OBJECTS AND SUMMARY OF THE DISCLOSURE

[0007] It is an object of the present disclosure to provide inactivated virus compositions in which an inactivated whole Zika virus is stabilized during storage. In particular, it is an object of the present disclosure to provide inactivated virus compositions in which an inactivated whole Zika virus is stabilized during storage at – 80 °C for extended periods of time, such as e.g. storage for at least 10 days until e.g. 6 months or a year. Such compositions intended to be stored frozen in general do not (yet) contain an aluminium-based adjuvant such as an aluminum salt such as alum/aluminum hydroxide – if so desired, such aluminium-based adjuvants can be added later on, when no more frozen storage is scheduled.

[0008] It is a further object of the present disclosure to provide inactivated virus compositions that are able to stabilize an inactivated whole Zika virus during one or multiple freeze thaw cycles.

[0009] The above objects are achieved by examples of the present disclosure as described and claimed herein.

[0010] The present disclosure is, therefore, directed to a liquid inactivated virus composition comprising:

a) an inactivated whole Zika virus,
b) at least one pharmaceutically acceptable buffer with a concentration of at least about 6.5 mM, and
c) optionally a polyol,

wherein the liquid inactivated virus composition preferably does not contain an adjuvant selected from aluminum salts, and
said at least one pharmaceutically acceptable buffer does not comprise phosphate ions.

[0010a] In one aspect, the present disclosure provides a liquid inactivated virus composition comprising:

a) an inactivated whole Zika virus,
b) at least one pharmaceutically acceptable buffer with a concentration of at least about 6.5 mM, and
c) optionally a polyol,

wherein the liquid inactivated virus composition preferably does not contain an adjuvant selected from aluminum salts and

said at least one pharmaceutically acceptable buffer does not comprise phosphate ions, optionally wherein the concentration of phosphate ions in the liquid inactivated virus composition is less than about 7 mM, or less than about 6 mM, or less than about 5 mM, or less than about 4 mM, or less than about 3 mM, or less than about 2 mM, or less than about 1 mM.

[0010b] In another aspect, the present disclosure provides a liquid vaccine comprising:

a) the inactivated virus composition as described herein, and

b) an adjuvant such as aluminum hydroxide, optionally 100μg/ml to 800μg/ml aluminum hydroxide, or 200μg/ml to 600μg/ml aluminum hydroxide, or 300μg/ml to 500μg/ml aluminum hydroxide, or about 400μg/ml aluminum hydroxide based on elemental aluminum.

[0010c] In another aspect, the present disclosure provides a unit dose of the liquid vaccine as described herein, optionally comprising from about 1 μg to about 15 μg, or about 10 μg, or about 5 μg, or about 2 μg of the inactivated whole Zika virus.

[0011] In a certain example, the concentration of phosphate ions within the liquid inactivated virus composition is less than about 7 mM, or less than about 6 mM, or less than about 5 mM, or less than about 4 mM, or less than about 3 mM, or less than about 2 mM, or less than about 1 mM.

[0012] The present disclosure is also directed to the use of an inactivated virus composition comprising:

a) an inactivated whole Zika virus,

b) at least one pharmaceutically acceptable buffer with a concentration of at least about 6.5 mM, and

c) optionally a polyol,

wherein the liquid inactivated virus composition preferably does not contain an adjuvant selected from aluminum salts and

wherein said at least one pharmaceutically acceptable buffer does not comprise phosphate ions, for stabilizing the inactivated whole Zika virus.

[0012a] In another aspect, the present disclosure provides a use of a composition comprising:

a) at least one pharmaceutically acceptable buffer with a concentration of at least about 6.5 mM, and

b) optionally a polyol,

wherein the inactivated virus composition does not contain an adjuvant selected from aluminum salts and said at least one pharmaceutically acceptable buffer does not comprise phosphate ions, for stabilizing an inactivated whole Zika virus.

[0012b] In another aspect, the present disclosure provides the unit dose of vaccine as described herein, for use in therapy. In yet another aspect, the present disclosure provides a use of a unit dose of vaccine as described herein, in the manufacture of a medicament for preventing a Zika virus infection in a human subject, in need thereof.

