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(54) **COMPOSITION FOR THE PRESERVATION OF VIRUSES**

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(57) **ABSTRACT**

The present invention relates to a composition for the preservation of a virus, the composition including a virus, a lipid and a cryoprotectant.

Figure 1

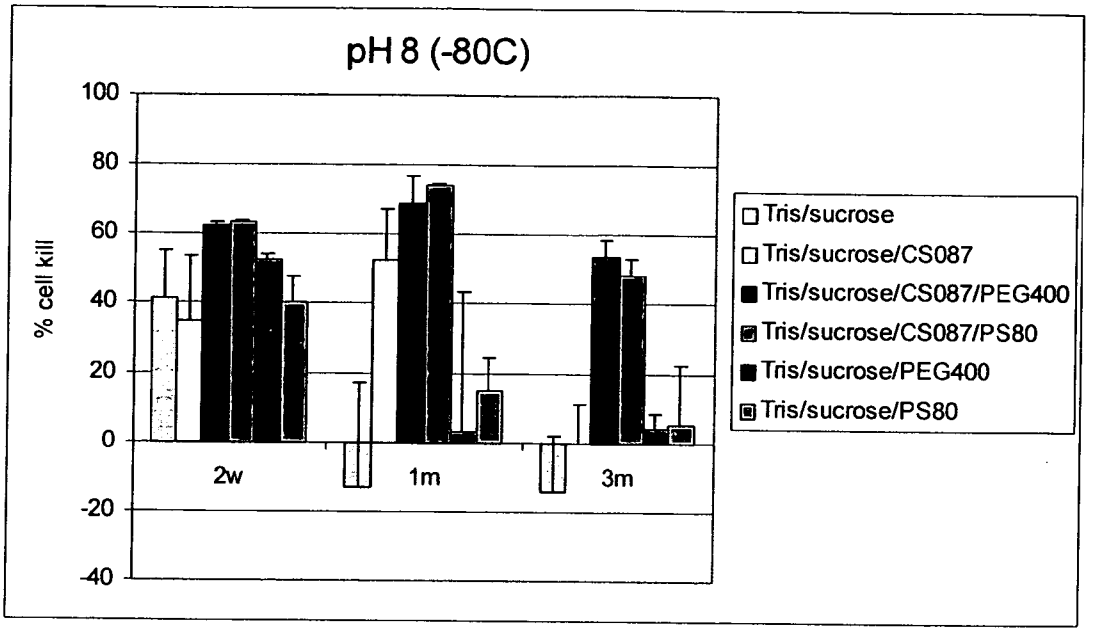

pH 8 (-80C)
% cell kill
100
80
60
40
20
0
-20
-40
2w
1m
3m
Tris/sucrose
Tris/sucrose/CS087
Tris/sucrose/CS087/PEG400
Tris/sucrose/CS087/PS80
Tris/sucrose/PEG400
Tris/sucrose/PS80

