What is claimed:

1. A method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition, the oxygen carrier-containing pharmaceutical composition including hemoglobin, the hemoglobin consisting essentially of non-polymeric crosslinked tetrameric hemoglobin having a beta-beta cross-linking of greater than 40%, the method comprising:

a) providing mammalian whole blood including at least red blood cells and plasma;

b) separating the red blood cells from the plasma in the mammalian whole blood;

c) filtering the red blood cells that were separated from the plasma to obtain a filtered red blood cell fraction;

d) washing the filtered red blood cell fraction to remove plasma protein impurities, resulting in washed red blood cells;

e) disrupting the washed red blood cells to create a solution comprising a lysate of disrupted red blood cells;

f) performing filtration to remove at least a portion of the waste retentate from the lysate;

g) extracting a first hemoglobin solution from the lysate;

h) performing at least one purification process to remove one or more of viruses, waste retentate, or protein impurities;

i) cross-linking the first hemoglobin solution by bis-3,5-dibromosalicyl fumarate to form crosslinked hemoglobin in an oxygenated environment wherein the crosslinked hemoglobin is non-polymeric crosslinked tetrameric hemoglobin having at least 40% beta-beta cross-linking;

j) removing any residual chemicals;

k) heat treating the crosslinked hemoglobin in a deoxygenated environment to denature and precipitate any residual non-stabilized/ non-crosslinked hemoglobin, any dimeric hemoglobin and any other protein impurities such that the resulting heat stable crosslinked tetrameric hemoglobin has an undetectable concentration of dimer and consists essentially of non-polymeric crosslinked tetrameric hemoglobin with a beta-beta cross-linking of at least 40% and an oxygen affinity greater than the oxygen affinity of native hemoglobin of the same species measured under substantially similar conditions;

l) removing precipitate by a centrifugation or a filtration to form a clear solution; and

m) adding the purified and heat stable crosslinked tetrameric hemoglobin to a pharmaceutically acceptable carrier.

2. The method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition according to claim 1 wherein the heat processing step is a high temperature short time (HTST) process conducted at approximately 70°C to 95°C for 30 seconds to 3 hours followed immediately by cooling and adding N-acetyl cysteine in an amount of 0.2 to 0.4% immediately following the cooling.

3. The method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition according to claim 1 wherein the whole blood is bovine whole blood and the beta-beta cross-linking is greater than 50% and the p50 value is less than approximately 23 mm Hg.

4. The method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition according to claim 1 wherein the whole blood is bovine whole blood and the beta-beta cross-linking is greater than 60% and the p50 value is less than approximately 23 mm Hg.

5. The method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition according to claim 1 wherein the purification is performed using chromatography, the chromatography including use of one or more cation-exchange columns or anion-exchange columns.

6. The method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition according to claim 5 wherein the chromatography column is one or more DEAE, CM and/or hydroxyapatite columns.

7. The method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition according to claim 1 wherein the whole blood is human blood.

8. The method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition according to claim 1 wherein the whole blood is porcine blood, equine blood, or canine blood.

9. A method for the preparation of a highly purified and heat stable oxygen carrier containing pharmaceutical composition according to claim 1 further comprising packaging the hemoglobin solution in a low oxygen permeability package such that hemoglobin solution has a shelf life on the order of two years.

10. A highly purified and heat stable oxygen carrier-containing pharmaceutical composition comprising hemoglobin, wherein the hemoglobin consisting essentially of non-polymeric crosslinked tetrameric hemoglobin having beta-beta cross-linking of greater than 40% formed by the process of claim 1.

11. A highly purified and heat stable oxygen carrier-containing pharmaceutical composition comprising hemoglobin, wherein the hemoglobin consisting essentially of non-polymeric crosslinked tetrameric hemoglobin having beta-beta cross-linking of greater than 60% formed by the process of claim 4.

12. The composition of claim 10 for oxygenating tissue, wherein said composition is administered either in vivo or ex vivo.

13. The composition of claim 11 for oxygenating tissue, wherein said composition is administered either in vivo or ex vivo.

