Claims

1. A method of preparing homogenously sized lipid vesicles, said method comprising dispersing a first liquid phase in a second liquid phase;

wherein said first liquid phase comprises a lipid phase and said second liquid phase comprises an aqueous phase; or said first liquid phase comprises an aqueous phase and said second liquid phase comprises a lipid phase;

said method comprising controlling provision of the first liquid phase in a first flow direction to a membrane, said membrane defining a plurality of pores; and controlling provision of the second liquid phase to the membrane in a crossflow to the first flow direction, via the plurality of pores, to form a lipid vesicle suspension;

characterised in that more than 90% (volume weighted) of the homogenously sized lipid vesicles are less than about 220 nm with a polydispersity index of ≤0.

2. The method according to claim 1 wherein the first liquid phase comprises a lipid phase and the second liquid phase comprises an aqueous phase.

3. The method according to claim 1 wherein the first liquid phase comprises an aqueous phase and the second liquid phase comprises a lipid phase.

4. The method according to any one of the previous claims wherein the lipid vesicles are liposomes or lipid nanoparticles (LNPs).

5. A method of preparing homogenously sized lipid vesicles, said method comprising dispersing a first liquid phase in a second liquid phase, wherein said first liquid phase comprises a lipid phase;

wherein said method uses a crossflow emulsification apparatus; said crossflow emulsification apparatus (AXF) comprising:

an outer tubular sleeve provided with a first inlet at a first end; a lipid vesicle suspension outlet; and a second inlet, distal from and inclined relative to the first inlet;

a tubular membrane provided with a plurality of pores and adapted to be positioned inside the tubular sleeve; and

optionally an insert adapted to be located inside the tubular membrane, said insert comprising an inlet end and an outlet end, each of the inlet end and an outlet end being provided with chamfered region; the chamfered region is provided with a plurality of orifices and a furcation plate; and

controlling provision of the first liquid phase to the tubular membrane; and controlling provision of a second liquid phase to the tubular membrane via the plurality of pores to form a lipid vesicle suspension;

characterised in that more than 90% (volume weighted) of the homogenously sized lipid vesicles are less than about 220 nm with a polydispersity index of ≤0.

6. The method according to claim 7 wherein the lipid vesicles are liposomes or lipid nanoparticles (LNPs).

7. The method according to any one of the preceding claims wherein the aqueous phase includes one or more active agents.

8. The method according to any one of the preceding claims wherein the aqueous phase comprises a buffered solution.

9. The method according to claim 8 wherein the aqueous phase buffers include, but shall not be limited to, MES (2-(*N*-morpholino)ethanesulfonic acid), citrate, phosphate, acetate, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), TRIS (tris(hydroxymethyl)aminomethane) and PBS (phosphate-buffered saline); and combinations thereof.

10. The method according to any one of claims 5 to 9 wherein when the one or more active agents is hydrophobic, then the one or more active agents may be included in the lipid phase.

11. The method according to any one of the preceding claims wherein the lipid vesicles are produced unloaded and loaded afterwards (active loading).

12. The method according to any one of claims 1 to 10 wherein the lipid vesicles are produced loaded (passive loading).

13. The method according to claim 7 wherein the one or more active agents is a bioactive agents, such as a therapeutic agent (drug), vaccine and the like.

14. The method according to claim 13 wherein the bioactive agent is a therapeutic nucleic acid such as one encoding for an antigen.

15. The method according to claim 14 wherein therapeutic nucleic acids include, e.g., messenger RNA (mRNA), antisense oligonucleotides, ribozymes, DNAzymes, plasmids, immune stimulating nucleic acids, antagomir, antimir, mimic, supermir and aptamers.

16. The method according to claim 1 wherein the lipid vesicles are LNPs and the LNPs are ionisable or cationic LNPs.

17. The method according to any one of claims 1 to 16 wherein the lipid vesicles comprise cationic lipid vesicles, such as, DDA (dimethyl dioctadecyl ammonium bromide) or DOTAP (1,2-dioleoyl-3-trimethylammonium-propane) may suitably be used.

18. The method according to claim 1 wherein the lipid vesicles comprise neutral lipid vesicles and the neutral lipid vesicles comprise sphingosylphosphorylcholine (SPC), L-α-hydrogenated phosphatidylcholine (HSPC), distearoylphosphatidylcholine (DSPC) and 1-palmitoyl-2-oleoylphosphatidylcholine, (POPC), and the like; and combinations thereof.

19. The method according to claim 1 wherein the lipid vesicles comprise a lipid having a pKa in the range of 5.0 to 7.6 and the lipids include a tertiary amine.

20. The method according to claim 19 wherein the lipid vesicles comprise 1,2-dilinoleyloxy-N,N-dimethyl-3-aminopropane or 1,2-dilinolenyloxy-N,N-dimethyl-3-

aminopropane. Another suitable lipid having a tertiary amine is 1,2-dioleyloxy-N,Ndimethyl-3-aminopropane.

21. The method according to any one of the preceding claims wherein the lipid vesicles comprise a pegylated lipid.

22. The method according to claim 21 wherein the pegylated lipids include, but shall not be limited to, 1-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG), pegylated diacylglycerol (PEG-DAG), e.g. l-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG), a pegylated phosphatidylethanoloamine (PEG-PE), a PEG succinate diacylglycerol (PEG- S-DAG), e.g. 4-O-(2',3'-di(tetradecanoyloxy)propyl- 1 -O-(ώ-methoxy(polyethoxy)ethyl)butanedioate (PEG-S-DMG), a pegylated ceramide (PEG-cer), or a PEG dialkoxypropylcarbamate, e.g. ώ-methoxy(polyethoxy)ethyl-N-(2,3-di(tetradecanoxy)propyl)carbamate, 2,3-di(tetradecanoxy)propyl-N-(ώ-methoxy(polyethoxy)ethyl)carbamate or distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol) 2000]; and combinations thereof.

23. A method according to claim 5 wherein the apparatus includes the insert.

24. A method according to claim 5 wherein the crossflow apparatus comprises a plurality of tubular membranes.

25. A method according to claim 5 wherein the apparatus is suitable for preparing lipid vesicles with a PDI of from about 0.02 to about 0.3.

26. Lipid vesicles prepared by the method according to any one of claims 1 to 25.