Figure 2

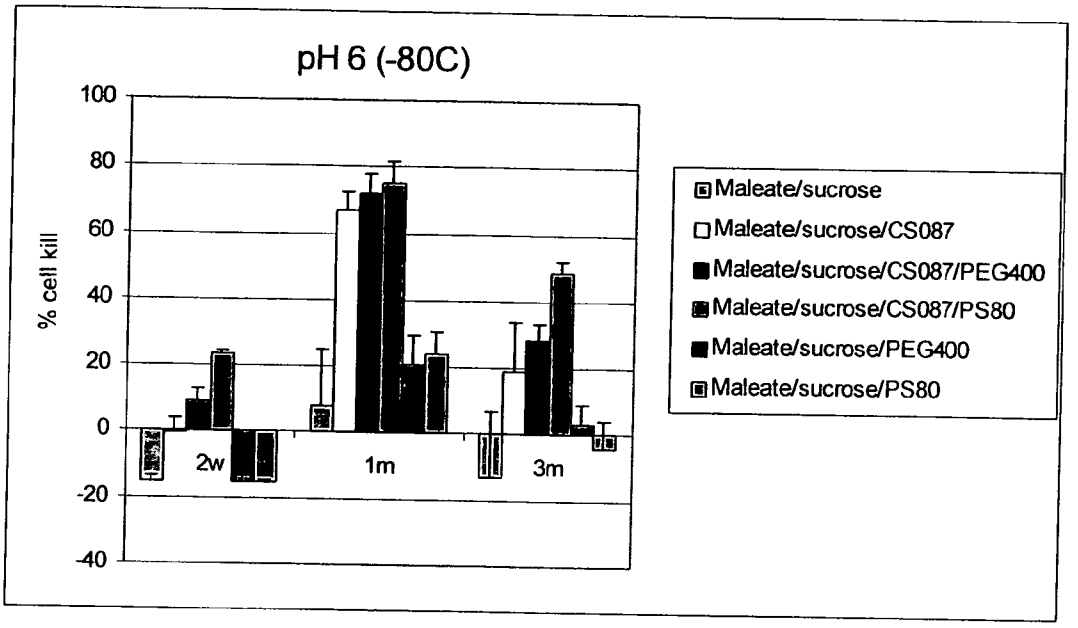

pH 6 (-80C)
% cell kill
100
80
60
40
20
0
-20
-40
2w
1m
3m
Maleate/sucrose
Maleate/sucrose/CS087
Maleate/sucrose/CS087/PEG400
Maleate/sucrose/CS087/PS80
Maleate/sucrose/PEG400
Maleate/sucrose/PS80

Figure 3

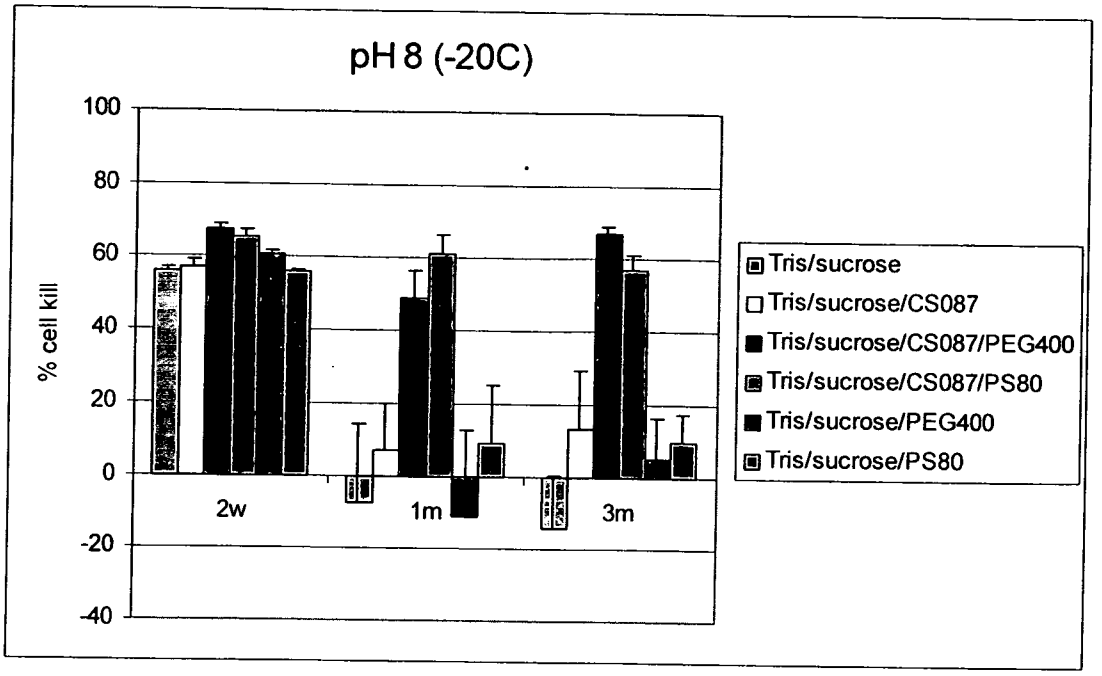

pH 8 (-20C)
% cell kill
100
80
60
40
20
0
-20
-40
2w
1m
3m
Tris/sucrose
Tris/sucrose/CS087
Tris/sucrose/CS087/PEG400
Tris/sucrose/CS087/PS80
Tris/sucrose/PEG400
Tris/sucrose/PS80

