met-hemoglobin, which cannot be used to oxygenate tissues. Further, such long term heat treatment processes are not compatible with commercial-scale production processes.

SUMMARY ~~OF THE INVENTION~~

[009] Disclosed herein is~~The present invention relates to~~ a method for separating unmodified hemoglobin from cross-linked hemoglobin in a hemoglobin solution including polymeric hemoglobin. The method involves contacting the hemoglobin solution with a high temperature short time apparatus wherein unmodified tetrameric hemoglobin is thermally denatured to form a precipitate. The denatured and precipitated hemoglobin dimers are then separated from the solution, while retaining the cross-linked hemoglobin in the solution.

[009a] In one aspect, there is provided a method for the preparation of an oxygen carrier-containing pharmaceutical composition, the oxygen carrier-containing pharmaceutical composition including hemoglobin, the hemoglobin including cross-linked polymeric hemoglobin, the method comprising:

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

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

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

(d) lysing the red blood cells to create a solution comprising a lysate of disrupted red blood cells;

(e) extracting a first hemoglobin solution from the lysate;

(f) performing one or more purification processes on the first hemoglobin solution;

(g) cross-linking hemoglobin tetramers in the first hemoglobin solution to create a second hemoglobin solution including cross-linked polymeric hemoglobin, the polymeric hemoglobin including two or more cross-linked hemoglobin tetramers;

(h) heat treating the second hemoglobin solution including the cross-linked polymeric hemoglobin in a deoxygenated environment at a temperature greater than 85°C and less than or equal to approximately 95°C for a period of less than approximately 40 minutes to denature and precipitate any residual non-reacted hemoglobin, non-stabilized hemoglobin (dimer), and any other impurities;

(i) cooling the heat-treated solution to a temperature approximately less than or equal to 25 °C in approximately two minutes or less to prevent formation of met-hemoglobin;

(j) removing precipitate by a centrifugation or a filtration apparatus to form a solution of hemoglobin including cross-linked polymeric hemoglobin.

[0010] The advantages of this disclosed method~~invention~~ include providing a blood-substitute with an improved intravascular retention time, a reduction or elimination of significant renal elimination of hemoglobin and the side effects associated therewith, a suitable oncotic pressure, and reduced hypertensive effects.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] FIG. 1 represents a schematic flow diagram of a high temperature short time apparatus method for denaturing unmodified hemoglobin from modified hemoglobin blood-substitute according to the present invention.

[0012] FIG. 2 depicts high performance liquid chromatography analysis for non-heat treated polymeric hemoglobin.