[0013] In a further aspect, the present disclosure is further directed to a method of preparing a liquid inactivated virus composition comprising:

a) an inactivated whole Zika virus,

b) a pharmaceutically acceptable buffer, wherein the said buffer is not phosphate buffer and wherein the concentration of said buffer is at least 6.5 mM; and

c) optionally a polyol,

wherein the inactivated virus composition does not contain an adjuvant selected from aluminum salts, the method comprising the following steps:

Step 1. isolating a Zika virus preparation from supernatants obtained from one or more non-human cells;

Step 2. purifying the Zika virus preparation;

Step 3. inactivating the virus preparation;

Step 4. transferring the Zika virus preparation into a pharmaceutically acceptable buffer to obtain the Zika virus drug substance.

[0014] The present disclosure is further directed to a liquid vaccine comprising:

a) the inactivated virus composition in accordance with the disclosure, and

b) an adjuvant such as aluminum hydroxide.

[0015] In a certain example, the liquid vaccine comprises from about 50 mM to about 200 mM NaCl, from about 8.5 mM to about 80 mM Tris and from about 0.4% w/v to about 4.7% w/v sucrose.

[0016] The present disclosure is also directed to a method of treating or preventing, in particular preventing a Zika virus infection in a human subject in need thereof, comprising administering to the subject a unit dose of the liquid vaccine in accordance with the present disclosure, as described above.

[0017] In a further aspect, the present disclosure is further directed to a method of preparing a liquid vaccine, the method comprising the following steps:

Step 1. providing the inactivated virus composition according to the present disclosure, as described above,

Step 2. adding an adjuvant, the adjuvant preferably being an aluminum salt, and optionally a further pharmaceutically acceptable buffered liquid to the inactivated virus composition.

[0018] In a further aspect, the present disclosure is also directed to a product obtainable by the method described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] **FIG. 1** shows bright field microscopy images of Vero cell monolayers mock infected (top) or infected with ZIKAV strain PRVABC59 (bottom).

[0020] **FIG. 2** shows growth kinetics of ZIKAV PRVABC59 P1 on Vero cell monolayers, as determined by $TCID_{50}$.

[0021] **FIG. 3** shows potency assay testing ($TCID_{50}$) of Zika virus PRVABC59 P5 clones a-f.

[0022] **FIG. 4** shows bright-field microscopy images depicting the cytopathic effect (CPE) of growth of Zika virus PRVABC59 P6 clones a-f on Vero cell monolayers.

[0023] **FIG. 5** shows potency assay testing ($TCID_{50}$) of Zika virus PRVABC59 P6 clones a-f

[0024] **FIG. 6** shows an amino acid sequence alignment comparing the envelope glycoprotein sequence of Zika virus near residue 330 from Zika virus strains PRVABC59 P6e (SEQ ID NO: 8) and PRVABC59 (SEQ ID NO: 9) with several other flaviviruses (WNV (SEQ ID NO: 10); JEV (SEQ ID NO: 11); SLEV (SEQ ID NO: 12); YFV (SEQ ID NO: 13); DENV 1 16007 (SEQ ID NO: 14); DENV 2 16681 (SEQ ID NO: 15); DENV 3 16562 (SEQ IDNO: 16); and DENV 4 1036 (SEQ ID NO: 17)).

CLAIMS

1. A liquid inactivated virus composition comprising:

a) an inactivated whole Zika virus,

b) at least one pharmaceutically acceptable buffer with a concentration of at least about 6.5 mM, and

c) optionally a polyol,

wherein the liquid inactivated virus composition preferably does not contain an adjuvant selected from aluminum salts and

said at least one pharmaceutically acceptable buffer does not comprise phosphate ions, optionally wherein the concentration of phosphate ions in the liquid inactivated virus composition is less than about 7 mM, or less than about 6 mM, or less than about 5 mM, or less than about 4 mM, or less than about 3 mM, or less than about 2 mM, or less than about 1 mM.

2. The liquid inactivated virus composition of claim 1, wherein the liquid inactivated virus composition comprises only one pharmaceutically acceptable buffer.

3. The liquid inactivated virus composition of claim 1, wherein the liquid inactivated virus composition comprises at least two different pharmaceutically acceptable buffers, wherein the molar ratio of the two most concentrated pharmaceutically acceptable buffers in the liquid inactivated virus composition is not between 1:2 to 2:1, or not between 1:5 to 5:1, or not between 8:1 to 1:8, or not between 10:1 to 1:10.