Figure 4

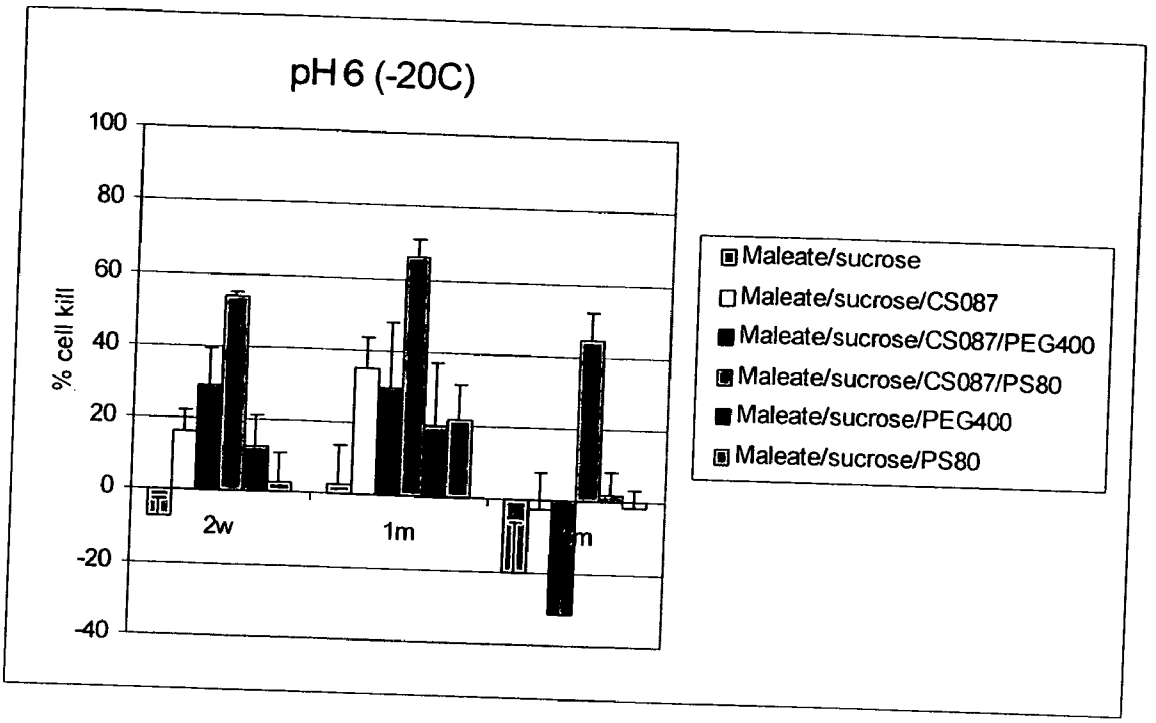

pH 6 (-20C)
% cell kill
100
80
60
40
20
0
-20
-40
2w
1m
Maleate/sucrose
Maleate/sucrose/CS087
Maleate/sucrose/CS087/PEG400
Maleate/sucrose/CS087/PS80
Maleate/sucrose/PEG400
Maleate/sucrose/PS80