14. A method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition, the oxygen carrier-containing pharmaceutical composition including hemoglobin, the hemoglobin consisting essentially of non-polymeric crosslinked tetrameric hemoglobin having a beta-beta cross-linking of greater than 50%, the method comprising:

a) providing mammalian whole blood including at least red blood cells and plasma;

b) separating the red blood cells from the plasma in the mammalian whole blood;

c) filtering the red blood cells that were separated from the plasma to obtain a filtered red blood cell fraction;

d) washing the filtered red blood cell fraction to remove plasma protein impurities, resulting in washed red blood cells;

e) disrupting the washed red blood cells to create a solution comprising a lysate of disrupted red blood cells;

f) performing filtration to remove at least a portion of the waste retentate from the lysate;

g) extracting a first hemoglobin solution from the lysate;

h) performing at least one purification process to remove one or more of viruses, waste retentate, or protein impurities;

i) cross-linking the first hemoglobin solution by bis-3,5-dibromosalicyl fumarate to form crosslinked hemoglobin in a deoxygenated environment wherein the crosslinked hemoglobin is non-polymeric crosslinked tetrameric hemoglobin having at least 50% beta-beta cross-linking;

j) removing any residual chemicals;

k) heat treating the crosslinked hemoglobin in a deoxygenated environment to denature and precipitate any residual non-stabilized/ non-crosslinked hemoglobin, any dimeric hemoglobin and any other protein impurities such that the resulting heat stable crosslinked tetrameric hemoglobin has an undetectable concentration of dimer and consists essentially of non-polymeric crosslinked tetrameric hemoglobin with a beta-beta cross-linking of at least 50% having an oxygen affinity less than the oxygen affinity of native hemoglobin of the same species measured under substantially similar conditions;

l) removing precipitate by a centrifugation or a filtration to form a clear solution; and

m) adding the purified and heat stable crosslinked tetrameric hemoglobin to a pharmaceutically acceptable carrier.

15. The method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition according to claim 14 wherein the whole blood is bovine whole blood and the oxygen affinity of the crosslinked tetrameric bovine hemoglobin has a p50 value on the order of approximately 38 to 50 mm Hg.

16. A method for the preparation of a highly purified and heat stable oxygen carrier containing pharmaceutical composition according to claim 14 further comprising packaging the hemoglobin solution in a low oxygen permeability package such that hemoglobin solution has a shelf life on the order of two years.

17. A highly purified and heat stable oxygen carrier-containing pharmaceutical composition comprising hemoglobin, wherein the hemoglobin consisting essentially of non-polymeric crosslinked tetrameric hemoglobin having beta-beta cross-linking of greater than 50% formed by the process of claim 14.

18. A highly purified and heat stable oxygen carrier-containing pharmaceutical composition comprising hemoglobin, wherein the hemoglobin consisting essentially of non-polymeric crosslinked tetrameric hemoglobin having beta-beta cross-linking of greater than 60% formed by the process of claim 15.

19. The composition of 17 for oxygenating tissue, wherein said composition is administered either in vivo or ex vivo.

20. The composition of 18 for oxygenating tissue, wherein said composition is administered either in vivo or ex vivo.

21. The method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition according to claim 1 wherein the heat processing step is a high temperature short time (HTST) process conducted at approximately 70°C to 95°C for 30 seconds to 3 hours followed immediately by cooling and adding N-acetyl cysteine in an amount of 0.025% to 0.4% immediately following the cooling.

22. The method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition according to claim 1 wherein the heat processing step is a high temperature short time (HTST) process conducted at approximately 70°C to 95°C for 30 seconds to 3 hours followed immediately by cooling and adding N-acetyl cysteine in an amount of 0.025% to 0.2% immediately following the cooling.

23. A method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition, the oxygen carrier-containing pharmaceutical composition including hemoglobin, the hemoglobin consisting essentially of non-polymeric

crosslinked tetrameric hemoglobin having a beta-beta cross-linking of greater than 40%, the method comprising:

a) providing mammalian whole blood including at least red blood cells and plasma;

b) separating the red blood cells from the plasma in the mammalian whole blood;

c) filtering the red blood cells that were separated from the plasma to obtain a filtered red blood cell fraction;

d) washing the filtered red blood cell fraction to remove plasma protein impurities, resulting in washed red blood cells;

e) disrupting the washed red blood cells to create a solution comprising a lysate of disrupted red blood cells;

f) performing filtration to remove at least a portion of the waste retentate from the lysate;

g) extracting a first hemoglobin solution from the lysate;

h) performing at least one purification process to remove one or more of viruses, waste retentate, or protein impurities;

i) cross-linking the first hemoglobin solution by bis-3,5-dibromosalicyl fumarate to form crosslinked hemoglobin in an oxygenated environment wherein the crosslinked hemoglobin is non-polymeric crosslinked tetrameric hemoglobin having at least 40% beta-beta cross-linking;

j) removing any residual chemicals;

k) heat treating the crosslinked hemoglobin in a deoxygenated environment to denature and precipitate any residual non-stabilized non-crosslinked hemoglobin, any dimeric hemoglobin and any other protein impurities such that the resulting heat stable crosslinked tetrameric hemoglobin has an undetectable concentration of dimer and consists essentially of non-polymeric crosslinked tetrameric hemoglobin with a beta-beta cross-linking of at least 40% and an oxygen affinity greater than the oxygen affinity of native hemoglobin of the same species measured under substantially similar conditions;

l) cooling the resulting heat stable crosslinked hemoglobin;

m) adding 0.025%-0.4% of N-acetyl cysteine;

n) removing precipitate by a centrifugation or a filtration to form a clear solution; and

o) adding the purified and heat stable crosslinked tetrameric hemoglobin to a pharmaceutically acceptable carrier.

