CLAIMS

1. A method of purifying haptoglobin and hemopexin from a solution containing both proteins, the method comprising:
   (i) providing a solution containing both haptoglobin and hemopexin;
   (ii) precipitating the haptoglobin from the solution by adding ammonium sulphate to the solution;
   (iii) separating the precipitated haptoglobin from the solution containing hemopexin; and
   (iv) separately purifying the haptoglobin and/or hemopexin in one or more steps.

2. The method of claim 1 comprising precipitating the haptoglobin from the solution by adding about 2.2M to about 2.5M ammonium sulphate to the solution.

3. The method of claim 2 comprising precipitating the haptoglobin from the solution by adding about 2.4M ammonium sulphate to the solution.

4. The method of any one of claims 1 to 3 comprising precipitating the haptoglobin from the solution at a pH of less than or equal to 8.

5. The method of claim 4 comprising precipitating the haptoglobin from the solution at a pH within the range of about 6 to about 8.

6. The method of claim 5 comprising precipitating the haptoglobin from the solution at a pH of about 7.

7. The method of any one of claims 1 to 6, wherein the solution containing both haptoglobin and hemopexin further comprises transferrin.

8. The method of any one of claims 1 to 7, wherein the solution is a human plasma fraction.

9. The method of claim 8, wherein the solution is a Cohn Fraction IV.

10. The method of claim 9, wherein the solution is a Cohn Fraction IV_4.

11. The method of any one of claims 1 to 10, wherein the method further comprises:
    (i) dissolving the precipitated haptoglobin in a buffer to obtain a haptoglobin solution;
    (ii) passing the haptoglobin solution through a anion exchange chromatographic resin under conditions such that the haptoglobin binds to the resin;
    (iii) eluting the haptoglobin from the resin; and
    (iv) recovering the eluted haptoglobin.

12. The method of claim 11, further comprising the step of exposing the haptoglobin solution to a lipid removal agent under conditions which allow lipid to bind to the agent.

13. The method of any one of claims 1 to 12, wherein the method further comprises:
    (i) passing the solution containing hemopexin through a hydrophobic interaction chromatographic resin under conditions that allow the hemopexin to bind to the resin;
    (ii) collecting the flow-through fraction from step (i);
    (iii) optionally washing the resin following step (ii) and collecting the flow-through wash fraction; and
    (iv) eluting the hemopexin from the resin following step (ii) and/or following step (iii); and
    (v) recovering the eluted hemopexin.

14. The method of claim 13, further comprising:
    (i) passing the eluted hemopexin through a metal ion affinity chromatographic resin under conditions that allow the hemopexin to bind to the resin;
    (ii) eluting the hemopexin from the resin; and

(iii) recovering the eluted hemopexin.

15. The method of any one of claims 7-14 further comprising:

(a) passing the solution containing hemopexin through a hydrophobic interaction chromatographic resin under conditions that allow the hemopexin to bind to the resin;

(b) collecting the flow-through fraction from step (a);

(c) optionally washing the resin following step (b) and collecting the flow-through wash fraction;

(d) passing the flow-through fraction from step (b) and/or the flow-through wash fraction from step (c) through a anion exchange chromatographic resin under conditions such that transferrin binds to the resin; and

(e) recovering the transferrin from the resin.

16. A composition comprising the haptoglobin recovered by the method of any one of claims 1 to 12.

17. A composition comprising the hemopexin recovered by the method of any one of claims 1 to 10, 13 and 14.

18. A composition comprising the transferrin recovered by the method of claim 15.

19. A formulation comprising the composition of any one of claims 16 to 18 and a pharmaceutically acceptable carrier.

20. A method of treating a condition associated with haemolysis, the method comprising administering to a subject in need thereof the composition of any one of claims 15 to 17 or the formulation of claim 19.

21. The method of claim 20, wherein the condition is selected from the group consisting of haemolytic anaemia, transfusion-induced haemolysis, haemolytic uraemic syndrome, an autoimmune disease, malaria infection and burns.