Figure 5

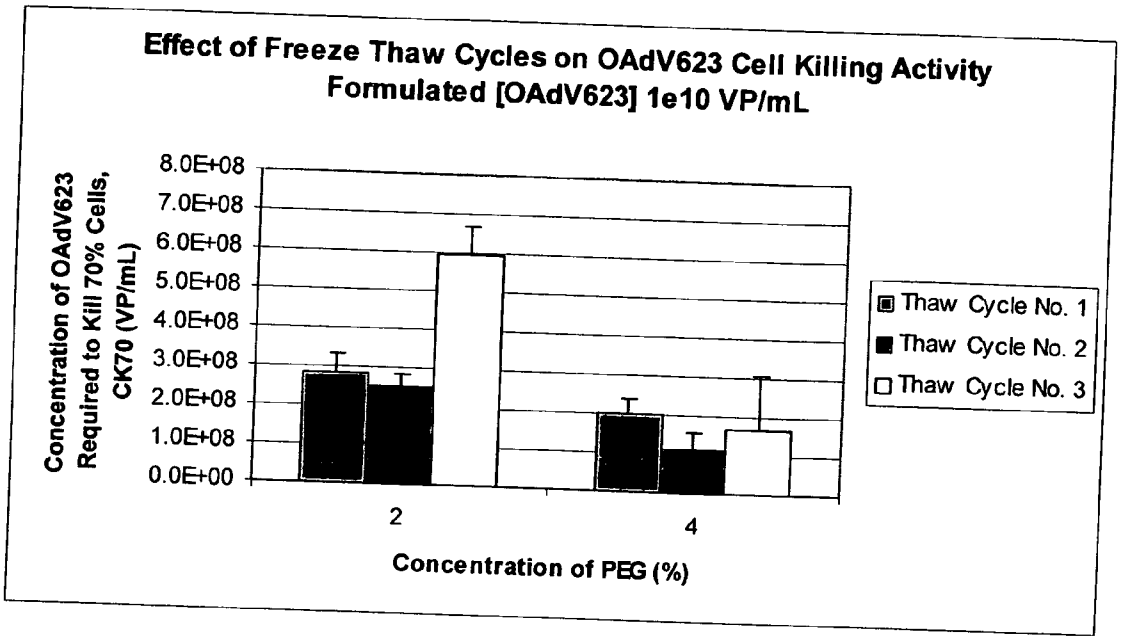

Effect of Freeze Thaw Cycles on OAdV623 Cell Killing Activity
Formulated [OAdV623] 1e10 VP/mL
Concentration of OAdV623 Required to Kill 70% Cells, CK70 (VP/mL)
8.0E+08
7.0E+08
6.0E+08
5.0E+08
4.0E+08
3.0E+08
2.0E+08
1.0E+08
0.0E+00
2
4
Concentration of PEG (%)
Thaw Cycle No. 1
Thaw Cycle No. 2
Thaw Cycle No. 3

