plasma supernatant with heparin, thereby reducing the amidolytic and pro-coagulant activities in the IgG.

[0009] Due to rising concerns over the limited supply of starting material for the IgG preparation and loss of a significant amount of IgG during the purification process, there exist an immediate need in the art to provide a method to increase the availability of a significant amount of alternative starting material for the manufacturing of IgG.

SUMMARY

[0010] The present disclosure solves these and other problems. In one example, the present disclosure is based on the finding that C1-INH depleted plasma supernatant can be used as a starting material for the preparation of Immunoglobulin G (IgG) enriched fraction, thus, making availability of another starting material for the preparation of IgG. Recent concerns over the amidolytic content of these compositions paired with the occurrence of thromboembolic events in patients being administered plasma-derived protein compositions, has highlighted a need in the art for a method for reducing serine proteases (*e.g.*, FXIa and FXIIa) and serine protease zymogens (*e.g.*, FXI and FXII) during the manufacturing of these biologics. Advantageously, the present disclosure is based, at least in part, on the surprising finding that heparin can be used to reduce the procoagulant and amidolytic activities to acceptable levels during the fractionation process. Also provided are therapeutic plasma-derived protein compositions having reduced serine protease activity, serine protease content, and/or serine protease zymogen content. Also provided are methods for treating or preventing disease by the administration of a composition of the disclosure.

[0010a] In one aspect, the present disclosure provides a method for preparing an Immunoglobulin G (IgG) enriched fraction from a C1-INH depleted supernatant fraction comprising IgG, the method comprising: (a) contacting the C1-INH depleted supernatant fraction with heparin, thereby forming a heparinized fraction; and (b) isolating IgG from the heparinized fraction, thereby forming an IgG enriched fraction.

[0011] In one example, the present disclosure provides a method for preparing an Immunoglobulin G (IgG) enriched fraction from a C1-INH depleted supernatant fraction comprising IgG. The method includes:

(a) contacting the C1-INH depleted supernatant fraction with heparin, thereby forming a heparinized C1-INH depleted fraction; and

(b) isolating IgG from the heparinized C1-INH depleted fraction, thereby forming an IgG enriched fraction.

[0012] In one example of the methods described herein, the supernatant fraction is a supernatant produced following C1-inhibitor adsorption.

[0013] In an exemplary example, the supernatant fraction is a plasma supernatant.

[0014] In one example, the plasma supernatant is a C1-INH depleted cryo-poor plasma.

[0015] In various examples, the plasma supernatant is derived from a double-depleted cryo-poor plasma (DDCPP).

[0016] In an exemplary example, the supernatant fraction is depleted of one or more other blood coagulation factor(s) selected from Factor II, VII, IX, X and a mixture thereof.

[0017] In one example, the supernatant fraction is concentrated to a protein value of normal plasma before further processing.

[0018] In an exemplary example, the heparin is added in an amount of from about 1 to about 20 Units per mL of supernatant fraction.

[0019] In an exemplary example, the heparin is added in an amount of from about 5 to about 10 Units per mL of supernatant fraction.

[0020] In one example, the heparin is added in an amount of about 5 Units per mL of supernatant fraction.

[0021] In various examples, the heparin is added in an amount of about 10 Units per mL of supernatant fraction.

[0022] In some examples, the method further comprises:

(c) removing C1-INH esterase inhibitor (C1-INH) from a cryo-poor plasma fraction containing C1-INH, thereby forming a C1-INH depleted supernatant fraction.

[0023] In one example, the IgG enriched fraction contains from about 60% to about 80% of the IgG content found in the supernatant fraction.

[0024] In one example, the IgG enriched fraction contains at least about 50% of the IgG content found in the supernatant fraction.

[0025] In one example of the methods described above, the purity of γ-globulins in the IgG enriched fraction is at least about 95%.

[0026] In one example of the methods described above, the purity of γ-globulins in the IgG enriched fraction is from about 95% to about 99.9%.

