CLAIMS

1. A method for producing a homogeneous adjuvant formulation comprising a liposome bilayer comprising (a) monophosphoryl lipid A (MPLA), (b) a saponin, and (c) a liposome composition comprising (i) at least one phospholipid selected from phosphatidylcholine (PC) and/or phosphatidylglycerol (PG), wherein the phospholipid is selected from the group consisting of dimyristoyl phosphatidylcholine (DMPC), dipalmitoyl phosphatidylcholine (DPPC), distearyl phosphatidylcholine (DSPC), dimyristoyl phosphatidylglycerol (DMPG), dipalmitoyl phosphatidylglycerol (DPPG), distearyl phosphatidylglycerol (DSPG), and a combination thereof, and (ii) cholesterol wherein the mole percent concentration of the cholesterol in the liposome composition is greater than 50% (mol/mol),

said method comprising the steps of:

(i) dissolving the phospholipids, cholesterol and MPLA in an organic solvent to form an organic phase;

(ii) injecting the organic phase of step (i) into an aqueous phase at a specific flowrate and at a specific ratio of the organic phase to the aqueous phase to form a liposome;

(iii) stirring the liposome of step (ii) to form an intermediate liposome;

(iv) removing the organic phase of the intermediate liposome of step (iii);

(v) concentrating the intermediate liposome of step (iv); and

(vi) compounding the intermediate liposome of step (v) with a saponin to form a final adjuvant formulation having a size range of about 30-400 nm with a polydispersity of 0.05 to 0.5,

thereby producing the homogeneous adjuvant formulation.

2. The method of claim 1, wherein:

(a) in step (i) the phospholipids, cholesterol and MPLA are dissolved in the organic solvent by sonication, heat or a combination thereof, wherein the organic solvent is ethanol or isopropyl alcohol; or

(b) the aqueous phase comprises water or a buffer; or

(c) the flowrate of step (ii) is 0.5 mL/min to 400 mL/min or quick addition; or
(d) the intermediate liposome of step (iii) is stirred at a rate of 700 rpm to 900 rpm; or
(e) the ratio of organic phase to aqueous phase of step (ii) ranges from 1:4 to 1:16.

3. The method of claim 2, wherein:
(a) the organic phase is heated to a temperature between 45°C to 65°C; or
(b) the buffer comprises 10 mM phosphate at pH 6.2 containing 150 mM NaCl; or
(c) the aqueous phase is at a temperature between 20°C to 60°C.

4. The method of claim 1, wherein:
(a) the size of the intermediate liposome in step (iv) is downsized by using a high pressure extruder or a microfluid homogenizer; or
(b) the organic solvent is removed before downsizing of step (iv) or after downsizing of step (iv); or
(c) removing the organic phase of the intermediate liposome of step (iv) is by Tangential Flow Filtration (TFF); or
(d) the concentrating of step (v) is by Ultrafiltration; or
(e) the compounding of step (vi) is at a mixing speed of 300 rpm; or
(f) the compounding of step (vi) ranges from 1 hour to 24 hours; or
(g) the compounding of step (vi) occurs at room temperature or from 2-8°C; or
(h) the injecting of step (ii) is by pump or syringe injection.

5. The method of claim 4, wherein:
(a) the size of the liposome is downsized in step (iv) by using membrane sizes ranging from 50 nm to 120 nm or homogenization pressures between 17000 PSI and 24000 PSI, or a combination of both; or
(b) the TFF is TFF diafiltration; or
(c) the TFF comprises membranes having a molecular weight cut-off (MWCO) ranging from 100-500 kDa; or
(d) the Ultrafiltration comprises a bioburden reduction filter and a sterile filter.

6. The method of claim 1, wherein the final adjuvant formulation has a size range of about 30-400 nm and a polydispersity of 0.05 to 0.5.

7. A method for producing a homogeneous adjuvant formulation comprising a liposome bilayer comprising (a) monophosphoryl lipid A (MPLA), (b) a saponin, and (c) a liposome composition comprising (i) at least one phospholipid selected from phosphatidylcholine (PC) and/or phosphatidylglycerol (PG), wherein the phospholipid is selected from the group consisting of dimyristoyl phosphatidylcholine (DMPC), dipalmitoyl phosphatidylcholine (DPPC), distearyl phosphatidylcholine (DSPC), dimyristoyl phosphatidylglycerol (DMPG), dipalmitoyl phosphatidylglycerol (DPPG), distearyl phosphatidylglycerol (DSPG), and a combination thereof, and (ii) cholesterol wherein the mole percent concentration of the cholesterol in the liposome composition is greater than 50% (mol/mol),

said method comprising the steps of:

(i) dissolving the phospholipids, cholesterol and MPLA in an organic solvent or a mixture of organic solvents to form an organic phase;

(ii) injecting the organic phase of step (i) into an aqueous phase at a specific flowrate and at a specific ratio of the organic phase to the aqueous phase to form a liposome;

(iii) concentrating the intermediate liposome of step (ii);

(iv) removing the organic phase of the intermediate liposome of step (iii);

(v) filtering the intermediate liposome of step (iv), and

(vi) compounding the intermediate liposome of step (v) with a saponin to form a final adjuvant formulation having a size range of about 30-400 nm with a polydispersity of 0.05 to 0.5,

thereby producing the homogeneous adjuvant formulation.

