution formed in step (m), in a third precipitation step, with between 22% and 28% alcohol at a pH of between 6.7 and 7.3 to form a third precipitate by:

(n) adjusting the pH of the solution of step (m) to 7.0 and adding ethanol to a final concentration of 25%, thereby forming a precipitate; and

(o) separating liquid and precipitate from the mixture of step (n);

(5) re-suspending the third precipitate to form a suspension by:

(p) dissolving the precipitate in an aqueous solution comprising a solvent or detergent and maintaining the solution for at least 60 minutes;

(6) separating the soluble fraction from the suspension formed in step (p), thereby forming an enriched IgG composition,

(7) passing the solution after step (p) through a cation exchange chromatography column and eluting proteins absorbed on the column in an eluate;

(8) passing the eluate from step (7) through an anion exchange chromatography column to generate an effluent;

(9) passing the effluent from step (8) through a nanofilter to generate a nanofiltrate;

(10) passing the nanofiltrate from step (9) through an ultrafiltration membrane to generate an ultrafiltrate; and

(11) diafiltrating the ultrafiltrate from step (10) against a diafiltration buffer to generate a diafiltrate having a protein concentration between 8% (w/v) and 12% (w/v), thereby obtaining a composition of concentrated IgG,

wherein at least one of the first precipitation step, second precipitation step, or third precipitation step comprises spray addition of the alcohol.

17. The method of claim 16, wherein the ethanol concentration is adjusted in at least one of steps (g) and (n) by introducing ethanol into the fraction by spraying.

18. The method of claim 16 or 17, wherein the pH is adjusted to the appropriate pH after the addition of ethanol in at least one of steps (g) and (n).

19. The method of claim 18, wherein the pH is maintained for the entire precipitation reaction by continuous adjustment of the pH in at least one of steps (g) and (n).

20. The method of any one of claims 16 to 19, wherein the pH is adjusted in at least one step by spraying a solution capable of adjusting the pH.

21. The method of claim 20, wherein pH is adjusted by spraying glacial acetic acid.

22. The method of any one of claims 16 to 21, wherein the method further comprises filtering the diafiltrate through a filter of 0.22 μm or less.

23. The method of any one of claims 16 to 22, wherein the plasma is human plasma.

24. The method of any one of claims 16 to 23, wherein the temperature of the mixture from step (g) is -7°C.

25. The method of any one of claims 16 to 24, wherein the precipitate formed in step (h) is re-suspended with between 12 L and 18 L of buffer per kg precipitate..

26. The method of claim 25, wherein the precipitate is re-suspended with 15 L of buffer per kg precipitate.

27. The method of any one of claims 16 to 26, wherein the amount of silicon dioxide added to the suspension in step (j) is between 0.02 grams per gram precipitate formed in step (h) and 0.06 grams per gram precipitate formed in step (h).

28. The method of claim 27, wherein the amount of silicon dioxide added to the suspension in step (j) is 0.04 grams per gram precipitate formed in step (h).

29. The method of any one of claims 16 to 28, wherein a filter aid is added to the mixture prior to filtration in step (k).

30. The method of claim 29, wherein the filter aid is diatomaceous earth.

31. The method of any one of claims 16 to 30, wherein the purity of the γ-globulins in the filtrate formed in step (k) is at least 85%.

32. The method of any one of claims 16 to 31, wherein the filtrate formed in step (k) contains less than 10 mg of fibrinogen per gram total protein.

33. The method of any one of claims 16 to 32, wherein the filtrate formed in step (k) contains less than 500 IU of PKA activity per gram total protein.

34. The method of any one of claims 16 to 33, wherein the detergent used in step (m) comprises between 0.1% (w/v) and 0.3% (w/v) polysorbate-80.

35. The method of any one of claims 16 to 34, wherein the aqueous solution used in step (p) comprises Triton-X 100, polysorbate-80, and TNBP.

36. The method of any one of claims 16 to 35, wherein the solution in step (p) is maintained at a temperature between 18°C and 25°C.