24. The method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition according to claim 14 wherein the heat processing step is a high temperature short time (HTST) process conducted at approximately 70°C to 95°C for 30 seconds to 3 hours followed immediately by cooling and adding N-acetyl cysteine in an amount of 0.025% to 0.4% immediately following the cooling.

25. The method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition according to claim 14 wherein the heat processing step is a high temperature short time (HTST) process conducted at approximately 70°C to 95°C for 30 seconds to 3 hours followed immediately by cooling and adding N-acetyl cysteine in an amount of 0.025% to 0.2% immediately following the cooling.

26. The method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition according to claim 14 wherein the heat processing step is a high temperature short time (HTST) process conducted at approximately 70°C to 95°C for 30 seconds to 3 hours followed immediately by cooling and adding N-acetyl cysteine in an amount of 0.2% to 0.4% immediately following the cooling.

27. A method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition, the oxygen carrier-containing pharmaceutical composition including hemoglobin, the hemoglobin consisting essentially of non-polymeric crosslinked tetrameric hemoglobin having a beta-beta cross-linking of greater than 50%, the method comprising:

a) providing mammalian whole blood including at least red blood cells and plasma;

b) separating the red blood cells from the plasma in the mammalian whole blood;

c) filtering the red blood cells that were separated from the plasma to obtain a filtered red blood cell fraction;

d) washing the filtered red blood cell fraction to remove plasma protein impurities, resulting in washed red blood cells;

e) disrupting the washed red blood cells to create a solution comprising a lysate of disrupted red blood cells;

f) performing filtration to remove at least a portion of the waste retentate from the lysate;

g) extracting a first hemoglobin solution from the lysate;

h) performing at least one purification process to remove one or more of viruses, waste retentate, or protein impurities;

i) cross-linking the first hemoglobin solution by bis-3,5-dibromosalicyl fumarate to form crosslinked hemoglobin in a deoxygenated environment wherein the crosslinked hemoglobin is non-polymeric crosslinked tetrameric hemoglobin having at least 50% beta-beta cross-linking;

j) removing any residual chemicals;

k) heat treating the crosslinked hemoglobin in a deoxygenated environment to denature and precipitate any residual non-stabilized/ non-crosslinked hemoglobin, any dimeric hemoglobin and any other protein impurities such that the resulting heat stable crosslinked tetrameric hemoglobin has an undetectable concentration of dimer and consists essentially of non-polymeric crosslinked tetrameric hemoglobin with a beta-beta cross-linking of at least 50% having an oxygen affinity less than the oxygen affinity of native hemoglobin of the same species measured under substantially similar conditions;

l) cooling the resulting heat stable crosslinked tetrameric hemoglobin;

m) adding 0.025%-0.4% N-acetyl cysteine;

n) removing precipitate by a centrifugation or a filtration to form a clear solution; and

o) adding the purified and heat stable crosslinked tetrameric hemoglobin to a pharmaceutically acceptable carrier.

What is claimed:

1. A method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition, the oxygen carrier-containing pharmaceutical composition including hemoglobin, the hemoglobin consisting essentially of non-polymeric crosslinked tetrameric hemoglobin having a beta-beta cross-linking of greater than 40%, the method comprising:

a) providing mammalian whole blood including at least red blood cells and plasma;

b) separating the red blood cells from the plasma in the mammalian whole blood;

c) filtering the red blood cells that were separated from the plasma to obtain a filtered red blood cell fraction;

d) washing the filtered red blood cell fraction to remove plasma protein impurities, resulting in washed red blood cells;

e) disrupting the washed red blood cells to create a solution comprising a lysate of disrupted red blood cells;

f) performing filtration to remove at least a portion of the waste retentate from the lysate;

g) extracting a first hemoglobin solution from the lysate;

h) performing at least one purification process to remove one or more of viruses, waste retentate, or protein impurities;

i) cross-linking the first hemoglobin solution by bis-3,5-dibromosalicyl fumarate to form crosslinked hemoglobin in an oxygenated environment wherein the crosslinked hemoglobin is non-polymeric crosslinked tetrameric hemoglobin having at least 40% beta-beta cross-linking;

