(i) provision of an aqueous suspension of said perfluorobutane microbubbles stabilised by hydrogenated egg phosphatidylserine in an aqueous solution;
(ii) adjusting the size of said microbubbles to a median size in the range 2.7 to 3.5 µm;
(iii) diluting the size-adjusted suspension from step (ii) with a sterile sucrose aqueous solution to give a final sucrose concentration of 5-20 % w/v;
(iv) dispensing an aliquot of the composition from step (iii) into a vial to give a filled vial, wherein multiple vials are dispensed in parallel;
(v) within a holding time period of 1.0 to 2.75 minutes for each filled vial after the dispensing of step (iv) is complete, cooling each such filled vial to a temperature of –40 ºC to –70 ºC, wherein multiple filled vials are cooled in parallel wherein cooling is achieved by loading into a freeze-drier apparatus, in which the shelves are pre-cooled to -60 ºC;
(vi) repeating steps (iv) and (v) as necessary, until the desired number of cooled, filled vials has been obtained;
(vii) freeze-drying the cooled, filled vials from steps (v) and (vi);
(viii) back-filling the headspace gas in the freeze-dried vials from step (vii) with perfluorobutane;
(ix) sealing the vials from step (viii) with a closure.

In another aspect, there is provided a method of preparation of a kit for the preparation of an ultrasound contrast agent which comprises a suspension of microbubbles of perfluorobutane stabilised by hydrogenated egg phosphatidylserine in a sterile, aqueous medium which comprises:

(i) carrying out the method as defined above, to give a commercial scale batch of 500 to 80,000 vials containing the contrast agent precursor as defined above; and
(ii) provision of a container of sterile, aqueous solution for each vial suitable for reconstituting said vials of precursor from step (i) to give said contrast agent.

In another aspect, there is provided a method of preparation of an ultrasound contrast agent which comprises a suspension of microbubbles of perfluorobutane stabilised by hydrogenated egg phosphatidylserine in a sterile, aqueous medium which comprises:
(i) carrying out the method as defined above, to give a commercial scale batch of 500 to 80,000 vials containing the contrast agent precursor as defined above; and

CLAIMS.

1. A method of preparation of a commercial scale batch of vials of an ultrasound contrast agent precursor which comprises a lyophilised composition of microbubbles of perfluorobutane stabilised by hydrogenated egg phosphatidylserine in sucrose wherein said batch size is in the range 500 to 80,000 vials;

wherein said process comprises:

(i) provision of an aqueous suspension of said perfluorobutane microbubbles stabilised by hydrogenated egg phosphatidylserine in an aqueous solution;

(ii) adjusting the size of said microbubbles to a median size in the range 2.7 to 3.5 μm;

(iii) diluting the size-adjusted suspension from step (ii) with a sterile sucrose aqueous solution to give a final sucrose concentration of 5-20 % w/v;

(iv) dispensing an aliquot of the composition from step (iii) into a vial to give a filled vial, wherein multiple vials are dispensed in parallel;

(v) within a holding time period of 1.0 to 2.75 minutes for each filled vial after the dispensing of step (iv) is complete, cooling each such filled vial to a temperature of –40 ºC to –70 ºC, wherein multiple filled vials are cooled in parallel wherein cooling is achieved by loading into a freeze-drier apparatus, in which the shelves are pre-cooled to -60 ºC;

(vi) repeating steps (iv) and (v) as necessary, until the desired number of cooled, filled vials has been obtained;

(vii) freeze-drying the cooled, filled vials from steps (v) and (vi);

(viii) back-filling the headspace gas in the freeze-dried vials from step (vii) with perfluorobutane;

(ix) sealing the vials from step (viii) with a closure.

2. The method of claim 1, where the lyophilized precursor composition is sterile.

3. The method of claim 1 or claim 2, where the sucrose concentration of step (iii) is 8-12% w/v.

4. The method of any one of claims 1 to 3, where the size adjustment of step (ii) is carried out by:

(a) one or more cycles of flotation and re-suspension; or

(b) differential centrifugation; or

(c) combinations thereof.

5. The method of any one of claims 1 to 4, where after step (iii), the size distribution and microbubble concentration is checked before proceeding to the dispending step (iv).

6. The method of any one of claims 1 to 5, where the holding time period of step (v) is the same for each filled vial.

7. A method of preparation of a kit for the preparation of an ultrasound contrast agent which comprises a suspension of microbubbles of perfluorobutane stabilised by hydrogenated egg phosphatidylserine in a sterile, aqueous medium which comprises:

(i) carrying out the method of any one of claims 1 to 6, to give a commercial scale batch of 500 to 80,000 vials containing the contrast agent precursor as defined in any one of claims 1 to 3; and

(ii) provision of a container of sterile, aqueous solution for each vial suitable for reconstituting said vials of precursor from step (i) to give said contrast agent.

8. The method of claim 7, where the sterile aqueous solution is water for injection which contains less than 100 μM total concentration of free aluminium, barium, magnesium, calcium and zinc ions.

9. A method of preparation of an ultrasound contrast agent which comprises a suspension of microbubbles of perfluorobutane stabilised by hydrogenated egg phosphatidylserine in a sterile, aqueous medium which comprises:

(i) carrying out the method of any one of claims 1 to 6, to give a commercial scale batch of 500 to 80,000 vials containing the contrast agent precursor as defined in any one of claims 1 to 3; and

(ii) reconstituting the vials of precursor from step (i) with a sterile aqueous solution.