37. The method of any one of claims 16 to 36, wherein the nanofilter of step (9) has a mean pore size of between 15 nm to 72 nm.

38. The method of claim 37, wherein the nanofilter has a mean pore size of between 19 nm and 35 nm.

39. The method of claim 38, wherein the nanofilter has a mean pore size of 35 nm.

40. The method of any one of claims 16 to 39, wherein the ultrafiltration membrane of step (10) has a nominal molecular weight cut off (NMWCO) of 100 kDa or less.

41. The method of any one of claims 16 to 40, wherein the protein concentration of the composition is 10% (w/v).

42. The method of any one of claims 16 to 41, wherein the solution formed in step (m) contains at least 85% of the IgG present in the cryo-poor plasma fraction of step (f).

43. The method of claim 42, wherein the solution formed in step (m) contains at least 90% of the IgG present in the cryo-poor plasma fraction of step (f).

WHAT IS CLAIMED IS:

1. A method for preparing an enriched IgG composition from plasma, the method comprising the steps of:

(a) precipitating a cryo-poor plasma fraction, in a first precipitation step, with between about 6% and about 10% alcohol at a pH of between about 7.0 and about 7.5 to obtain a first precipitate and a first supernatant;

(b) precipitating IgG from the first supernatant, in a second precipitation step, with between about 20% and about 25% alcohol at a pH of between about 6.7 and about 7.3 to form a second precipitate;

(c) re-suspending the second precipitate to form a suspension;

(d) precipitating IgG from the suspension formed in step (c), in a third precipitation step, with between about 22% and about 28% alcohol at a pH of between about 6.7 and about 7.3 to form a third precipitate;

(e) re-suspending the third precipitate to form a suspension; and

(f) separating the soluble fraction from the suspension formed in step (e), thereby forming an enriched IgG composition,

wherein at least one of the first precipitation step, second precipitation step, or third precipitation step comprises spray addition of the alcohol.

2. The method of claim 1, wherein all of the first precipitation step, second precipitation step, and third precipitation step comprise spray addition of the alcohol.

3. The method of claim 1 or 2, wherein the pH of at least one of the first precipitation step, second precipitation step, or third precipitation step is achieved by addition of a pH modifying solution after addition of the alcohol.

4. The method of any one of claims 1 to 3, wherein the pH of all of the first precipitation step, second precipitation step, and third precipitation step is achieved by addition of a pH modifying solution after addition of the alcohol.

5. The method of claim 3 or 4, wherein the addition of a pH modifying solution comprises spray addition of the pH modifying solution.

6. The method of any one of claims 3 to 5, wherein the pH of the precipitation step is modified before and after the addition of alcohol, during and after the addition of alcohol, or before, during, and after the addition of alcohol.

7. The method of any one of claims 1 to 6, wherein the pH of at least one precipitation step is maintained for the entire precipitation step by continuous adjustment of the pH.

8. The method of any one of claims 1 to 7, wherein the method further comprises an ion exchange chromatography purification step.

9. The method of claim 8, wherein the method comprises both an anion exchange chromatography purification step and a cation exchange chromatography step.

10. The method of any one of claims 1 to 9, wherein the method further comprises a nanofiltration step and/or an ultrafiltration/diafiltration step.

11. The method of any one of claims 1 to 10, wherein the enriched IgG composition obtained in step (f) contains at least 85% of the IgG content found in the cryo-poor plasma fraction used in step (a).

12. The method of claim 11, wherein the enriched IgG composition obtained in step (f) contains at least 90% of the IgG content found in the cryo-poor plasma fraction used in step (a).

13. The method of any one of claims 1 to 12, wherein the purity of γ-globulins in the final IgG composition is at least about 95%.

