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 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 liposome dispersion has a pH of between 2 and 5.5, 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 still another aspect, the present invention refers to a pharmaceutical composition prepared by the method of the present invention. In yet another aspect, the present invention refers to a method of chemically stabilizing an aqueous liposome dispersion comprising at least one lipid having one or more bonds selected from the group

consisting of ester bonds, thioester bonds and amide bonds, the method comprising adjusting the pH of the aqueous liposome dispersion to a pH of between 2 and 5.5. 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.

In one example, the aqueous formulation has a pH of between 2 and 5, preferably of between 2.5 and 5, more preferably of between 3 and 4.5, more preferably of between 3 and 4, and even more preferably of between 3.5 and 4.

In one example, 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.

In one example, 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.

In one example, 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.

In one example, 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).

In one example, 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).

In one example, the aqueous formulation further comprises at least one lipid not having any ester bonds, thioester bonds or amide bonds.

In one example, 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.

In one example, 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.

In one example, 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).

In one example, the non-cationic lipid is an anionic lipid.

In one example, the aqueous formulation comprises at least one cationic lipid and at least one non-cationic lipid.

In one example, 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.

In one example, the molar fraction of the at least one cationic lipid with respect to total lipid is at least 5%, preferably at least 10%, more preferably at least 20%.

In one example, the at least one pH adjusting agent comprises an acid and/or an acidic buffer.

In one example, the acid is a linear, branched or cyclic C_1-C_{28}, preferably C_1-C_{22}, carboxylic acid.

In one example, 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).

In one example, the acidic buffer is based on an acid as defined above.

In one example, the acidic buffer is selected from the group consisting of acetate buffer, citrate buffer, phosphate buffer and carbonate buffer.

In one example, the at least one pH adjusting agent comprises acetic acid and/or acetate buffer.

In one example, 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.

In one example, 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.

In one example, 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.

In one example, the lipids present in the aqueous formulation form liposomes.

In one example, the at least one pH adjusting agent is associated with the liposomes.

Also described herein is a method of preparing an aqueous formulation as defined above, the method comprising

- forming the liposomes in an aqueous solution comprising the at least one pH adjusting agent and having a pH of between 2 and 5.5 or
- adding the at least one pH adjusting agent to an aqueous solution comprising liposomes in order to adjust the pH of the aqueous solution to a pH of between 2 and 5.5.

Also described herein is a kit comprising an aqueous formulation as defined above.

In one example, the kit further comprises, in a separate container, a pharmaceutically active compound, wherein, preferably, the pharmaceutically active compound comprises a nucleic acid, preferably DNA or RNA.

In one example, the nucleic acid is provided in a buffered solution having a pH of between 6 and 8.

In yet another example, described herein is a method of preparing a pharmaceutical composition, the method comprising

- providing an aqueous formulation as defined above; and
- mixing the aqueous formulation with a pharmaceutically active compound.

In one example, the pharmaceutically active compound comprises a nucleic acid, preferably DNA or RNA, wherein, preferably, the nucleic acid is provided in a buffered solution having a pH of between 6 and 8.

In a further example, described herein is a pharmaceutical composition prepared by the method as defined above.

In another example, described herein is a method of chemically stabilizing 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, the method comprising

- adjusting the pH of the aqueous formulation to a pH of between 2 and 5.5.

In one example, the chemical stabilization occurs by inhibition of ester bond, thioester bond and/or amid bond hydrolysis.

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 as defined above.

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

In one example, the pH is adjusted to a pH of between 2 and 5, preferably of between 2.5 and 5, more preferably of between 3 and 4.5, more preferably of between 3 and 4, and even more preferably of between 3.5 and 4.

In one example, the pH of the aqueous lipid formulation is adjusted by adding at least one pH adjusting agent, preferably at least one pH adjusting agent as defined above.

In one example, 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 above.

In one example, the lipids present in the aqueous formulation form liposomes.

Brief Description of the Figures

Figure 1 shows the percentage of DOPE recovered from DOTMA/DOPE liposome dispersions prepared in dispersion solutions having different pH values. The liposome dispersion was stored at 37°C, and samples were taken at various time points.