4. The liquid inactivated virus composition of any one of the preceeding claims, wherein the concentration of potassium ions in the liquid inactivated virus composition is less than about 4 mM, or less than about 3 mM, or less than about 2 mM, or less than about 1.5 mM, or less than about 0.5 mM, or less than about 0.1 mM, or about 0 mM.

5. The liquid inactivated virus composition of any one of the preceeding claims, wherein the liquid inactivated virus composition is substantially free or free of protamine sulphate.

6. The liquid inactivated virus composition of any one of the preceeding claims,

wherein the pH of the liquid inactivated virus composition is from about pH 6.0 to about pH 9.0 or from about pH 6.5 to about pH 8.0, or from about pH 6.8 to about pH 7.8, or about pH 7.6, as determined at room temperature, and/or

wherein the concentration of the pharmaceutically acceptable buffer is at least about 7 mM, or at least about 7.5 mM, or at least about 8 mM, or at least about 8.5 mM, or at least about 9 mM, or at least about 10 mM, optionally wherein the concentration of the pharmaceutically acceptable buffer is from about 7 mM to about 200 mM, or from about 7.5 mM to about 200 mM, or from about 8 mM to about 200 mM, or from about 8.5 mM to about 200 mM, or from about 9 mM to about 100 mM, or from about 9 mM to about 60 mM, or about 10 mM, or about 20 mM, or about 50 mM.

7. The liquid inactivated virus composition of any one of the preceeding claims, wherein the pharmaceutically acceptable buffer comprises an amino group-containing molecule, optionally wherein the pharmaceutically acceptable buffer is Tris or Histidine buffer.

8. The liquid inactivated virus composition of any one of the preceding claims, wherein the composition further comprises at least one polyol, optionally wherein the liquid inactivated virus composition comprises from about 1% w/v to about 60% w/v of the polyol, or from about 6% w/v to about 50% w/v of the polyol, or from about 6% w/v to about 40% w/v of the polyol, or from about 6% w/v to about 35% w/v of the polyol, or from about 6% w/v to about 30% w/v of the polyol, or from about 6% w/v to about 25% w/v of the polyol, or from about 6% w/v to about 20% w/v of the polyol, or from about 6% w/v to about 15% w/v of the polyol, or from about 6% w/v to about 12% w/v of the polyol, or about 7% w/v of the polyol, or about 10% w/v of the polyol.

9. The liquid inactivated virus composition of claim 8, wherein the liquid inactivated virus composition comprises a pharmaceutically acceptable buffer comprising an amino group-containing molecule, and from about 6% w/v to about 15% w/v of a polyol, optionally wherein the liquid inactivated virus composition comprises Tris, and from about 6% w/v to about 15% w/v of a polyol.

10. The liquid inactivated virus composition of claim 8 or 9, wherein the polyol is a sugar, optionally wherein the sugar is a disaccharide such as sucrose.

11. The liquid inactivated virus composition of claim 10, wherein the liquid inactivated virus composition comprises from about 5% w/v to about 20% w/v sucrose, or from about 6% w/v to about 15% w/v sucrose, or from about 6% w/v to about 8% w/v sucrose, such as about 7% w/v sucrose.

12. The liquid inactivated virus composition of claim 11, wherein the liquid inactivated virus composition comprises from about 8.5 mM to about 50 mM Tris and from about 6% to about 15% w/v sucrose, wherein the pH of the inactivated virus composition is from about pH 7.0 to about pH 8.0, when measured at room temperature.

13. The liquid inactivated virus composition of claim 8, wherein the polyol is glycerol, optionally wherein the liquid inactivated virus composition comprises from about 1% v/v to about 60% v/v glycerol, or from about 7% v/v to about 15% v/v glycerol, or about 10% v/v of glycerol.

14. The liquid inactivated virus composition of claim 13, wherein the inactivated virus composition comprises from about 8.5 mM to about 50 mM Tris and from about 6% v/v to about 15% v/v glycerol, wherein the pH of the inactivated virus composition is from about pH 7.0 to about pH 8.0, when measured at room temperature.

