CLAIMS

1. A method of producing a lipid-encapsulated RNA nanoparticle, comprising the steps

a) flowing an aqueous solution comprising an RNA through a 1st tube having an inner diameter (ID) of between 0.1" and 0.132″;

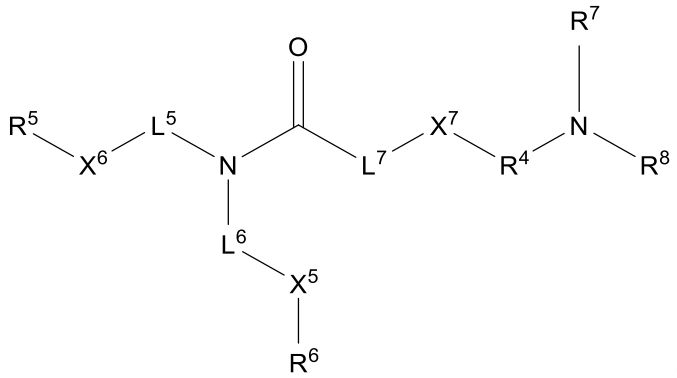
b) flowing an ethanol solution comprising lipids through a 2nd tube having an ID of between 0.005" and 0.02” at one third the flow rate of the aqueous solution through the 1st tube, wherein the lipids comprise a cationic lipid; and

c) mixing the ethanol solution with the aqueous solution by flowing the ethanol solution and the aqueous solution into a mixing module consisting of the 2nd tube perpendicularly joined to the 1st tube;

wherein the mixing produces an output solution flowing in the 1st tube comprising a turbulent flow of the RNA and the lipids in between 10% to 75% ethanol v/v;

wherein the lipid-encapsulated RNA nanoparticles have a bilayer structure; and,

wherein the cationic lipid has a pKa of 6 to 7 and a structure of Formula I:



(I)

or a pharmaceutically acceptable salt or solvate thereof, wherein

R^5 and R^6 are each independently selected from the group consisting of a linear or branched C_1-C_{31} alkyl, C_2-C_{31} alkenyl or C_2-C_{31} alkynyl and cholesteryl;

L^5 and L^6 are each independently selected from the group consisting of a linear C_1-C_{20} alkyl and C_2-C_{20} alkenyl;

X^5 is -C(O)O- or -OC(O)-;

X^6 is -C(O)O- or -OC(O)-;

X^7 is S or O;

L^7 is absent or lower alkyl;

R^4 is a linear or branched C_1-C_6 alkyl; and

R^7 and R^8 are each independently selected from the group consisting of a hydrogen and a linear or branched C_1-C_6 alkyl.

2. The method of claim 1, wherein the output has a flow rate of at least 200 ml/min.

3. The method of claim 1, wherein the flow rate of the output has a Reynolds number of at least 2,000.

4. The method of claim 1, wherein the aqueous solution is pumped through the 1st tube by a 1st HPLC pump with a back pressure of at least 10 psi, 25 psi, 50 psi, 75 psi, or 100 psi and the ethanol solution is pumped through the 2nd tube by a 2nd HPLC pump with a back pressure of at least 40 psi, 80 psi, 150 psi, 300 psi, or 400 psi.

5. The method of claim 1, wherein the 1st tube has an ID of 0.132" and the 2nd tube has an ID of 0.007", 0.01", or 0.02".

6. The method of claim 1, wherein the aqueous solution is pumped at a flow rate of at least 30 ml/min, 45 ml/min, 60 ml/min, 75 ml/min, 90 ml/min, 105 ml/min, 120 ml/min, 150 ml/min, 225 ml/min, 262.5 ml/min, 300 ml/min, or 450 ml/min; and the ethanol solution is pumped at a flow rate of at least 10 ml/min, 15 ml/min, 20 ml/min, 25 ml/min, 30 ml/min, 35 ml/min, 40 ml/min, 50 ml/min, 60 ml/min, or 75 ml/min, 87.5 ml/min, 100 ml/min, or 150 ml/min.

