Claims

1. A process of obtaining Apo A-I from an Apo A-I containing protein fraction (A), comprising:

   (a) suspending the Apo A-I containing protein fraction (A) in a buffer solution (B) comprising 15 to 30% of a linear or branched C_1 to C_4 alcohol (w/w), wherein the suspended Apo A-I containing protein fraction (A) has a pH in the range from 6.4 to 10.0,

   (b) removing impurities from the suspension while keeping the Apo A-I proteins solubilized,

   (c) precipitating Apo A-I from the suspension, and

   (d) collecting the Apo A-I precipitate.

2. The process of claim 1, wherein the buffer solution (B) comprises 20% of the linear or branched C_1 to C_4 alcohol (w/w).

3. The process according to claim 1 or claim 2, wherein the suspended Apo A-I containing protein fraction (A) has a pH in the range from 7.2 to 8.8.

4. The process according to any one of claims 1 to 3, wherein the suspended Apo A-I containing protein fraction (A) has a pH in the range from 8.1 to 8.5.

5. The process according to claim 1 or claim 2, wherein the suspended Apo A-I containing protein fraction (A) has a pH in the range from 7.0 to 7.6.

6. The process according to any one of claims 1 to 5, wherein the buffer solution (B) comprises 5 mM to 35 mM $NaHCO_3$.

7. The process according to any one of claims 1 to 6, wherein the linear or branched C_1 to C_4 alcohol is ethanol.

8. The process according to any one of claims 1 to 7, wherein in step (a), the volume ratio of Apo A-I protein fraction (A) to buffer solution (B) is from 1:1 to 1:5.

9. The process according to any one of claims 1 to 8, wherein the conductivity of the suspension formed at step (a) is less than 5 mS/cm.

10. The process according to any one of claims 1 to 9, wherein in step (b), impurities are removed by precipitation by adding linear or branched C_1 to C_4 alcohol to an alcohol concentration of 55 to 65% (w/w).

11. The process according to any one of claims 1 to 10, wherein in step (b) the linear or branched C_1 to C_4 alcohol added to the suspension has a temperature of about -5°C to 15 °C.

12. The process according to any one of claims 1 to 11, wherein in step (b), impurities are removed by precipitation followed by filtration, centrifugation, decantation, ultrafiltration and/or sedimentation.

13. The process according to any one of claims 1 to 12, wherein in step (c), the pH of the suspension is adjusted in the range from 4.6 to 5.6.

14. The process according to any one of claims 1 to 13, wherein in step (c), the temperature of the

suspension is adjusted to -2 to 20°C to precipitate Apo A-I.

15. The process according to any one of claims 1 to 14, further comprising step (e), comprising delipidating the collected Apo A-I precipitate through addition of ethanol in the range from 40 to 96% (w/w).

16. Apo A-I obtained by a process according to any one of the preceding claims.

17. A purified Apo A-I comprising less than 0.3 mg of IgA per gram of Apo A-I.

18. A pharmaceutical composition comprising Apo A-I according to claim 16 or claim 17 and a pharmaceutically acceptable carrier or diluent.

19. A method of producing a pharmaceutical composition including producing Apo A-I according to the method of any one of claims 1 to 15 and combining the Apo A-I with a pharmaceutically acceptable carrier or diluent

20. Reconstituted HDL comprising Apo A-I according to claim 16 or claim 17.

21. A pharmaceutical composition comprising reconstituted HDL according to claim 20 and a pharmaceutically acceptable carrier or diluent.

22. A method of producing reconstituted HDL including producing Apo A-I according to the method of any one of claims 1 to 15 and combining the Apo A-I with a lipid to thereby produce reconstituted HDL.

23. A method of producing a pharmaceutical composition including producing Apo A-I according to the method

of any one of claims 1 to 15; combining the Apo A-I with a lipid to thereby produce reconstituted HDL; and combining the reconstitued HDL with a pharmaceutically acceptable carrier or diluent to thereby produce the pharmaceutical composition.

Claims

1. A preparation of purified, human plasma-derived Apolipoprotein A-I (Apo A-I), wherein the Apo A-I preparation contains IgA, wherein the IgA is present in the Apo A-I preparation in an amount less than 0.3 mg of IgA per gram of Apo A-I.

2. The Apo A-**1** preparation of claim 1, wherein the preparation comprises:

(a) less than 0.7 mg of IgG per gram of Apo A-I;

(b) less than 0.05 mg of IgM per gram of Apo A-I;

(c) less than 4.9 mg of haptoglobin per gram of Apo A-I;

(d) less than 2.7 mg of hemopexin per gram of Apo A-I;

(e) less than 6.4 mg of fibrinogen per gram of Apo A-I;

(f) less than 0.9 mg of ceruloplasmin per gram of Apo A-I;

(g) less than 14.6 mg of albumin per gram of Apo A-I;

(h) less than 2.3 mg of alpha-2-macroglobulin per gram of Apo A-I;

(i) less than 12 mg of alpha-1-antitrypsin per gram of Apo A-I; and/or

(j) less than 3.9 mg of transferrin per gram of Apo A-I.

3. A pharmaceutical composition comprising the Apo A-1 preparation of claim 1.

4. A reconstituted high density lipoprotein (rHDL) formulation comprising the Apo A-I preparation of claim 1.

5. The rHDL formulation of claim 4, further comprising one or more of a lipid, a detergent, and a stabilizer.

6. The rHDL formulation of claim 4, further comprising a lipid selected from phospholipids, cholesterol, cholesterol-esters, fatty acids and triglycerides.

7. The rHDL formulation of claim 6, wherein the lipid is a phospholipid.

8. The rHDL formulation of claim 7, wherein the phospholipid is selected from phosphatidylcholine, phosphatidic acid, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, phosphatidylinositol, and sphingomyelin.

9. The rHDL formulation of claim 4, further comprising a detergent selected from cationic detergents, anionic detergents, Zwitterionic detergents, and non-ionic detergents.

10. The rHDL formulation of claim 9, wherein the detergent is selected from bile acid and salts thereof.

11. The rHDL formulation of claim 10, wherein the detergent comprises sodium cholate.

12. The rHDL formulation of claim 4, further comprising a carbohydrate stabilizer.

13. The rHDL formulation of claim 12, wherein the carbohydrate stabilizer is selected from sugars and sugar alcohols.

14. The rHDL formulation of claim 13, wherein the carbohydrate stabilizer is one or more selected from sucrose, mannitol, and sorbitol.

15. The rHDL formulation of claim 4, further comprising a phospholipid, a detergent selected from a bile acid or salt thereof, and a carbohydrate stabilizer.

16. A pharmaceutical composition comprising the rHDL formulation of claim 4.

17. A pharmaceutical composition comprising the rHDL formulation of claim 15.

18. A process of obtaining the Apo A-I preparation of claim 1 from an Apo A-I containing protein fraction (A), comprising:

(a) suspending the Apo A-I containing protein fraction (A) in a buffer solution (B) comprising 15 to 30% (w/w) of a linear or branched C1 to C4 alcohol to form a suspension comprising solubilized Apo A-I, wherein the suspension has a pH in the range from 6.4 to 10.0;

(b) removing impurities from the suspension while keeping the Apo A-I solubilized;

(c) precipitating Apo A-I from the suspension, and

(d) collecting the Apo A-I precipitate.

19. The process of claim 18, wherein step (a) comprises suspending 50 kg or more of the Apo A-I containing protein fraction (A) in buffer solution (B) to form a suspension comprising solubilized Apo A-I obtained from 50 kg or more of a starting fraction.