CLAIMS

1. A method of manufacturing a freeze-dried composition suitable for the preparation of a suspension of stabilized gas-filled microbubbles, said composition comprising: (i) an amphiphilic material comprising a phospholipid and a fatty acid; and (ii) a polyethylene glycol as freeze-drying protecting component; which comprises:

a. preparing a liquid mixture comprising said amphiphilic material and said freeze-drying protecting component in a solvent;

b. freeze-drying the liquid mixture to remove said solvent and obtain a freeze-dried product; and

c. after completion of the freeze-drying of step b, heating said freeze-dried product at ambient pressure at a temperature higher than 35°C and up to 50°C, for a period of time of from eight to twenty hours.

2. The method according to claim 1 wherein said liquid mixture comprises said amphiphilic material and said freeze-drying protecting component dispersed in an organic solvent.

3. The method according to claim 1 wherein said liquid mixture comprises said amphiphilic material and said freeze-drying protecting component dispersed in an aqueous emulsion of a water immiscible solvent and water.

4. The method according to claim 1 wherein said heating step is carried out at a temperature of 38°C or higher.

5. The method according to claim 1 wherein said heating step is carried out at a temperature of 40°C or higher.

6. The method according to claim 1 wherein said phospholipid comprises dilauroyl-phosphatidylcholine (DLPC), dimyristoyl-phosphatidylcholine (DMPC), dipalmitoyl-phosphatidylcholine (DPPC), diarachidoyl-phosphatidylcholine (DAPC),

distearoyl-phosphatidylcholine (DSPC), dioleoyl-phosphatidylcholine (DOPC), 1,2 Distearoyl-*sn*-glycero-3-Ethylphosphocholine (Ethyl-DSPC), dipentadecanoyl--phosphatidylcholine (DPDPC), 1-myristoyl-2-palmitoyl-phosphatidylcholine (MPPC), 1-palmitoyl-2-myristoyl-phosphatidylcholine (PMPC), 1-palmitoyl-2-stearoyl-phosphatidylcholine (PSPC), 1-stearoyl-2-palmitoyl-phosphatidylcholine (SPPC), 1-palmitoyl-2-oleylphosphatidylcholine (POPC), 1-oleyl-2-palmitoyl-phosphatidylcholine (OPPC), dilauroyl-phosphatidylglycerol (DLPG) and its alkali metal salts, diarachidoylphosphatidyl-glycerol (DAPG) and its alkali metal salts, dimyristoylphosphatidylglycerol (DMPG) and its alkali metal salts, dipalmitoylphosphatidylglycerol (DPPG) and its alkali metal salts, distearoylphosphatidylglycerol (DSPG) and its alkali metal salts, dioleoyl-phosphatidylglycerol (DOPG) and its alkali metal salts, dimyristoyl phosphatidic acid (DMPA) and its alkali metal salts, dipalmitoyl phosphatidic acid (DPPA) and its alkali metal salts, distearoyl phosphatidic acid (DSPA), diarachidoylphosphatidic acid (DAPA) and its alkali metal salts, dimyristoyl-phosphatidylethanolamine (DMPE), dipalmitoylphosphatidylethanolamine (DPPE), distearoyl phosphatidyl-ethanolamine (DSPE), dioleylphosphatidyl-ethanolamine (DOPE), diarachidoylphosphatidyl-ethanolamine (DAPE), dilinoleylphosphatidylethanolamine (DLPE), dimyristoyl phosphatidylserine (DMPS), diarachidoyl phosphatidylserine (DAPS), dipalmitoyl phosphatidylserine (DPPS), distearoylphosphatidylserine (DSPS), dioleoylphosphatidylserine (DOPS), dipalmitoyl sphingomyelin (DPSP), and distearoylsphingomyelin (DSSP), dilauroyl-phosphatidylinositol (DLPI), diarachidoylphosphatidylinositol (DAPI), dimyristoylphosphatidylinositol (DMPI), dipalmitoylphosphatidylinositol (DPPI), distearoylphosphatidylinositol (DSPI) or dioleoyl-phosphatidylinositol (DOPI).

7. The method according to claim 1 wherein said fatty acid comprises capric (n-decanoic), lauric (n-dodecanoic), myristic (n-tetradecanoic), palmitic (n-hexadecanoic), stearic (n-octadecanoic), arachidic (n-eicosanoic), behenic (n-docosanoic) or n-tetracosanoic acid.

8. The method according to claim 1, wherein said amphiphilic material comprises DSPC, DPPG and palmitic acid.

9. The method according to claim 1, wherein said amphiphilic material comprises DSPC, DPPE-PEG5000 and palmitic acid.

10. The method according to claim 8, wherein said heating step is carried out at a temperature of from 40°C to 48°C.

11. The method according to claim 9, wherein said heating step is carried out at a temperature of from 36°C to 45°C.

12. The method according to claim 1, wherein said heating in step c is performed for at least twelve hours.

13. The method according to any of the preceding claims wherein said liquid mixture is sampled into glass vials which are loaded into a freeze-dryer.

14. The method according to claim 13 which comprises, at the completion of step b, saturating the headspace of the vials containing the freeze-dried product with a physiologically acceptable gas and then stoppering and sealing the vials.

15. A method of manufacturing a suspension of gas-filled microvesicles which comprises obtaining a freeze-dried product according to the method of any of claims 1 to 14 and reconstituting said freeze-dried product with a pharmaceutically acceptable liquid carrier in the presence of a physiologically acceptable gas under gentle agitation.

16. Use of the~~A method of diagnosing which comprises:~~

~~(i) manufacturing a~~ suspension of gas-filled microvesicles manufactured according to the method of claim 15 in the manufacture of a medicament for diagnostic~~;~~

~~(ii) administering to a patient said suspension of gas-filled microvesicles; and~~

~~(iii) detecting an~~ ultrasound imaging~~signal from a region of interest in said patient~~.

17. The use~~A method~~ according to claim 16, wherein the medicament is to be used for~~which comprises~~ ultrasound imaging of a~~the~~ heart.

18. The use~~A method~~ according to claim 16, wherein the medicament is to be used for~~which comprises~~ ultrasound imaging of a~~the~~ liver.

19. The use~~A method~~ according to claim 16, wherein the medicament is to be used for~~which comprises~~ ultrasound imaging of a urinary tract.

20. The suspension of gas-filled microvesicles manufactured according to the method of claim 15 for use in diagnostic ultrasound imaging.

21. The suspension of gas-filled microvesicles for use according to claim 20, wherein the diagnostic ultrasound imaging comprises ultrasound imaging of a heart, a liver or a unitary tract.