8. The method of claim 7, wherein:

(a) in step (i) the phospholipids, cholesterol and MPLA are dissolved in the organic solvent by sonication, heat, stirring or a combination thereof, wherein the organic solvent is ethanol or isopropyl alcohol; or

(b) the aqueous phase comprises water or a buffer; or
(c) the flowrate of step (ii) is 12 mL/min.

9. The method of claim 8, wherein:
(a) the organic phase is heated to a temperature between 45°C to 65°C; or
(b) the buffer comprises 10 mM phosphate at pH 6.2 containing 150 mM NaCl; or
(c) the aqueous phase is at a temperature between 20°C to 65°C.

10. The method of claim 7, wherein:
(a) the intermediate liposome of step (vi) is stirred at a rate of 100 rpm to 1000 rpm; or
(b) the ratio of organic phase to aqueous phase of step (ii) ranges from 1:2 to 1:16; or
(c) removing the organic phase of the intermediate liposome of step (iv) is by Tangential Flow Filtration (TFF); or
(d) the concentrating of step (iii) is by Ultrafiltration; or
(e) the filtering of step (v) comprises a bioburden reduction filter and a sterile filter; or
(f) the compounding of step (vi) is at a mixing speed of 350 rpm for 1 hour at RT; or
(g) the injecting of step (ii) is by pump or syringe injection.

11. The method of claim 10, wherein:
(a) the ratio of organic phase to aqueous phase of step (ii) is 4:7; or
(b) the TFF is TFF diafiltration; or
(c) the TFF comprises membranes having a molecular weight cut-off (MWCO) ranging from 100-500 kDa.

12. The method of claim 7, wherein the final adjuvant formulation has a size range of about 50-200 nm and a polydispersity of 0.05 to 0.5.

13. A method for producing a heterogeneous adjuvant formulation comprising a liposome bilayer comprising (a) monophosphoryl lipid A (MPLA), (b) a saponin, and (c) a liposome composition comprising (i) at least one phospholipid selected from at least one phosphatidylcholine (PC) and/or phosphatidylglycerol (PG), wherein the phospholipid is

selected from the group consisting of dimyristoyl phosphatidylcholine (DMPC), dipalmitoyl phosphatidylcholine (DPPC), distearyl phosphatidylcholine (DSPC), dimyristoyl phosphatidylglycerol (DMPG), dipalmitoyl phosphatidylglycerol (DPPG), distearyl phosphatidylglycerol (DSPG), and a combination thereof, and (ii) cholesterol wherein the mole percent concentration of the cholesterol in the liposome composition is greater than 50% (mol/mol),

said method comprising the steps of:

(i) dissolving the phospholipids, cholesterol and MPLA in an organic solvent to form a lipid solution as an organic phase;

(ii) simultaneously injecting the organic phase of step (i) and an aqueous phase together at specific flowrates and at a specific ratio of the organic solvent phase to the aqueous phase to form a liposome;

(iii) concentrating the intermediate liposome of step (ii);

(iv) removing the organic phase of the intermediate liposome of step (iii);

(v) treating the intermediate liposome of step (iv) by microfluidizer for 10 passes at ~17500 psi;

(vi) filtering the intermediate liposome of step (v) using 0.22 um membrane; and

(vii) compounding the intermediate liposome of step (vi) with a saponin,

thereby producing the heterogeneous adjuvant formulation.

14. The method of claim 13, wherein:

(a) in step (i) the phospholipids, cholesterol and MPLA are dissolved in the organic solvent by sonication, heat, stirring or a combination thereof, wherein the organic solvent is ethanol or isopropyl alcohol or their mixture; or

(b) the aqueous phase comprises water or a buffer; or

(c) in step (ii) the organic phase has a flowrate of 1.333 ml/min and the aqueous phase has a flowrate of 10.667 ml/min (organic:aqueous = 1:8, two syringes used); or

(d) the intermediate liposome of step (iii) is stirred at a rate of 100 rpm to 900 rpm; or

(e) the ratio of organic phase to aqueous phase of step (ii) ranges from 1:4 to 1:8; or

(f) removing the organic phase of the intermediate liposome of step (iii) and step (iv) is by Tangential Flow Filtration (TFF); or

(g) the organic solvent is removed before microfluidizing of step (v); or
(h) the concentrating of step iii) is by Ultrafiltration; or
(i) a buffer is added to the compounding of step (vi); or
(j) the compounding of step (vii) is at a mixing speed of 350 rpm for 1 hour to 48 at RT or agitated for 1hr; or
(k) the injecting of step (ii) is by Nanoassemblr or pump or syringe injection.