22. The method of claim 20, wherein the condition is selected from the group consisting of sickle cell anaemia, hereditary spherocytosis, hereditary elliptocytosis, thalassemia, congenital dyserythropoietic anemia and Paroxysmal nocturnal hemoglobinuria, systemic lupus erythematosus and chronic lymphocytic leukemia.

23. Use of the composition of any one of claims 16 to 18 or the formulation of claim 19 in the manufacture of a medicament for treating a condition associated with haemolysis.

24. Use of claim 23, wherein the condition is selected from the group consisting of haemolytic anaemia, transfusion-induced haemolysis, haemolytic uraemic syndrome, an autoimmune disease, malaria infection and burns.

25. Use of claim 23, wherein the condition is selected from the group consisting of sickle cell anaemia, hereditary spherocytosis, hereditary elliptocytosis, thalassemia, congenital dyserythropoietic anemia and Paroxysmal nocturnal hemoglobinuria, systemic lupus erythematosus and chronic lymphocytic leukemia.

Claims:

1. A method of purifying haptoglobin and hemopexin from a solution containing both proteins, the method comprising:

   (i) providing a solution containing both haptoglobin and hemopexin;

   (ii) precipitating the haptoglobin from the solution by adding ammonium sulfate to the solution;

   (iii) separating the precipitated haptoglobin from the solution containing hemopexin; and

   (iv) separately purifying the haptoglobin and/or hemopexin in one or more steps,

   wherein the haptoglobin is precipitated from the solution, at a pH of 6 to 8, by adding 2.3 M to 2.5 M ammonium sulfate to the solution.

2. The method of claim 1, wherein the haptoglobin is precipitated from the solution by adding about 2.4M ammonium sulfate to the solution.

3. The method of claim 1, wherein the haptoglobin is precipitated from the solution at a pH of about 7.

4. The method of claim 1, wherein the solution containing both haptoglobin and hemopexin further comprises transferrin.

5. The method of claim 1, wherein the solution is a human plasma fraction.

6. The method of claim 5, wherein the solution is a Cohn Fraction IV.

7. The method of claim 6, wherein the solution is a Cohn Fraction IV_4.

8. The method of claim 1, wherein the method further comprises:

   (i) dissolving the precipitated haptoglobin in a buffer to obtain a haptoglobin solution;

   (ii) passing the haptoglobin solution through a strong anion exchange chromatographic resin under conditions such that the haptoglobin binds to the resin;

   (iii) eluting the haptoglobin from the resin; and

   (iv) recovering the eluted haptoglobin.

9. The method of claim 8, further comprising exposing the haptoglobin solution to a lipid removal agent under conditions that allow lipid to bind to the agent.

10. The method of claim 1, wherein the method further comprises:

(a) passing the solution containing hemopexin through a hydrophobic interaction chromatographic resin under conditions that allow the hemopexin to bind to the resin;

(b) collecting the flow-through fraction from step (a);

(c) optionally washing the resin following step (b) and collecting the flow-through wash fraction; and

(d) eluting the hemopexin from the resin following step (b) and/or following step (c); and

(e) recovering the eluted hemopexin.

11. The method of claim 10, further comprising:

(i) passing the eluted hemopexin through a metal ion affinity chromatographic resin under conditions that allow the hemopexin to bind to the resin;

(ii) eluting the hemopexin from the resin; and

(iii) recovering the eluted hemopexin.

12. The method of claim 4, further comprising:

(a) passing the solution containing hemopexin through a hydrophobic interaction chromatographic resin under conditions that allow the hemopexin to bind to the resin;

(b) collecting the flow-through fraction from step (a);

(c) optionally washing the resin following step (b) and collecting the flow-through wash fraction;

(d) passing the flow-through fraction from step (b) and/or the flow-through wash fraction from step (c) through a weak anion exchange chromatographic resin under conditions such that transferrin binds to the resin; and

(e) recovering the transferrin from the resin.