[0027] In an exemplary example, the present disclosure provides a method for isolating IgG from the heparinized fraction comprising one or more of the following steps in any order or combination:

(i) precipitating the heparinized fraction with from about 6% to about 10% ethanol, e.g., aqueous ethanol, at a pH of from about 7.0 to about 7.5 to obtain a Fraction I precipitate and a Fraction I supernatant; and

(ii) precipitating IgG from the Fraction I supernatant with from about 18% to about 27% ethanol, e.g., aqueous ethanol, at a pH of from about 6.7 to about 7.3 to form a Fraction II+III precipitate.

[0028] In one example of the methods described above, the method further comprises precipitating IgG from the heparinized fraction with from about 18% to about 27% ethanol, e.g., aqueous ethanol, at a pH of from about 6.7 to about 7.3 to form a Fraction I+II+III precipitate.

[0029] In one example, the method further comprises one or more of the following steps in any order or combination:

(iii) suspending the Fraction II+III or Fraction I+II+III precipitate in a suspension buffer, thereby forming an IgG suspension;

(iv) mixing finely divided silicon dioxide (SiO_2) with the IgG suspension, e.g., for at least about 30 minutes;

(v) filtering the IgG suspension, thereby forming a filtrate and a filter cake.

[0030] In one example, the method further comprises one or more of the following steps in any order or combination:

(vi) washing the filter cake with at least about 1 filter press dead volume of a wash buffer having a pH of from about 4.9 to about 5.3, thereby forming a wash solution;

(vii) combining the filtrate with the wash solution, thereby forming a combined solution, and treating the combined solution with a detergent;

(viii) adjusting the pH of the combined solution of step (vii) to about 7.0 and adding thereto ethanol to a final concentration of from about 20% to about 30%, thereby forming a Precipitate G precipitate;

(ix) dissolving the Precipitate G precipitate in an aqueous solution comprising a member selected from a solvent, a detergent and a combination thereof, and

incubating the solution, e.g., for at least about 60 minutes, forming an incubated solution;

(x) passing the incubated solution through a cation exchange chromatography column and eluting proteins absorbed on the column in an eluate;

(xi) passing the eluate through an anion exchange chromatography column to generate a flow-through fraction;

(x) passing the flow through fraction through a nanofilter to generate a nanofiltrate;

(xi) concentrating the nanofiltrate by ultrafiltration to generate a first ultrafiltrate;

(xii) diafiltering the first ultrafiltrate against a diafiltration buffer to generate a diafiltrate; and

(xiii) concentrating the diafiltrate by ultrafiltration to generate a second ultrafiltrate having a protein concentration of from about 8% (w/v) to about 22% (w/v), thereby forming an IgG enriched fraction.

[0031] In one example, the method comprises adding SiO_2 to a final concentration of from about 0.02 to about 0.10 grams of SiO_2 per gram of the Fraction II+III or Fraction I+II+III precipitate.

[0032] In one example, the method comprises washing the filter cake with at least about 3 filter press dead volumes of a wash buffer.

[0033] In one example, the method comprises washing the filter cake with at least about 2 filter press dead volumes of a wash buffer.

[0034] In one example, the method comprises eluting at least one protein with at least about 35 mM sodium dihydrogen phosphate dihydrate.

[0035] In one example, the diafiltration buffer comprises from about 200 mM to about 300 mM glycine.

[0036] In one example, the method further comprises treating an IgG solution with a solvent and/or detergent in at least one viral inactivation or removal step.

[0037] In one example of the methods described above, the method further comprises an incubation step at low pH, from about 4.0 to about 5.2.

[0038] In one example of the methods described above, the method further comprises an incubation step at low pH, from about 4.4 to about 4.9.