15. The method of claim 14, wherein:
(a) the lipid formulation organic phase is heated to a temperature of 45- 65°C; or
(b) the buffer comprises 10 mM phosphate at pH 6.2 containing 150 mM NaCl; or
(c) the aqueous phase is at a temperature between 45-60°C; or
(d) the size of the intermediate liposome in step (iv) is treated by using a microfluidizer; or
(e) the TFF is TFF ultrafiltration and diafiltration; or
(f) the TFF comprises membranes having a molecular weight cut-off (MWCO) ranging from 100-500 kDa; or
(g); following the compounding of step (vii) the intermediate liposome is stored at RT without stirring for up to 48 hours.

16. The method of claim 13, wherein the final adjuvant formulation has a size of about 300 to 1000 nm and a polydispersity of 0.4-1.

17. A method for producing a heterogeneous adjuvant formulation comprising a liposome bilayer comprising (a) monophosphoryl lipid A (MPLA), (b) a saponin, and (c) a liposome composition comprising (i) at least one phospholipid selected from phosphatidylcholine (PC) and/or phosphatidylglycerol (PG), wherein the phospholipid is selected from the group consisting of dimyristoyl phosphatidylcholine (DMPC), dipalmitoyl phosphatidylcholine (DPPC), distearyl phosphatidylcholine (DSPC), dimyristoyl phosphatidylglycerol (DMPG), dipalmitoyl phosphatidylglycerol (DPPG), distearyl phosphatidylglycerol (DSPG), and a combination thereof, and (ii) cholesterol wherein the

mole percent concentration of the cholesterol in the liposome composition is greater than 50% (mol/mol),

said method comprising the steps of:

(i) dissolving the phospholipids, cholesterol and MPLA in an organic solvent to form a lipid formulation an organic phase;

(ii) injecting the organic phase of step (i) into an aqueous phase at a specific flowrate and at a specific ratio of the organic phase to the aqueous phase to form a liposome;

(iii) stirring the liposome of step (ii) to form an intermediate liposome;

(iv) concentrating the intermediate liposome of step (iii);

(v) removing the organic phase of the intermediate liposome of step (iv); and

(vi) compounding the intermediate liposome of step (vi) with a saponin,

thereby producing the heterogeneous adjuvant formulation.

18. The method of claim 17, wherein:

(a) in step (i) the phospholipids, cholesterol and MPLA are dissolved in the organic solvent by sonication, heat, stirring or a combination thereof, and wherein the organic solvent comprises ethyl acetate and isopropyl alcohol; or

(b) the aqueous phase comprises water or a buffer; or

(c) the flowrate of step (ii) is 20 mL/min; or

(d) the intermediate liposome of step (iii) is stirred at a rate of 100 rpm to 900 rpm; or

(e) the ratio of organic phase to aqueous phase of step (ii) ranges from 1:4 to 1:8; or

(f) the organic solvent is removed before compounding of step (vi); or

(g) removing the organic phase of the intermediate liposome of step (iv) and step (v) is by Tangential Flow Filtration (TFF); or

(h) the concentrating of step (vi) is by Ultrafiltration; or

(i) a buffer is added to the compounding of step (vi); or

(j) the compounding of step (vi) is at a mixing speed of 300 rpm for 1 hour at RT; or

(k) the injecting of step (ii) is by pipette, pump or syringe injection.

19. The method of claim 18, wherein:

(a) the lipid formulation organic phase is heated to a temperature from 50°C to 65°C: or

(b) the buffer comprises 10 mM phosphate at pH 6.2 containing 150 mM NaCl; or

(c) the aqueous phase is at a temperature of 20-25°C; or

(d) the TFF is TFF ultrafiltration and diafiltration; or

(e) the TFF comprises membranes having a molecular weight cut-off (MWCO) ranging from 100-500 kDa; or

(f) following the compounding of step (vii) the intermediate liposome is stored at RT without stirring for 24 hours.

20. The method of claim 17, wherein the final adjuvant formulation has a size range of 300 nm to 1000 nm and a polydispersity of 0.4 to 1.0.

