lyophilization of liposomes are reported in the literature. However, these additional technical steps make manufacturing also more complicated and more expensive. In many cases, it is required that cryoprotectants are added, which may not be possible or desirable for certain products. For example, the presence of cryoprotectants and/or freezing/lyophilization itself may affect the product properties in an undesired way. Therefore, the long-term stabilization of liquid liposome preparations is still an unmet need. In this context, there is a considerable interest in techniques to minimize hydrolysis of liposomes or, more generally speaking, colloidally dispersed lipids in the liquid (aqueous) phase. This is particularly the case if the liposomes are intended for use as pharmaceutical products because in that case the stabilization method must fulfill the regulatory and technological requirements for such products. Most challenging in this context are products for parenteral (e.g. intravenous) administration, where, inter alia, certain criteria relating to sterility, selection of excipients, ion and pH conditions or particulate composition must be fulfilled.

The present invention aims at providing methods and means to increase the stability, particularly the chemical stability of lipids and/or liposomes formulated in aqueous formulations, thereby increasing the shelf-life stability of these formulations.

Summary

In one aspect, the present invention refers to a kit comprising (i) an aqueous liposome dispersion comprising liposomes comprising at least one lipid having one or more bonds selected from the group consisting of ester bonds, thioester bonds and amide bonds said liposomes being cationic at physiological pH, and at least one pH adjusting agent, wherein the aqueous liposome dispersion has a pH of between 2 and 4.5, and (ii) in a separate container, a pharmaceutically active nucleic acid, wherein the pharmaceutically active nucleic acid is provided in a buffered solution having a pH of between 6 and 8. In another aspect, the present invention refers to a method of preparing a pharmaceutical composition, the method comprising providing an aqueous liposome dispersion according to the present invention; and
- mixing the aqueous liposome dispersion with a pharmaceutically active nucleic acid, wherein the pharmaceutically active nucleic acid is provided in a buffered solution having a pH of between 6 and 8. In yet another aspect, the present invention refers to a method of chemically stabilizing an aqueous liposome dispersion

for increasing its shelf-life stability, said aqueous liposomes dispersion comprising liposomes comprising at least one lipid having one or more bonds selected from the group consisting of ester bonds, thioester bonds and amide bonds, said liposomes being cationic at physiological pH, the method comprising adjusting the pH of the aqueous liposome dispersion to a pH of between 2 and 4.5. Also described herein is an aqueous formulation comprising

- at least one lipid having one or more bonds selected from the group consisting of ester bonds, thioester bonds and amide bonds, and
- at least one pH adjusting agent,

wherein the aqueous formulation has a pH of between 2 and 5.5.

In one example, at least one of the lipids present in the aqueous formulation is a cationic lipid. In one example, the cationic lipid is a cationic lipid as defined herein.

In one example, the overall net charge of the lipids present in the aqueous formulation is positive.

Claims

1. A kit comprising

(i) an aqueous liposome dispersion comprising

- liposomes comprising at least one lipid having one or more bonds selected from the group consisting of ester bonds, thioester bonds and amide bonds, said liposomes being cationic at physiological pH, and
- at least one pH adjusting agent,

wherein the aqueous liposome dispersion has a pH of between 2 and 4.5, and

(ii) in a separate container, a pharmaceutically active nucleic acid, wherein the pharmaceutically active nucleic acid is provided in a buffered solution having a pH of between 6 and 8.

2. The kit according to claim 1, wherein the aqueous liposome dispersion has a pH of between 3 and 4.5, more preferably of between 3 and 4, and even more preferably of between 3.5 and 4.

3. The kit according to claim 1 or 2, wherein the at least one lipid having one or more bonds selected from the group consisting of ester bonds, thioester bonds and amide bonds comprises a glycerolipid and/or a glycerophospholipid.

4. The kit according to any of claims 1 to 3, wherein the at least one lipid having one or more bonds selected from the group consisting of ester bonds, thioester bonds and amide bonds comprises a cationic lipid and/or a non-cationic lipid.

5. The kit according to claim 4, wherein the cationic lipid is selected from the group consisting of 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP), 1,2-dioleoyloxy-3-dimethylammoniumpropane (DODAP) and analogues of these molecules having a different composition of the acyl chain moiety.

6. The kit according to claim 4 or 5, wherein the non-cationic lipid is a neutral lipid, wherein, preferably, the neutral lipid is selected from the group consisting of 1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dioleoyl-sn-glycero-3-

phosphocholine (DOPC), phosphatidylcholine (PC) and dimyristoyl phosphatidylcholine (DMPC).

7. The kit according to claim 4 or 5, wherein the non-cationic lipid is an anionic lipid, wherein, preferably, the anionic lipid is selected from the group consisting of phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidic acid (PA), phosphatidylglyerol (PG) and dimyristoyl phosphatidylglyerol (DMPG).

8. The kit according to any of claims 1 to 7, wherein the liposomes further comprise at least one lipid not having any ester bonds, thioester bonds or amide bonds.

9. The kit according to claim 8, wherein the at least one lipid not having any ester bonds, thioester bonds or amide bonds comprises a cationic lipid and/or a non-cationic lipid.

10. The kit according to claim 9, wherein the cationic lipid is selected from the group consisting of 1,2-di-O-octadecenyl-3-trimethylammoniumpropane (DOTMA), 1,2-dioleyloxy-N,N-dimethylaminopropane (DODMA), dioctadecyldi-methylammonium (DODA(Br)/DDAB), dioctadecyldimethylammoniumchloride (DODAC), 1,2-dimyristoyloxypropyl-1,3-dimethylhydroxyethylammonium (DMRIE), 2,3-dioleoyloxy-N-[2(spermine carboxamide)ethyl]-N,N-dimethyl-1-propanamium trifluoroacetate (DOSPA) analogues of these molecules having a different composition of the acyl chain moiety.

