In another aspect, there is provided a method for preparing an ultrasound contrast agent, comprising the following steps:

(i) Homogenising perfluorocarbon continuously in a sterile aqueous dispersion of phospholipid to generate phospholipid-stabilised microbubbles of perfluorocarbon dispersed in an aqueous dispersion;

(ii) Adjusting the size distribution of microbubbles in the aqueous dispersion to a median size in the range of from 1 to 6 µm, preferably from 2 to 5 µm;

(iii) Optionally adding a tonicity agent to the aqueous dispersion;

(iv) Adding a buffering agent to the aqueous dispersion to adjust the bulk pH of the aqueous dispersion to a pH of from about 7.5 or above, preferably about 8.5 or above;

(v) Adjusting the concentration of microbubbles in the aqueous dispersion to achieve a target concentration of microbubbles of about 6-10 µl/ml;

(vi) Dispensing the aqueous dispersion into a vial and flushing the headspace of the vial with perfluorocarbon;

wherein the buffering agent is tris(hydroxymethyl)aminomethane (Tris); and

wherein the perfluorocarbon is perfluorobutane and the phospholipid is hydrogenated egg phosphatidyl serine.

In another aspect, there is provided an ultrasound contrast agent prepared according to the method as defined above.

Further, the present disclosure is directed to a method for improving the contrast of an ultrasonic image of tissue in a subject, a method for in vivo imaging of tissue in a subject, and a method for diagnosing of a subject, which methods comprise injecting an ultrasound contrast agent as described above into the subject.

~~In another aspect, there is provided a use of method for improving the contrast of an ultrasonic image of tissue in a subject, comprising injecting the ultrasound contrast agent as defined above into said subject and carrying out an ultrasound scan of said tissue.~~

~~In another aspect, there is provided a method for *in vivo* imaging of tissue in a subject, comprising injecting the ultrasound contrast agent as defined above into said subject, carrying out an ultrasound scan of said tissue and generating an image of said tissue.~~

~~In another aspect, there is provided a method for diagnosing of a subject, comprising injecting the ultrasound contrast agent as defined above into said subject, carrying out an ultrasound scan of a region of interest in said subject, generating an image of said region of interest and assessing said image in order to make a diagnosis.~~

In another aspect, there is provided the use of the ultrasound contrast agent as defined above in the manufacture of a medicament for improving the contrast of an ultrasonic image of tissue in a subject, the use further comprising an ultrasound scan of said tissue to be carried out.

In another aspect, there is provided the use of the ultrasound contrast agent as defined above in the manufacture of a medicament for *in vivo* imaging of tissue in a subject, the use further comprising an ultrasound scan of said tissue to be carried out and an image of said tissue to be generated.

In another aspect, there is provided the use of the ultrasound contrast agent as defined above in the manufacture of a medicament for diagnosing of a subject, the use further comprising an ultrasound scan of a region of interest in said subject to be carried out, an image of said region of interest to be generated and said image to be assessed in order to make a diagnosis.

In another aspect, there is provided an ultrasound contrast agent for use in a method as defined above.

In another aspect, there is provided the use of an ultrasound contrast agent as defined above for the manufacture of a medicament for use in a method as defined above.

The present disclosure also relates to an ultrasound contrast agent for use in a method as described herein.

CLAIMS

1. An ultrasound contrast agent comprising:
(a) microbubbles of perfluorocarbon, which microbubbles are stabilised by a membrane of phospholipid; and
(b) a buffering agent;
wherein the ultrasound contrast agent has a bulk pH of from about 7.5 or above, preferably about 8.5 or above;
wherein the buffering agent is tris(hydroxymethyl)aminomethane (Tris); and
wherein the perfluorocarbon is perfluorobutane and the phospholipid is hydrogenated egg phosphatidyl serine.