21. A method for producing a heterogeneous adjuvant formulation comprising a liposome bilayer comprising (a) monophosphoryl lipid A (MPLA), (b) a saponin, and (c) a liposome composition comprising (i) at least one phospholipid selected from phosphatidylcholine (PC) and/or phosphatidylglycerol (PG), wherein the phospholipid is selected from the group consisting of dimyristoyl phosphatidylcholine (DMPC), dipalmitoyl phosphatidylcholine (DPPC), distearyl phosphatidylcholine (DSPC), dimyristoyl phosphatidylglycerol (DMPG), dipalmitoyl phosphatidylglycerol (DPPG), distearyl phosphatidylglycerol (DSPG), and a combination thereof, and (ii) cholesterol wherein the mole percent concentration of the cholesterol in the liposome composition is greater than 50% (mol/mol),

said method comprising the steps of:

(i) preparing a lyopilized organic phase comprising the phospholipids, cholesterol and MPLA;

(ii) rehydrating the lyophilized organic phase of step (i) with an aqueous phase to form an intermediate liposome;

(iii) Downsizing of the intermediate liposome of step (ii) using microfluidizer;

(iv) compounding the intermediate liposome of step (iii) with a saponin,

thereby producing the heterogeneous adjuvant formulation.

22. The method of claim 21, wherein:

(a) in step (i) the phospholipids, cholesterol and MPLA are dissolved in the organic solvent by sonication, heat, stirring or a combination thereof, and wherein the organic solvent comprises tert-butyl alcohol (TBA) or its mixture; or

(b) the aqueous phase comprises water or a buffer; or

(c) the intermediate liposome of step (ii) is stirred at a rate of 100-1000 rpm; or

(d) the size of the intermediate liposome in step (iii) is downsized with a microfluidizer and pressure of 18640 PSI; or

(e) a buffer is added to the compounding of step (iv); or

(f) the compounding of step (iv) is at a mixing speed of 300 rpm or by agitation for 1 hour at RT.

23. The method of claim 22, wherein:

(a) the lipid organic phase is heated to a temperature from 25°C to 65°C and wherein the organic phase solution is lyophilized; or

(b) the buffer comprises 10 mM phosphate at pH 6.2 containing 150 mM NaCl; or

(c) the aqueous phase is at a temperature from 20°C to 70°C; or

(d) following the compounding of step (iv) the intermediate liposome is stored at RT without stirring for 24 hours; or

(e) the organic solvent is removed before rehydration of step (ii) by lyophilization.

24. The method of claim 21, wherein the final adjuvant formulation has a size > 300nm and a polydispersity >0.4.

25. An adjuvant formulation produced by any one of the methods according to claims 1-24.

26. The adjuvant formulation of claim 25, wherein said saponin in the adjuvant formulation is QS-21.

27. An adjuvant formulation comprising monophosphoryl 3-deacyl lipid A phosphorylated hexaacyl disaccharide (3D-PHAD), QS-21, 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dimyristoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (DMPG), and cholesterol, wherein said adjuvant formulation has a size between about 30 nm and about 400 nm and a polydispersity between about 0.05 and about 0.5.

28. The adjuvant formulation of claim 27, wherein the adjuvant formulation comprises 3D-PHAD, QS-21, DMPC, DMPG, cholesterol, phosphate buffer, and sodium chloride.

29. The adjuvant formulation of claim 27, wherein the adjuvant formulation comprises 3D-PHAD at a concentration of about 0.4 mg/ml or about 0.8 mg/ml.

30. The adjuvant formulation of claim 27, wherein the adjuvant formulation comprises DMPC at a concentration of about 14 mg/ml or about 28 mg/ml.

31. The adjuvant formulation of claim 27, wherein the adjuvant formulation comprises DMPG at a concentration of about 1.6 mg/ml or about 3.2 mg/ml.

32. The adjuvant formulation of claim 27, wherein the adjuvant formulation comprises cholesterol at a concentration of about 11 mg/ml or about 22 mg/ml.

33. The adjuvant formulation of claim 27, wherein the adjuvant formulation comprises QS-21 at a concentration of about 0.2 mg/ml or about 0.4 mg/ml.

34. The adjuvant formulation of claim 27, wherein the adjuvant formulation comprises DMPC and DMPG at a mole ratio of DMPC to DMPG (mol/mol) of between about 10:1 and about 8:1.

35. The adjuvant formulation of claim 27, wherein the adjuvant formulation has a mole ratio of cholesterol: phospholipids between about 55:50 and about 55:40.

36. The adjuvant formulation of claim 27, wherein the adjuvant formulation has a 3D-PHAD:phospholipid mole ratio between about 1:80 and about 1:95.