[0039] In an exemplary example, the present disclosure provides a supernatant after C1-inhibitor adsorption fraction comprising IgG, wherein said fraction is a cryo-poor plasma fraction depleted of C1-INH by at least about 70% of total present in the cryo-poor plasma fraction.

[0039a] In another aspect, the present disclosure provides a supernatant after C1-inhibitor adsorption of a cryo-poor plasma fraction comprising IgG, wherein said supernatant fraction comprised no more than 30 % of the total amount of C1-inhibitor present in the cryo-poor plasma fraction prior to C1-inhibitor adsorption and from 1 to 20 IU heparin per mL of supernatant.

[0040] In a fourth example, the present disclosure provides a pharmaceutical composition comprising an IgG enriched fraction prepared according to the present disclosure.

[0041] In one example, the composition comprises at least about 80 to 220 grams of IgG per liter of the composition.

[0042] In one example, the pH of the pharmaceutical composition is from about 4.4 to about 4.9.

DETAILED DESCRIPTION OF THE INVENTION

A. Introduction

[0043] Unlike other biologics that are produced via recombinant expression of DNA vectors in host cell lines, plasma-derived proteins are fractionated from human blood and plasma donations. Thus, the supply of these products cannot be increased by simply increasing the volume of production. Rather the level of commercially available blood products is limited by the available supply of blood and plasma donations. This dynamic results in a shortage in the availability of raw human plasma for the manufacture of new plasma-derived blood factors that have lesser established commercial markets, including Complement Factor H (CFH) and inter-alpha-trypsin inhibitor proteins (IαIp).

[0044] Concerns over the amidolytic content of plasma-derived compositions has highlighted a need in the art for a method for reducing serine proteases (*e.g.*, FXIa and FXIIa) and serine protease zymogens (*e.g.*, FXI and FXII) during manufacturing of IgG, and other biologics.

[0045] C1-inhibitor (C1-INH, C1 esterase inhibitor) is the most important physiological inhibitor of plasma kallikrein, Factor XIa and Factor XIIa. Depletion of C1-inhibitor can result in accumulation of these factors in starting materials for the manufacture of commercial IgG therapeutics such as GAMMAGARD® LIQUID (GGL), making it challenging to produce IgG preparations for intravenous administration without elevated risk of thromboembolic events.

WHAT IS CLAIMED IS:

1. A method for preparing an Immunoglobulin G (IgG) enriched fraction from a C1-INH depleted supernatant fraction comprising IgG, the method comprising:

(a) contacting the C1-INH depleted supernatant fraction with heparin, thereby forming a heparinized fraction; and

(b) isolating IgG from the heparinized fraction, thereby forming an IgG enriched fraction.

2. The method of claim 1, wherein the supernatant fraction is a supernatant after C1-inhibitor adsorption.

3. The method of claim 1 or 2, wherein the supernatant fraction is a plasma supernatant.

4. The method of claim 3, wherein the plasma supernatant is a C1-INH depleted cryo-poor plasma.

5. The method of any one of claims 1-4, wherein the supernatant fraction is depleted of one or more of other blood coagulation factors selected from Factor II, VII, IX and X or a mixture thereof.

6. The method of any one of claims 1-5, wherein the supernatant fraction is concentrated to a protein value of normal plasma before further processing.

7. The method of any one of claims 1-6, wherein the heparin is added in an amount of 1 to 20 units per ml of supernatant fraction.

8. The method of claim 7, wherein the heparin is added in an amount of about 5 units per ml of supernatant fraction.

9. The method of claim 7, wherein the heparin is added in an amount of about 10 units per ml of supernatant fraction.

10. The method of any one of claims 1-9, further comprising, prior to step (a), removing C1-INH esterase inhibitor (C1-INH) from a cryo-poor plasma fraction containing C1-INH, thereby forming the C1-INH depleted supernatant fraction.

11. The method of any one of claims 1-10, wherein the IgG enriched fraction contains at least 50% of the IgG content found in the supernatant fraction.