Figure 2 shows the percentage of DOPE recovered from DOTMA/DOPE liposome dispersions with different pH values prepared in the presence of acetic acid (HAc) or various concentrations of acetic acid buffers (AcB). The dispersion were stored for 5 weeks at 40°C.

Figure 3 shows the percentage of DOPE recovered from stressed DOTMA/DOPE liposome dispersions after 5 or 6 weeks as a function of the pH value. Results from 3 independent experiments are shown.

Figure 4 shows the percentage of lipid recovery from DOTAP/DOPE liposomes after 2 weeks at 40°C as a function of the pH value.

Figure 5 shows the percentage of DOPE recovery from DOTMA/DOPE liposomes in water-for-injection with our without 5 mM acetic acid (HAc) after storage for 3 months at 5°C, 25°C or 40°C.

Claims

1. A kit comprising

(i) an aqueous liposome dispersion 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 liposome dispersion has a pH of between 2 and 5.5, 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 at least one of the lipids present in the aqueous liposome dispersion is a cationic lipid.

3. The kit according to claim 1 or 2, wherein the overall net charge of the lipids present in the aqueous liposome dispersion is positive.

4. The kit according to any of claims 1 to 3, wherein the aqueous liposome dispersion has a pH of between 2 and 5, preferably of between 2.5 and 5, more preferably of between 3 and 4.5, more preferably of between 3 and 4, and even more preferably of between 3.5 and 4.

5. The kit according to any of claims 1 to 4, 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.

6. The kit according to any of claims 1 to 5, 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.

7. The kit according to claim 6, 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.

8. The kit according to claim 6 or 7, 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).

9. The kit according to claim 6 or 7, 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).

10. The kit according to any of claims 1 to 9, wherein the aqueous liposome dispersion further comprises at least one lipid not having any ester bonds, thioester bonds or amide bonds.

11. The kit according to claim 10, 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.

12. The kit according to claim 11, 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.

13. The kit according to claim 11 or 12, 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).

14. The kit according to claim 11 or 12, wherein the non-cationic lipid is an anionic lipid.

15. The kit according to any of claims 1 to 14, wherein the aqueous liposome dispersion comprises at least one cationic lipid and at least one non-cationic lipid.

16. The kit according to claim 15, 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.

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

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

19. The kit according to claim 17 or 18, 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).

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

21. The kit according to any of claims 17 to 20, wherein the acidic buffer is selected from the group consisting of acetate buffer, citrate buffer, phosphate buffer and carbonate buffer.

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

23. The kit according to any of claims 1 to 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 does not exceed 100:1.

24. The kit according to claim 23, 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.

25. The kit according to any of claims 1 to 24, 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.

26. The kit according to any of claims 1 to 25, wherein the at least one pH adjusting agent is associated with the liposomes.

27. A method of preparing a pharmaceutical composition, the method comprising
- providing an aqueous liposome dispersion as defined in any of claims 1 to 26; 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.

28. A pharmaceutical composition prepared by the method according to claim 27.

29. A method of chemically stabilizing an aqueous liposome dispersion comprising at least one lipid having one or more bonds selected from the group consisting of ester bonds, thioester bonds and amide bonds, the method comprising
- adjusting the pH of the aqueous liposome dispersion to a pH of between 2 and 5.5.

30. The method according to claim 29, wherein the chemical stabilization occurs by inhibition of ester bond, thioester bond and/or amid bond hydrolysis.

31. The method according to claim 29 or 30, wherein at least one of the lipids present in the aqueous liposome dispersion is a cationic lipid.

32. The method according to any of claims 29 to 31, wherein the overall net charge of the lipids present in the aqueous liposome dispersion is positive.

33. The method according to any of claims 29 to 32, wherein the pH is adjusted to a pH of between 2 and 5, preferably of between 2.5 and 5, more preferably of between 3 and 4.5, more preferably of between 3 and 4, and even more preferably of between 3.5 and 4.

34. The method according to any of claims 29 to 33, 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 17 to 24.

35. The method according to any of claims 29 to 34, 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 5 to 9.