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 ~~OF THE INVENTION~~

[0013] In one aspect, the present ~~invention~~ disclosure 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 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.

[0016] In one specific embodiment, the pH of the first precipitation step is adjusted after the addition of alcohol by spray addition of a pH modifying agent. In another embodiment, the pH of the second precipitation step is adjusted after the addition of alcohol by spray addition of a pH modifying agent. In yet another embodiment, the pH of the third precipitation step is adjusted after the addition of alcohol by spray addition of a pH modifying agent.

[0017] Additionally, the preparatory methods provided herein may further comprise an ion exchange chromatography step (*i.e.*, anion exchange and/or cation exchange chromatography), a nanofiltration step, an ultrafiltration/diafiltration step, or any other suitable purification technique to further enhance the purity or quality of the IVIG preparations.

[0018] In another ~~aspect~~embodiment, a method is provided for preparing an enriched IgG composition from plasma comprising the steps of adjusting the pH of a cryo-poor plasma fraction to at or about 7.0, (b) adjusting the ethanol concentration of the cryo-poor plasma fraction of step (a) to at or about 25% (v/v) at a temperature between at or about -7°C and at or 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 at or about 600 ml of glacial acetic acid per 1000L of buffer, thereby forming a suspension, (e) mixing finely divided silicon dioxide (SiO_2) 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 at or about 150 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 at or about 7.0 and adding ethanol to a final concentration of at or 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.

[0019] ~~In another aspect, t~~The present ~~invention~~ disclosure also provides aqueous IgG compositions prepared by the methods described herein. Generally, the IgG compositions have high purity (*e.g.*, at least 95%, 98%, 99%, or higher IgG contents), contain protein concentrations between about 20 g/L and about 200 g/L, and contain extremely low levels of common IVIG contaminants, such as IgG, IgM, Fibrinogen, Transferrin, ACA, amidolytic activity, PKA, and the like.

[0020] In yet another ~~aspect~~embodiment, pharmaceutical IgG compositions and formulations suitable for use in IVIG therapies are provided. The pharmaceutical formulations have high purity (*e.g.*, at least 98%, 99%, or higher IgG contents), contain protein concentrations between about 20 g/L and about 200 g/L, and contain extremely low levels of common IVIG contaminants, such as IgG, IgM, Fibrinogen, Transferrin, ACA, amidolytic activity, PKA, and the like. Generally, the pharmaceutical compositions are appropriately formulated for intravenous administration (*i.e.*, for IVIG therapy), subcutaneous administration, or intramuscular administration.

[0021] ~~In a~~Another ~~aspect, the present invention~~ embodiment provides methods for treating an immunodeficiency, autoimmune disease, or acute infection in a human in need thereof, the method comprising administering a pharmaceutical composition described herein. Non, limiting examples of diseases and conditions that may be treated or managed through the methods provided herein include, allogeneic bone marrow transplant, chronic lymphocytic leukemia, idiopathic thrombocytopenic purpura (ITP), pediatric HIV, primary immunodeficiencies, Kawasaki disease, chronic inflammatory demyelinating polyneuropathy (CIDP), kidney transplant with a high antibody recipient or with an ABO incompatible donor, 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, hypogammaglobulinemia, primary immune deficiency, RRMS, Alzheimer's disease, and Parkinson's disease.

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.

[0244][~~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.