15. The liquid inactivated virus composition of any one of the preceding claims, wherein the liquid inactivated virus composition further comprises sodium chloride, optionally wherein the concentration of sodium chloride in the liquid inactivated virus composition is from about 5 mM to about 500 mM sodium chloride, or from about 10 mM to about 200 mM sodium chloride, or from about 10 mM to about 40 mM, or from about 10 mM to about 30 mM, such as about 20 mM.

16. The liquid inactivated virus composition of any one of the preceding claims, wherein the Zika virus is an African lineage virus or an Asian lineage virus, optionally wherein the Zika virus is derived from strain PRVABC59.

17. The liquid inactivated virus composition of any one of the preceding claims, wherein the Zika virus is chemically inactivated, optionally wherein the Zika virus is an inactivated whole virus obtainable from a method wherein the Zika virus is treated with

formaldehyde in an amount that ranges from about 0.001% w/v to about 3.0% w/v from 5 to 15 days at a temperature that ranges from about 15 °C to about 37 °C.

18. A liquid vaccine comprising:

a) the inactivated virus composition according to any one of the preceding claims, and

b) an adjuvant such as aluminum hydroxide, optionally 100µg/ml to 800µg/ml aluminum hydroxide, or 200µg/ml to 600µg/ml aluminum hydroxide, or 300µg/ml to 500µg/ml aluminum hydroxide, or about 400µg/ml aluminum hydroxide based on elemental aluminum.

19. The liquid vaccine of claim 18, wherein the concentration of sodium chloride in the liquid vaccine is from about 50 mM to about 200 mM, or from about 60 mM to about 150 mM, such as about 84 mM, optionally wherein the liquid vaccine comprises from about 8.5 mM to about 80 mM Tris and from about 60 mM to about 150 mM NaCl, and wherein the pH of the liquid inactivated virus composition is from about pH 7.0 to about pH 8.0, when measured at room temperature and wherein the liquid vaccine comprises from about 0.4% (w/v) to 4.7% (w/v) sucrose.

20. A unit dose of the liquid vaccine according to claim 18 or 19, optionally comprising from about 1 µg to about 15 µg, or about 10 µg, or about 5 µg, or about 2 µg of the inactivated whole Zika virus.

21. Use of a composition comprising:

a) at least one pharmaceutically acceptable buffer with a concentration of at least about 6.5 mM, and

b) optionally a polyol,

wherein the inactivated virus composition does not contain an adjuvant selected from aluminum salts and said at least one pharmaceutically acceptable buffer does not comprise phosphate ions,

for stabilizing an inactivated whole Zika virus.

22. The use of claim 21, for stabilizing the inactivated whole Zika virus during storage at 5 ± 3 °C for at least 10 days, or for stabilizing the inactivated whole Zika virus during storage at -80 °C for at least 10 days or at least 6 months or at least 12 months.

23. The use of claim 21 or 22, for stabilizing the inactivated whole Zika virus during one or multiple freeze thaw cycles, such as at least 2 freeze thaw cycles, or at least 4 freeze thaw cycles.

24. The unit dose of vaccine of claim 20, for use in therapy.

25. Use of a unit dose of vaccine according to claim 20, in the manufacture of a medicament for preventing a Zika virus infection in a human subject, in need thereof.

26. A method of preparing a liquid inactivated virus composition comprising:

a) an inactivated whole Zika virus,
b) a pharmaceutically acceptable buffer, wherein the said buffer is not phosphate buffer and wherein the concentration of said buffer is at least 6.5mM; and
c) optionally a polyol;

wherein the inactivated virus composition preferably does not contain an adjuvant selected from aluminum salts; the method comprising the following steps:

Step 1. isolating a Zika virus preparation from supernatants obtained from one or more non-human cells,
Step 2. purifying the Zika virus preparation;
Step 3. inactivating the virus preparation;
Step 4. transferring the Zika virus preparation into a pharmaceutically acceptable buffer to obtain the inactivated virus composition.

27. A method of preparing a liquid vaccine, the method comprising the following steps:

Step 1. providing the inactivated virus composition of claims 1 to 17,
Step 2. adding an adjuvant preferably an aluminum salt and optionally a further pharmaceutically acceptable buffered liquid to the inactivated virus composition.

28. A product obtainable by the method according to claim 26 or 27.