12. The method of any one of claims 1-11, wherein the purity of IgG in the IgG enriched fraction is at least 95%.

13. The method of any one of claims 1-12, wherein said isolating IgG from the heparinized fraction in b) comprises:

(i) precipitating the heparinized fraction with from 6% to 10% ethanol at a pH of from 7.0 to 7.5 to obtain a Fraction I precipitate and a Fraction I supernatant; and

(ii) precipitating IgG from the Fraction I supernatant with from 18% to 27% alcohol at a pH of from 6.7 to 7.3 to form a Fraction II+III precipitate.

14. The method of any one of claims 1-12, wherein said isolating IgG from the heparinized fraction in b) comprises:

(i) precipitating IgG from the heparinized fraction with from 18% to 27% alcohol at a pH of from 6.7 to 7.3 to form a Fraction I+II+III precipitate.

15. The method of claim 13 or 14, further comprising:

(iii) suspending the Fraction II+III or Fraction I+II+III precipitate in a suspension buffer, thereby forming an IgG suspension;

(iv) mixing finely divided silicon dioxide (SiO_2) with the IgG suspension for at least 30 minutes;

(v) filtering the IgG suspension, thereby forming a filtrate and a filter cake.

16. The method of claim 15, further comprising:

(vi) washing the filter cake with at least 1 filter press dead volume of a wash buffer having a pH of from 4.9 to 5.3, thereby forming a wash solution;

(vii) combining the filtrate with the wash solution, thereby forming a solution, and treating the solution with a detergent;

(viii) adjusting the pH of the solution of step (vii) to about 7.0 and adding ethanol to a final concentration of from 20% to 30%, thereby forming a Precipitate G precipitate;

(ix) dissolving the Precipitate G precipitate in an aqueous solution comprising a solvent and/or detergent/detergents and maintaining the solution for at least 60 minutes;

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

(xi) passing the eluate through an anion exchange chromatography column to generate a generate a flow-through effluent;

(xii) passing the effluent through a nanofilter to generate a nanofiltrate;

(xiii) concentrating the nanofiltrate by ultrafiltration to generate a first ultrafiltrate;

(xiv) diafiltering the first ultrafiltrate against a diafiltration buffer to generate a diafiltrate; and

(xv) concentrating the diafiltrate by ultrafiltration to generate a second ultrafiltrate having a protein concentration between 8% (w/v) and 22% (w/v), thereby forming an IgG enriched fraction.

17. The method of claim 15 or 16, wherein (iv) comprises adding SiO_2 to a final concentration of from 0.02 to 0.10 grams per gram of the Fraction II+III or Fraction I+II+III precipitate.

18. The method of claim 16 or 17, wherein (vi) comprises washing the filter cake with at least 2 filter press dead volumes of a wash buffer.

19. The method of any one of claims 16-18, wherein (x) comprises eluting the proteins with at least 35 mM sodium dihydrogen phosphate dihydrate.

20. The method of any one of claims 16-19, wherein the diafiltration buffer in (xiv) comprises from 200 mM to 300 mM glycine.

21. The method of any one of claims 16-20, wherein treating the solution with a solvent and/or detergent/detergents in (vii) comprises at least one viral inactivation or removal step.

22. The method of claim 21, wherein the viral inactivation is a solvent/detergent (S/D) viral inactivation step.

23. The method of any one of claims 16-22, wherein the method further comprises an incubation step at a low pH of from 4.0 to 5.2.

24. The method of any one of claims 16-22, wherein the method further comprises an incubation step at a low pH of from 4.4 to 4.9.

25. A supernatant after C1-inhibitor adsorption of a cryo-poor plasma fraction comprising IgG, wherein said supernatant fraction comprised no more than 30 % of the total amount of C1-inhibitor present in the cryo-poor plasma fraction prior to C1-inhibitor adsorption and from 1 to 20 IU heparin per mL of supernatant.