11. The kit according to claim 9 or 10, wherein the non-cationic lipid is a neutral lipid, wherein, preferably, the neutral lipid is selected from the group consisting of cholesterol (Chol) and sphingomyelin (SM).

12. The kit according to claim 9 or 10, wherein the non-cationic lipid is an anionic lipid.

13. The kit according to any of claims 1 to 12, wherein the liposomes comprise at least one cationic lipid and at least one non-cationic lipid.

14. The kit according to claim 13, wherein the molar ratio of the at least one cationic lipid to the at least one non-cationic lipid is from 1:4 to 4:1, preferably from 1:2 to 4:1.

15. The kit according to any of claims 1 to 14, wherein the liposomes comprise 1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine (DOPE) and at least one cationic lipid selected from the group consisting of 2-di-O-octadecenyl-3-trimethylammoniumpropane (DOTMA) and 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP).

16. The kit according to any of claims 1 to 15, wherein the at least one pH adjusting agent comprises an acid and/or an acidic buffer.

17. The kit according to claim 16, wherein the acid is a linear, branched or cyclic C_1-C_{28}, preferably C_1-C_{22}, carboxylic acid.

18. The kit according to claim 16 or 17, wherein the acid is selected from the group consisting of acetic acid, ascorbic acid, citric acid, hydrochloric acid, phosphoric acid, branched or unbranched, saturated, monounsaturated or polyunsaturated C_{12}-C_{28} fatty acids, preferably C_{12}-C_{22} fatty acids (e.g. oleic acid).

19. The kit according to any of claims 16 to 18, wherein the acidic buffer is based on an acid as defined in claim 16 or 17.

20. The kit according to any of claims 16 to 19, wherein the acidic buffer is selected from the group consisting of acetate buffer, citrate buffer, phosphate buffer and carbonate buffer.

21. The kit according to any of claims 1 to 20, wherein the at least one pH adjusting agent comprises acetic acid and/or acetate buffer.

22. The kit according to any of claims 1 to 21, wherein the at least one pH adjusting agent is present in an amount such that the molar ratio of total lipid to the at least one pH adjusting agent does not exceed 100:1.

23. The kit according to claim 22, wherein the at least one pH adjusting agent is present in an amount such that the molar ratio of total lipid to the at least one pH adjusting agent is from 10:1 to 1:10, preferably from 5:1 to 1:5, more preferably from 2:1 to 1:2, more preferably from 1.5:1 to 1:1.5, even more preferably about 1:1.

24. The kit according to any of claims 1 to 23, wherein the hydrolysis rate of the at least one lipid having one or more bonds selected from the group consisting of ester bonds, thioester bonds and amide bonds is reduced as compared to its hydrolysis rate at a pH of between 6 and 7.

25. The kit according to any of claims 1 to 24, wherein the at least one pH adjusting agent is bound to or forms part of the liposomes.

26. A method of preparing a pharmaceutical composition, the method comprising
- providing an aqueous liposome dispersion as defined in any of claims 1 to 25; and
- mixing the aqueous liposome dispersion with a pharmaceutically active nucleic acid, wherein the pharmaceutically active nucleic acid is provided in a buffered solution having a pH of between 6 and 8.

27. A method of chemically stabilizing an aqueous liposome dispersion for increasing its shelf-life stability, said aqueous liposome dispersion comprising liposomes comprising at least one lipid having one or more bonds selected from the group consisting of ester bonds, thioester bonds and amide bonds, said liposomes being cationic at physiological pH, the method comprising
- adjusting the pH of the aqueous liposome dispersion to a pH of between 2 and 4.5.

28. The method according to claim 27, wherein the chemical stabilization occurs by inhibition of ester bond, thioester bond and/or amid bond hydrolysis.

29. The method according to claim 27 or 28, wherein the pH is adjusted to a pH of between 3 and 4.5, more preferably of between 3 and 4, and even more preferably of between 3.5 and 4.

30. The method according to any of claims 27 to 29, wherein the pH of the aqueous liposome dispersion is adjusted by adding at least one pH adjusting agent, preferably at least one pH adjusting agent as defined in any of claims 16 to 23.

31. The method according to any of claims 27 to 30, wherein the at least one lipid having one or more bonds selected from the group consisting of ester bonds, thioester bonds and amide bonds is as defined in any of claims 3 to 7.

32. The method according to any of claims 27 to 31, wherein the liposomes further comprise at least one lipid not having any ester bonds, thioester bonds or amide bonds, wherein, preferably, the at least one lipid not having any ester bonds, thioester bonds or amide bonds is as defined in any of claims 9 to 12.

33. The method according to any of claims 27 to 32, wherein the liposomes comprise at least one cationic lipid and at least one non-cationic lipid.

34. The method according to claim 33, wherein the molar ratio of the at least one cationic lipid to the at least one non-cationic lipid is from 1:4 to 4:1, preferably from 1:2 to 4:1.

35. The method according to any of claims 27 to 34, wherein the liposomes comprise 1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine (DOPE) and at least one cationic lipid selected from the group consisting of 2-di-O-octadecenyl-3-trimethylammoniumpropane (DOTMA) and 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP).

36. The method according to any of claims 27 to 35, wherein the hydrolysis rate of the at least one lipid having one or more bonds selected from the group consisting of ester bonds, thioester bonds and amide bonds is reduced as compared to its hydrolysis rate at a pH of between 6 and 7.

37. The method according to any of claims 27 to 36, wherein the at least one pH adjusting agent is bound to or forms part of the liposomes.