14. The method of claim 13, wherein the purity of γ-globulins in the final IgG composition is at least about 98%.

15. The method of claim 13, wherein the purity of γ-globulins in the final IgG composition is at least about 99%.

16. The method of claim 1, wherein the method comprises the steps of:

(a) cooling a mixture of pooled plasma donations to a temperature between about 2°C and about 10°C;

(b) separating liquid and precipitate from the mixture of step (a) by centrifugation;

(c) admixing ethanol into the liquid supernatant formed in step (b) to a final concentration of between about 5% (v/v) to about 10% (v/v) ethanol, thereby forming a mixture;

(d) cooling the mixture formed in step (c) to between about -4°C and about 0°C;

(e) separating liquid and precipitate from the mixture of step (d) by centrifugation; and isolating the supernatant, thereby forming a cryo-poor plasma fraction.

(f) adjusting the pH of the first plasma fraction to about 7.0;

(g) adjusting the ethanol concentration of the first plasma fraction of step (f) to about 25% (v/v) at a temperature between about -7°C and about -9°C, thereby forming a mixture;

(h) separating liquid and precipitate from the mixture of step (g);

(i) re-suspending the precipitate of step (h) with a buffer containing phosphate and acetate, wherein the pH of the buffer is adjusted with between 300 mL and 700 mL of glacial acetic acid per 1000L of buffer, thereby forming a suspension;

(j) mixing finely divided silicon dioxide (SiO_2) with the suspension from step (i) for at least about 30 minutes;

(k) filtering the suspension with a filter press, thereby forming a filtrate;

(l) washing the filter press with at least 3 filter press dead volumes of a buffer containing phosphate and acetate, wherein the pH of the buffer is adjusted with between 50 mL and 200 mL of glacial acetic acid per 1000L of buffer, thereby forming a wash solution;

(m) combining the filtrate of step (k) with the wash solution of step (l), thereby forming a solution, and treating the solution with a detergent;

(n) adjusting the pH of the solution of step (m) to about 7.0 and adding ethanol to a final concentration of about 25%, thereby forming a precipitate;

(o) separating liquid and precipitate from the mixture of step (n);

(p) dissolving the precipitate in an aqueous solution comprising a solvent or detergent and maintaining the solution for at least 60 minutes;

(q) passing the solution after step (p) through a cation exchange chromatography column and eluting proteins absorbed on the column in an eluate;

(r) passing the eluate from step (q) through an anion exchange chromatography column to generate an effluent;

(s) passing the effluent from step (r) through a nanofilter to generate a nanofiltrate;

(t) passing the nanofiltrate from step (s) through an ultrafiltration membrane to generate an ultrafiltrate; and

(u) diafiltrating the ultrafiltrate from step (t) against a diafiltration buffer to generate a diafiltrate having a protein concentration between about 8% (w/v) and about 12% (w/v), thereby obtaining a composition of concentrated IgG.

17. The method of claim 16, wherein the ethanol concentration is adjusted in at least one of steps (g) and (n) by introducing ethanol into the fraction by spraying.

18. The method of claim 16 or 17, wherein the pH is adjusted to the appropriate pH after the addition of ethanol in at least one of steps (g) and (n).

19. The method of claim 18, wherein the pH is maintained for the entire precipitation reaction by continuous adjustment of the pH in at least one of steps (g) and (n).

20. The method of any one of claims 16 to 19, wherein the pH is adjusted in at least one step by spraying a solution capable of adjusting the pH.

21. The method of claim 20, wherein pH is adjusted by spraying glacial acetic acid.

22. The method of any one of claims 16 to 21, wherein the method further comprises filtering the diafiltrate through a filter of 0.22 μm or less.

23. The method of any one of claims 16 to22, wherein the plasma is human plasma.

24. The method of any one of claims 16 to 23, wherein the temperature of the mixture from step (g) is about -7°C.

25. The method of any one of claims 16 to 24, wherein the precipitate formed in step (h) is re-suspended with between about 12 L and about 18 L of buffer per kg precipitate..

26. The method of claim 25, wherein the precipitate is re-suspended with about 15 L of buffer per kg precipitate.

27. The method of any one of claims 16 to 26, wherein the amount of silicon dioxide added to the suspension in step (j) is between about 0.02 grams per gram precipitate formed in step (h) and about 0.06 grams per gram precipitate formed in step (h).