2. The ultrasound contrast agent according to claim 1, which is for long-term storage.

3. The ultrasound contrast agent according to any preceding claim, which is ready to use in a clinical setting, such as for *in vivo* imaging, diagnosing and/or treatment of a subject.

4. The ultrasound contrast agent according to any preceding claim, wherein the buffering agent has a concentration of from about 1 mM to about 10 mM.

5. The ultrasound contrast agent according to any preceding claim, wherein the membrane of phospholipid has a net negative charge.

6. The ultrasound contrast agent according to any preceding claim, wherein the ultrasound contrast agent further comprises a tonicity agent.

7. The ultrasound contrast agent according to any preceding claim, wherein the ultrasound contrast agent further comprises a viscosity agent, and/or a flotation-reducing agent.

8. A method for preparing an ultrasound contrast agent, comprising the following steps:
(i) Homogenising perfluorocarbon continuously in a sterile aqueous dispersion of phospholipid to generate phospholipid-stabilised microbubbles of perfluorocarbon dispersed in an aqueous dispersion;
(ii) Adjusting the size distribution of microbubbles in the aqueous dispersion to a median size in the range of from 1 to 6 µm, preferably from 2 to 5 µm;

(iii) Optionally adding a tonicity agent to the aqueous dispersion;
(iv) Adding a buffering agent to the aqueous dispersion to adjust the bulk pH of the aqueous dispersion to a pH of from about 7.5 or above, preferably about 8.5 or above;
(v) Adjusting the concentration of microbubbles in the aqueous dispersion to achieve a target concentration of microbubbles of about 6-10 µl/ml;
(vi) Dispensing the aqueous dispersion into a vial and flushing the headspace of the vial with perfluorocarbon;
wherein the buffering agent is tris(hydroxymethyl)aminomethane (Tris); and
wherein the perfluorocarbon is perfluorobutane and the phospholipid is hydrogenated egg phosphatidyl serine.

9. The method according to claim 8, wherein steps (iii) and (iv) are performed in any order.

10. The method according to any one of claims 8-9, wherein step (v) is performed before or after any one of steps (ii), (iii) and (iv), with the proviso that step (v) is performed after step (i) and before step (vi).

11. The method according to any one of claims 8-10, wherein the ultrasound contrast agent is for long-term storage and/or is ready to use in a clinical setting, such as for *in vivo* imaging, diagnosing and/or treatment of a subject.

12. The method according to any one of claims 8-11, which does not include a step of freeze-drying.

13. An ultrasound contrast agent prepared according to the method as defined in any one of claims 8-12.

14. Use of ~~A method for improving the contrast of an ultrasonic image of tissue in a subject, comprising injecting~~ the ultrasound contrast agent according to any one of claims 1-7 or 13 in the manufacture of a medicament for improving the contrast of an ultrasonic image of tissue in a subject, the use further comprising ~~into said subject and carrying out~~ an ultrasound scan of said tissue to be carried out.

15. ~~A method for *in vivo* imaging of tissue in a subject, comprising injecting~~Use of the ultrasound contrast agent according to any one of claims 1-7 or 13 in the manufacture of a medicament for *in vivo* imaging of tissue in a subject~~into said subject~~, the use further comprising ~~carrying out~~ an

ultrasound scan of said tissue to be carried out and ~~generating~~ an image of said tissue to be generated.

16. Use of ~~A method for diagnosing of a subject, comprising injecting~~ the ultrasound contrast agent according to any one of claims 1-7 or 13 in the manufacture of a medicament for diagnosing of a subject~~into said subject~~, the use further comprising ~~carrying out~~ an ultrasound scan of a region of interest in said subject to be carried out, ~~generating~~ an image of said region of interest to be generated and ~~assessing~~ said image to be assessed in order to make a diagnosis.

17. An ultrasound contrast agent for use in a method according to any one of claims 14-16.

18. Use of an ultrasound contrast agent according to any one of claims 1-7 or 13 for the manufacture of a medicament for use in a method according to any one of claims 14-16.