j) removing any residual chemicals;

k) heat treating the crosslinked hemoglobin in a deoxygenated environment to denature and precipitate any residual non-stabilized/non-crosslinked hemoglobin, any dimeric hemoglobin and any other protein impurities such that the resulting heat stable crosslinked tetrameric hemoglobin has an undetectable concentration of dimer and consists essentially of non-polymeric crosslinked tetrameric hemoglobin with a beta-beta cross-linking of at least 40% and an oxygen affinity greater than the oxygen affinity of native hemoglobin of the same species measured under substantially similar conditions;

l) removing precipitate by a centrifugation or a filtration to form a clear solution; and

m) adding the purified and heat stable crosslinked tetrameric hemoglobin to a pharmaceutically acceptable carrier.

2. The method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition according to claim 1 wherein the heat processing step is a high temperature short time (HTST) process conducted at approximately 70.degree. C. to 95.degree. C. for 30 seconds to 3 hours followed immediately by cooling and adding N-acetyl cysteine in an amount of 0.2 to 0.4% immediately following the cooling.

3. The method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition according to claim 1 wherein the whole blood is bovine whole blood and the beta-beta cross-linking is greater than 50% and the p50 value is less than approximately 23 mm Hg.

4. The method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition according to claim 1 wherein the whole blood is bovine whole blood and the beta-beta cross-linking is greater than 60% and the p50 value is less than approximately 23 mm Hg.

5. The method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition according to claim 1 wherein the purification is performed using chromatography, the chromatography including use of one or more cation-exchange columns or anion-exchange columns.

6. The method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition according to claim 5 wherein the chromatography column is one or more DEAE, CM and/or hydroxyapatite columns.

7. The method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition according to claim 1 wherein the whole blood is human blood.

8. The method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition according to claim 1 wherein the whole blood is porcine blood, equine blood, or canine blood.

9. A method for the preparation of a highly purified and heat stable oxygen carrier containing pharmaceutical composition according to claim 1 further comprising packaging the hemoglobin solution in a low oxygen permeability package such that hemoglobin solution has a shelf life on the order of two years.

10. A method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition, the oxygen carrier-containing pharmaceutical composition including hemoglobin, the hemoglobin consisting essentially of non-polymeric crosslinked tetrameric hemoglobin having a beta-beta cross-linking of greater than 50%, the method comprising:

a) providing mammalian whole blood including at least red blood cells and plasma;

b) separating the red blood cells from the plasma in the mammalian whole blood;

c) filtering the red blood cells that were separated from the plasma to obtain a filtered red blood cell fraction;

d) washing the filtered red blood cell fraction to remove plasma protein impurities, resulting in washed red blood cells;

e) disrupting the washed red blood cells to create a solution comprising a lysate of disrupted red blood cells;

f) performing filtration to remove at least a portion of the waste retentate from the lysate;

g) extracting a first hemoglobin solution from the lysate;

h) performing at least one purification process to remove one or more of viruses, waste retentate, or protein impurities;

i) cross-linking the hemoglobin by bis-3,5-dibromosalicyl fumarate to form crosslinked hemoglobin in a deoxygenated environment wherein the crosslinked hemoglobin is non-polymeric crosslinked tetrameric hemoglobin having at least 50% beta-beta cross-linking;

j) removing any residual chemicals;

k) heat treating the crosslinked hemoglobin in a deoxygenated environment to denature and precipitate any residual non-stabilized/non-crosslinked hemoglobin, any dimeric hemoglobin and any other protein impurities such that the resulting heat stable crosslinked tetrameric hemoglobin has an undetectable concentration of dimer and consists essentially of non-polymeric crosslinked tetrameric hemoglobin with a beta-beta cross-linking of at least 50%

having an oxygen affinity less than the oxygen affinity of native hemoglobin of the same species measured under substantially similar conditions;

l) removing precipitate by a centrifugation or a filtration to form a clear solution; and

m) adding the purified and heat stable crosslinked tetrameric hemoglobin to a pharmaceutically acceptable carrier.

11. The method for the preparation of a highly purified and heat stable oxygen carrier-containing pharmaceutical composition according to claim 10 wherein the whole blood is bovine whole blood and the oxygen affinity of the crosslinked tetrameric bovine hemoglobin has a p50 value on the order of approximately 38 to 50 mm Hg.

12. A method for the preparation of a highly purified and heat stable oxygen carrier containing pharmaceutical composition according to claim 10 further comprising packaging the hemoglobin solution in a low oxygen permeability package such that hemoglobin solution has a shelf life on the order of two years.