7. The method of claim 1, wherein the aqueous, ethanol, output solutions are maintained at 15-20 °C.

8. The method of claim 1, wherein the mixing module is made of stainless steel and consists of the 2nd tube mounted perpendicularly on the 1st tube, wherein the 1st tube has an opening through a wall, wherein the opening is the size of the outside diameter of the 2nd tube, and wherein

the 2nd tube is fitted over the opening to permit continuous movement of the ethanol solution in the 2nd tube into the aqueous solution in the 1st tube.

9. The method of claim 1, further comprising pumping a dilution buffer through a 3rd tube, and mixing the dilution buffer with the output solution by introducing the dilution buffer to the output solution in the region of a Y-connector connecting the 3rd tube to the 1st tube to produce a diluted output solution.

10. The method of claim 9, wherein the dilution buffer comprises

a) between 10 mM and 20 mM Tris buffer, between 45 mM and 55 mM NaCl, and between 8% and 10% sucrose, at a pH between 7.4 and 8.5; or

b) between 40 mM and 90 mM phosphate buffer, at a pH between 6.0 and 6.5; and the 3rd dilution buffer comprises between 20 mM; and, then

c) between 20 mM and 50 mM HEPES buffer, between 50 mM and 300 mM NaCl, between 0% and 15% sucrose, at a pH between 7.4 and 8.5.

11. The method of claim 9, wherein the diluted output solution comprises 6.25% ethanol; 8.25% ethanol; 8.3% ethanol; or 12.5% ethanol.

12. The method of claim 9, wherein the dilution buffer is pumped through the 3rd tube at a flow rate of 400-900 mL/min.

13. The method of claim 9, wherein the 3rd tube has an ID of 0.25".

14. The method of claim 1, wherein the lipid-encapsulated RNA nanoparticle has an average particle size of less than 100 nm.

15. The method of claim 1, wherein the lipid portion of the lipid-encapsulated RNA nanoparticle comprises 48 mol% to 66 mol% of a cationic lipid, 2 mol% to 12 mol% DSPC, 25 mol% to 42 mol% cholesterol, and 0.5 mol% to 3 mol% PEG2000-DMG.

16. The method of claim 1, wherein the lipid-encapsulated RNA nanoparticle has a total lipid:RNA weight ratio of 50:1 to 3:1.

17. The method of claim 1, wherein the lipid-encapsulated RNA nanoparticle comprises a cationic lipid:DOTAP (l,2-dioleoyl-3-trimethylammonium-propane):DSPC:cholesterol:PEG at molar percentages of 25:25:10:38.5:1.5, 25:25:10:37:3, 25:25:10:35:5, 20:20:7:51.5:1.5, 25:20:10:42:3, 20:30:13:32:5, 25:20:10:40:5, 25:25:13:35.5:1.5, 25:30:7:35:3, 30:20:13:34:3, 30:25:7:33:3, 30:30:10:25.8:1.5, 15:20:13:49:3, 20:20:13:44:3, 20:25:13:39:3, 15:25:13:44:3, 20:25:13:39:3, 25:25:13:34:3, 30:20:13:34:3, or 30:30:13:29:3.

18. The method of claim 1, wherein the lipid-encapsulated RNA nanoparticle comprises between 20 w/w% and 60 w/w% of the cationic lipid, between 5 w/w% and 30 w/w% of a helper lipid, between 0 w/w% and 60 w/w% of a cholesterol, and between 0.5 w/w% and 15 w/w% of a polyethylene glycol-lipid conjugate (PEG).

19. The method of claim 1, wherein the RNA is selected from the group consisting of a tRNA (transfer RNA), snRNA (small nuclear RNA), rRNA (ribosomal RNA), mRNA (messenger RNA), antisense RNA, siRNA (small interfering RNA) and self-replicating RNA.