of steam to the headspace specifically allows for rapid heating of extremities such as inlet ports, sterile filters and tank tops and hence reduces the total heat load on the bulk material. Utilising headspace steam heating offers the following advantages:

Property	Advantage
Rapid, even heating through latent heat transfer	Improved heating of system extremities with ensuing lowering of the heat load inflicted on the bulk material, decreased degradation and improved product quality and productivity
Pressure control temperature	Target temperature can be quickly and precisely established
High heat transfer coefficient	Smaller required heat transfer surface area, enabling reduced initial equipment outlay.

Summary

In a first aspect, the present disclosure provides a method of sterilisation of a phospholipid suspension, which comprises:

(i) mixing said phospholipid together with propyleneglycol in an aqueous biocompatible carrier in a jacketed vessel to give an aqueous phospholipid suspension; and

(ii) autoclaving the aqueous phospholipid suspension from step (i), wherein said autoclaving enables reaching F_0 values > 15 in all parts of the sterilization system, and wherein heating, in addition to sensible heat from heating the jacket of said vessel, comprises the addition of steam to:

(a) the headspace of the vessel of step (i); or

(b) the aqueous phospholipid suspension of step (i); or

(c) a combination of (a) and (b);

(iii) cooling the hot suspension from step (ii) to 15 to 30 °C to give the sterile phospholipid suspension;

wherein said phospholipid is hydrogenated egg phosphatidylserine (H-EPS).

solvents. The aqueous solvent is suitably a biocompatible carrier. By the term "biocompatible carrier" is meant a fluid, especially a liquid, such that the composition is physiologically tolerable, i.e. can be administered to the mammalian body without toxicity or undue discomfort. The biocompatible carrier is suitably an injectable carrier liquid such as sterile, pyrogen-free water for injection; an aqueous solution such as saline (which may advantageously be balanced so that the final product for injection is isotonic); an aqueous buffer solution comprising a biocompatible buffering agent (e.g. phosphate buffer); an aqueous solution of one or more tonicity-adjusting substances (e.g. salts of plasma cations with biocompatible counterions), sugars (e.g. glucose or sucrose), sugar alcohols (e.g. sorbitol or mannitol), glycols (e.g. glycerol), or other non-ionic polyol materials (e.g. polyethyleneglycols, propylene glycols and the like). Preferably the biocompatible carrier is pyrogen-free water for injection (WFI), isotonic saline or phosphate buffer. Hence the aqueous suspension suitably excludes water-immiscible organic solvents.

The terms "comprising" or "comprises" have their conventional meaning throughout this application and imply that the agent or composition must have the essential features or components listed, but that others may be present in addition. The term 'comprising' includes as a preferred subset "consisting essentially of" which means that the composition has the components listed without other features or components being present.

In another aspect, there is provided 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 method comprises:

(i) carrying out the method as defined above, to obtain a sterile aqueous suspension of hydrogenated egg phosphatidylserine;

(ii) forming microbubbles of perfluorobutane in the suspension from step (i) to give an aqueous suspension of perfluorobutane microbubbles stabilised by hydrogenated egg phosphatidylserine;

(iii) diluting the 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;

(v) freeze-drying the filled vials from step (iv);

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

(vii) sealing each of the vials from step (vi) 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:

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

(ii) provision of a container of sterile, aqueous medium suitable for reconstituting said vial of precursor from step (i) to give said contrast agent;

wherein said ultrasound contrast agent comprises a suspension of microbubbles of perfluorobutane stabilised by hydrogenated egg phosphatidylserine in said sterile, aqueous medium.

In another aspect, there is provided a method of preparation of an ultrasound contrast agent which comprises:

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

(ii) reconstituting the vial of precursor from step (i) with a sterile aqueous solution;

wherein said ultrasound contrast agent comprises a suspension of microbubbles of perfluorobutane stabilised by hydrogenated egg phosphatidylserine in said sterile, aqueous medium.

Preferred embodiments.

In the method of the first aspect, the addition of steam is preferably to the headspace of the vessel of step (i). Thus, whilst steam injection directly into the liquid suspension is possible, addition to the headspace confers a much more controlled heating effect - since injection into the liquid could lead to local, pronounced degradation.

The method of the first aspect is preferably carried out such that the F_0 values reached are at least 15 and not more than 25. Thus, the present inventors have established that more prolonged autoclave heating times where F_0 reaches 30 lead to undue degradation of the phospholipid. That in turn has an adverse effect on the stability of the PFB microbubbles prepared therefrom.