FRACTION I-IV-1 PRECIPITATION OF IMMUNOGLOBINS FROM PLASMA

CROSS REFERENCES TO APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 61/602,488 filed February 23, 2012.

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

[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, and WO/2011/150284~~; 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; and WO/2011/150284~~; 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~~, 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.

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), 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