manufacturing methods that produce yields that are at least 6 to 10% higher than currently achievable, as well as IVIG compositions provided there from.

BRIEF SUMMARY

[0012a] One aspect of the present invention relates to 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 lower temperature and 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, and (h) performing at least one ion exchange chromatography fractionation thereby preparing a composition of concentrated IgG, wherein the method further comprises treating the suspension formed in step (c) with finely divided silica dioxide (SiO_2) and filtering the solution prior to step (d).

[0013] ~~In one aspect, t~~The present disclosure also provides methods for preparing an enriched IgG compositions (*e.g.*, IVIG compositions) from plasma. Advantageously, the methods provided herein provide significant improvements over current state of the art manufacturing methods for preparing IVIG compositions. For example, the methods provided herein allow for increased yields of IgG in the final bulk composition without losing the purity required for intravenous administration.

[0014] ~~In one aspect, a~~A method is provided for preparing an enriched IgG composition from plasma 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. In one embodiment, alcohol is added in the first precipitation step by spraying. In another embodiment, alcohol is added in the second precipitation step by spraying. In yet another embodiment, alcohol is added in the third precipitation step by spraying.

[0015] In certain embodiments, the pH of one or more solution may be adjusted by the addition of a pH modifying agent by spraying. In related embodiments, 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, or before and after the addition of alcohol, during and after the addition of alcohol, or before, during, and after the addition of alcohol. In yet another related embodiment, the pH of a precipitation step may be maintained for the entirety of the precipitation reaction by continuously adjusting the pH.

CLAIMS

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

(a) precipitating a cryo-poor plasmid 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 □-globulins in the final IgG composition is at least about 95%.

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

15. The method of claim 13, wherein the purity of □-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) 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 -7°C and about -9°C, thereby forming a mixture;

(c) separating liquid and precipitate from the mixture of step (b);

(d) re-suspending the precipitate of step (c) 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;

(e) mixing finely divided silicon dioxide (SiO2) with the suspension from step (d) for at least about 30 minutes;

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

(g) 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;

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

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

(j) separating liquid and precipitate from the mixture of step (i);

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

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

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

(n) passing the effluent from step (m) through a nanofilter to generate a nanofiltrate;

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

(p) diafiltrating the ultrafiltrate from step (o) 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 (b) and (i) 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 (b) and (i).

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 (b) and (i).

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 □m 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 cryo-poor plasma fraction is formed by a method comprising the steps of:

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

(ii) separating liquid and precipitate from the mixture of step (i) by centrifugation;

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

49. The aqueous IgG composition of any one of claims 45 to 48, wherein the composition contains less than about 35 □g/mL IgA.

50. A pharmaceutical composition comprising an aqueous IgG composition prepared according to a method of any one of claims 1 to 44.

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

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

53. The pharmaceutical composition of any one of claims 50 to 52, wherein the pH of the composition is between about 4.6 and about 5.1.

54. The pharmaceutical composition of any one of claims 50 to 53, wherein the osmolarity of the composition is between about 240 mOsmol/kg and about 300 mOsmol/kg.

55. The pharmaceutical composition of any one of claims 50 to 54, wherein the composition is stable at room temperature for at least about 9 months.

56. The pharmaceutical composition of any one of claims 50 to55, wherein the composition is stable at a temperature between about 2°C and about 8°C for at least about 36 months.

57. The pharmaceutical composition of any one of claims 50 to 56, wherein the composition is formulated for intravenous administration.

58. A method of treating an immunodeficiency, autoimmune disease, or acute infection in a human in need thereof, the method comprising administering a pharmaceutical composition of any one of claims 50 to 57.

CLAIMS

1. 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 lower temperature and 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, and

(h) performing at least one ion exchange chromatography fractionation thereby preparing a composition of concentrated IgG,

wherein the method further comprises treating the suspension formed in step (c) with finely divided silica dioxide (SiO_2) and filtering the solution prior to step (d).

2. The method of claim 1, wherein in step (b), prior to or concomitant with alcohol addition, the solution is cooled to:

(i) between about -7°C and about -9°C, or to

(ii) a temperature at or about -7°C.

3. The method of claim 1 or 2, wherein the alcohol concentration of step (b) is

(i) between about 23% and about 27%, or

(ii) between about 24% and about 26%, or

(iii) at or about 25%.

4. The method of any one of claims 1 to 3, wherein in step (b), after completion of the alcohol addition, the pH of the solution is immediately adjusted to

(i) between about 6.8 and about 7.0, or to

(ii) at or about 6.9.

5. The method of any one of claims 1 to 4, wherein the pH of the solution is maintained

(i) at between about 6.8 to about 7.0, or

(ii) at about 6.9

continuously during the precipitation incubation period of step (b).

6. The method of any one of claims 1 to 5, wherein a cold extraction buffer is used in step (c) to re-suspend the precipitate at a ratio of

(i) 1 part precipitate to 15 parts of extraction buffer, or at ratios

(ii) from about 1:8 to about 1:30, or

(iii) from about 1:10 to about 1:20, or

(iv) from about 1:12 to about 1:18, or

(v) from about 1:13 to about 1:17, or

(vi) from about 1:14 to about 1:16.

7. The method of claim 6, wherein the extraction buffer has a pH between about 4.0 and about 5.5.

8. The method of claim 7, wherein the extraction buffer contains 5 mM monobasic sodium phosphate, 5 mM acetate, and 0.051% to 0.06 % glacial acetic acid (v/v).

9. The method of claim 7 or 8, wherein the extraction buffer contains 5 mM monobasic sodium phosphate and 5 mM acetate at a pH of at or about 4.5 ± 0.2 and conductivity of at or about 0.7 to 0.9 mS/cm.

10. The method of any one of claims 1 to 9, wherein the finely divided silica dioxide (SiO_2) is fumed silica.

11. The method of claim 10, wherein the fumed silica treatment includes addition of from about 0.1 kg/kg II+III paste to about 0.07 kg/kg II+III paste, or from about 0.2 kg/kg II+III paste to about 0.06 kg/kg II+III paste, or from about 0.3 kg/kg II+III paste to about 0.05 kg/kg II+III paste, or about 0.2, 0.3, 0.4, 0.5, 0.6, or 0.7 kg/kg II+III paste, and the mixture is incubated for between about 50 minutes and about 70 minutes, or about 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, or more minutes at a temperature between about 2°C and about 8°C.

12. The method of claim 10, wherein the fumed silica treatment comprises a 40 to 80 minute incubation period during which the suspension is constantly mixed.

13. The method of claim 10 or 12, wherein the fumed silica treatment is performed at between about 0°C and about 10°C, or between about 2°C and about 8°C.

14. The method of any one of claims 1 to 13, wherein the filtering of the solution prior to step (d) uses depth filtration.

15. The method of claim 14, further comprising washing the depth filter with between about 3.5 volumes and about 4.5 volumes, or about 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0 volumes of the filter dead volume after completing the filtration step.

16. The method of any one of claims 1 to 15, wherein one or more of the precipitation steps (a), (b) and (e) are performed by the spray addition of alcohol.

17. The method of any one of claims 1 to 16, wherein the pH of a solution is continuously adjusted during one or more of the alcohol precipitation steps (a), (b) and (e).

18. The method of any one of claims 1 to 17, wherein the pH of one or more solution is adjusted by addition of a pH modifying agent by spraying.