There is still a need for ultrasound lyophilised precursor preparation methods which give control over the vial-to-vial variation of the microbubble concentration and size distribution, yield and other important characteristics of the contrast agent. Such methods need to be amenable to commercial scale manufacture, and deliver precursor products with a uniformity of vial content and safety profile which satisfy the regulatory requirements (e.g. U.S. Pharmacopeia and ICH Guidelines).

The Present Disclosure.

Conventional microbubble ultrasound contrast agent preparation methods such as sonication, and high shear emulsification provide good yields and low cost production, with the capability of scale-up to commercial manufacture. They suffer from the disadvantage of difficulty in controlling the size distribution of the microbubbles. Whilst other preparation techniques have been developed which permit the preparation of such microbubbles with a tighter size distribution, these alternative methods are either lower-yielding, involve high cost equipment or are too slow to scale-up to commercial production methodology.

The present disclosure seeks to provide methods which are amenable to commercial scale manufacture, but which also give adequate control over the vial-to-vial variation of the microbubble concentration, size distribution, yield and other important characteristics of the contrast agent.

In an aspect, there is provided 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 the method comprising:

(i) removing microbubbles from an aqueous suspension of said perfluorobutane microbubbles stabilised by hydrogenated egg phosphatidylserine, wherein at least a portion of the removed microbubbles have a size below 2 μm and/or a size above 4 μm, and the resulting suspension has a median size of microbubbles in the range of 2 to 5 μm;

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

(iii) dispensing an aliquot of the composition from step (ii) into a vial to give a filled vial;

(iv) within a holding time period of less than 5 minutes after the dispensing of step (iii) is complete, cooling each such filled vial to a temperature of –40 °C to –70 °C;

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

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

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

(viii) sealing the vials from step (vii) 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:

(a) carrying out the method as defined above, to give a vial containing the contrast agent precursor; and

(b) provision of a container of sterile, aqueous solution suitable for reconstituting said vials of precursor from step (a) 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:

(a) carrying out the method as defined above, to give a vial containing the contrast agent precursor; and

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

The present inventors have found that, in production of batch sizes of thousands of vials of microbubble contrast agents, two key parameters are important to control variability: (a) microbubble size range prior to lyophilisation; and (b) the elapsed time between dispensing into a given vial and when the vial contents are frozen at -40 to -70 °C ("holding time").

The present disclosure provides a method of controlling both parameters, with consequent advantages of:

(i) optimal yield and concentration of microbubbles in each vial;

(ii) control over uniformity of content from vial to vial.

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;

the method comprising:

(i) removing microbubbles from an aqueous suspension of said perfluorobutane microbubbles stabilised by hydrogenated egg phosphatidylserine, wherein at least a portion of the removed microbubbles have a size below 2 μm and/or a size above 4 μm, and the resulting suspension has a median size of microbubbles in the range of 2 to 5 μm;

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

(iii) dispensing an aliquot of the composition from step (ii) into a vial to give a filled vial;

(iv) within a holding time period of less than 5 minutes after the dispensing of step (iii) is complete, cooling each such filled vial to a temperature of –40 °C to –70 °C;

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

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

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

(viii) sealing the vials from step (vii) 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 (ii) is 8-12% w/v.

4. The method of any one of claims 1 to 3, where the size adjustment of step (i) 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 (ii), the size distribution and microbubble concentration is checked before proceeding to the dispensing step (iii).

6. The method of any one of claims 1 to 5, where in step (iii), multiple vials are dispensed in parallel.

7. The method of any one of claims 1 to 6, where in step (iv), multiple filled vials are cooled in parallel.

8. The method of any one of claims 1 to 7, where the holding time period of step (iv) is less than 3 minutes.

9. The method of any one of claims 1 to 8, where the holding time period of step (iv) is the same for each filled vial.

10. The method of any one of claims 1 to 9, wherein after removing microbubbles in step (i) at least 89% of the microbubbles have a diameter between 2 and 4 μm.

11. 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:

(a) carrying out the method of any one of claims 1 to 10, to give a vial containing the contrast agent precursor; and

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

12. The method of claim 11, 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.

13. 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:

(a) carrying out the method of any one of claims 1 to 10, to give a vial containing the contrast agent precursor; and

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