28. The method of claim 27, wherein the amount of silicon dioxide added to the suspension in step (j) is about 0.04 grams per gram precipitate formed in step (h).

29. The method of any one of claims 16 to 28, wherein a filter aid is added to the mixture prior to filtration in step (k).

30. The method of claim 29, wherein the filter aid is diatomaceous earth.

31. The method of any one of claims 16 to 30, wherein the purity of the γ-globulins in the filtrate formed in step (k) is at least about 85%.

32. The method of any one of claims 16 to 31, wherein the filtrate formed in step (k) contains less than about 10 mg of fibrinogen per gram total protein.

33. The method of any one of claims 16 to 32, wherein the filtrate formed in step (k) contains less than about 500 IU of PKA activity per gram total protein.

34. The method of any one of claims 16 to 33, wherein the detergent used in step (m) comprises between about 0.1% (w/v) and about 0.3% (w/v) polysorbate-80.

35. The method of any one of claims 16 to 34, wherein the aqueous solution used in step (p) comprises Triton-X 100, polysorbate-80, and TNBP.

36. The method of any one of claims 16 to 35, wherein the solution in step (p) is maintained at a temperature between about 18°C and about 25°C.

37. The method of any one of claims 16 to 36, wherein the nanofilter of step (s) has a mean pore size of between about 15 nm to about 72 nm.

38. The method of claim 37, wherein the nanofilter has a mean pore size of between about 19 nm and about 35 nm.

39. The method of claim 38, wherein the nanofilter has a mean pore size of about 35 nm.

40. The method of any one of claims 16 to 39, wherein the ultrafiltration membrane of step (t) has a nominal molecular weight cut off (NMWCO) of about 100 kDa or less.

41. The method of any one of claims 16 to 40, wherein the protein concentration of the composition is about 10% (w/v).

42. The method of any one of claims 16 to 41, wherein the solution formed in step (m) contains at least about 85% of the IgG present in the cryo-poor plasma fraction of step (f).

43. The method of claim 42, wherein the solution formed in step (m) contains at least about 90% of the IgG present in the cryo-poor plasma fraction of step (f).

44. An aqueous IgG composition prepared by a method of any one of the preceding claims.

45. The aqueous IgG composition of claim 44, wherein the composition comprises at least about 80 grams of protein per liter of the composition.

46. The aqueous IgG composition of claim 44 or 45, wherein at least 95% of the protein is IgG.

47. The aqueous IgG composition of claim 46, wherein at least 98% of the protein is IgG.

48. The aqueous IgG composition of any one of claims 44 to 47, wherein the composition contains less than about 35 μg/mL IgA.

49. A pharmaceutical composition comprising an aqueous IgG composition prepared according to a method of any one of claims 1 to 43.

50. The pharmaceutical composition of claim 49, wherein the composition comprises about 100 grams of protein per liter of the composition.

51. The pharmaceutical composition of claim 49 or 50, wherein the composition further comprises glycine at a concentration from between about 200 mM to about 300 mM.

52. The pharmaceutical composition of any one of claims 49 to 51, wherein the pH of the composition is between about 4.6 and about 5.1.

53. The pharmaceutical composition of any one of claims 49 to 52, wherein the osmolarity of the composition is between about 240 mOsmol/kg and about 300 mOsmol/kg.

54. The pharmaceutical composition of any one of claims 49 to 53, wherein the composition is stable at room temperature for at least about 9 months.

55. The pharmaceutical composition of any one of claims 49 to 54, wherein the composition is stable at a temperature between about 2°C and about 8°C for at least about 36 months.

56. The pharmaceutical composition of any one of claims 49 to 55, wherein the composition is formulated for intravenous administration.

57. Use of a pharmaceutical composition of any one of claims 49 to 56 in the manufacture of a medicament for treating an immunodeficiency, autoimmune disease, or acute infection in a human in need thereof.