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.

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 ºC ("holding time").

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 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 to 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

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

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.

The contrast agent precursors of the present disclosure have also been found to be less sensitive towards multivalent cations.

Without wishing to be bound by theory, the present inventors believe that, due to the high density increment between the liquid matrix (e.g. sucrose solution) and the gas-filled microbubbles, the microbubbles tend to segregate rapidly to the top of the liquid volume ("creaming"). It is this creaming that needs to be minimized and controlled in order to provide benefits (i) and (ii) described above. It is believed likely that a high concentration of microbubbles in the surface layer impairs the drying of the cake during lyophilisation, and/or that too high a local concentration depletes the survival rate of microbubbles, as they are trapped partially outside the final sucrose structure i.e. as holes in the surface rather than fully embedded gas pockets.

Detailed Description of the Invention.

In a first aspect, the present invention provides a method of preparation of an ultrasound contrast agent precursor which comprises a lyophilised composition of microbubbles of perfluorobutane stabilised by hydrogenated egg phosphatidylserine in sucrose; 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 to 5 μm;

(iii) diluting the size-adjusted suspension from step (ii) with a sterile aqueous sucrose 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;

(v) within a holding time period of less than 5 minutes after the dispensing of step (iv) is complete, cooling each such filled vial to a temperature of –40 ºC to –70 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 (viii) with perfluorobutane;

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