Figure 6

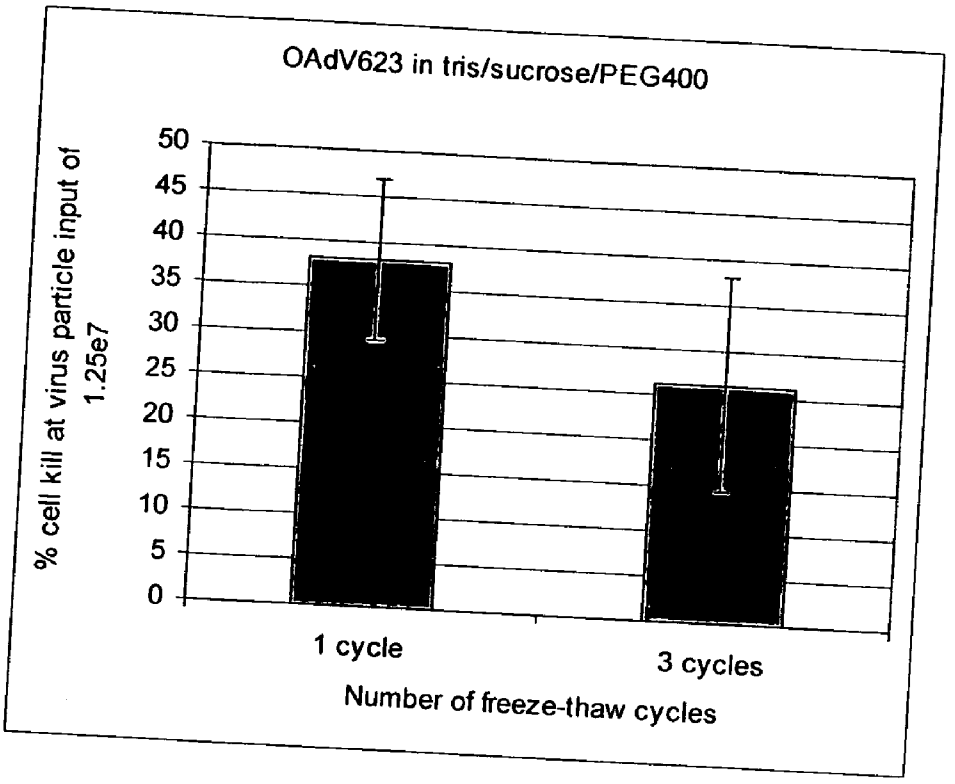

OAdV623 in tris/sucrose/PEG400
% cell kill at virus particle input of 1.25e7
50
45
40
35
30
25
20
15
10
5
0
1 cycle
3 cycles
Number of freeze-thaw cycles

Figure 7

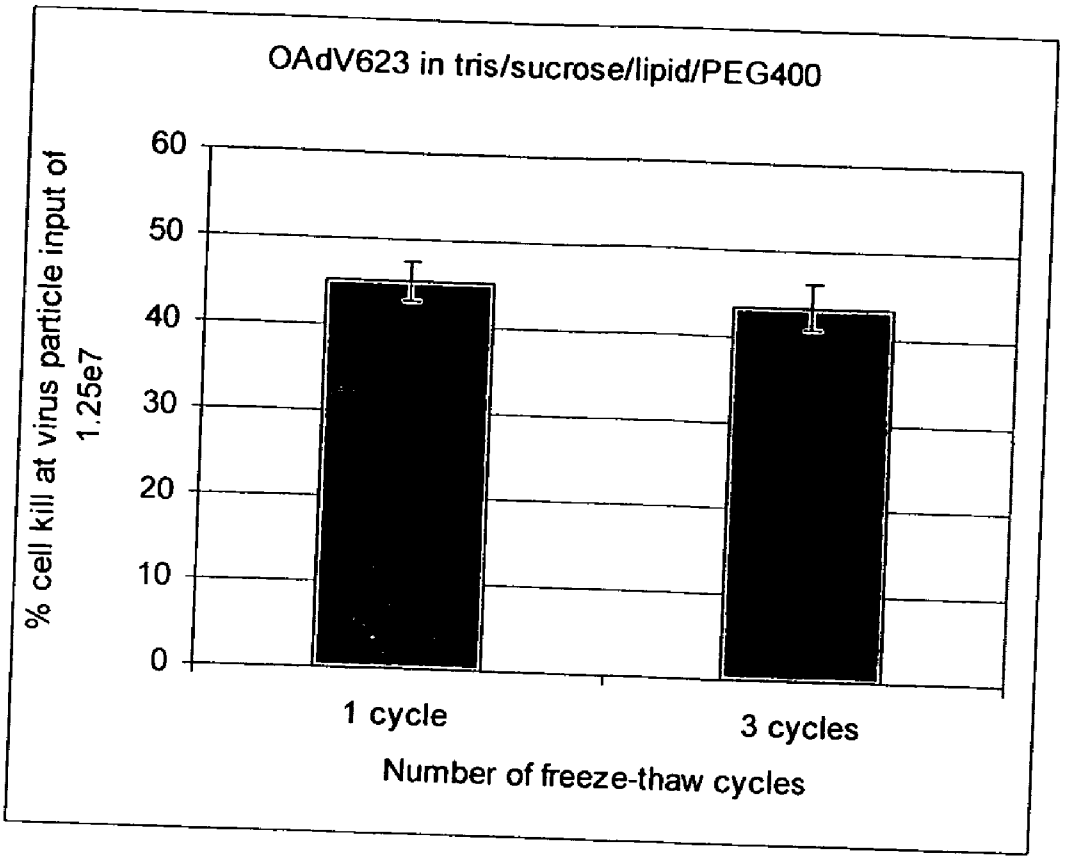

OAdV623 in tris/sucrose/lipid/PEG400
% cell kill at virus particle input of 1.25e7
60
50
40
30
20
10
0
1 cycle
3 cycles
Number of freeze-thaw cycles

Figure 8

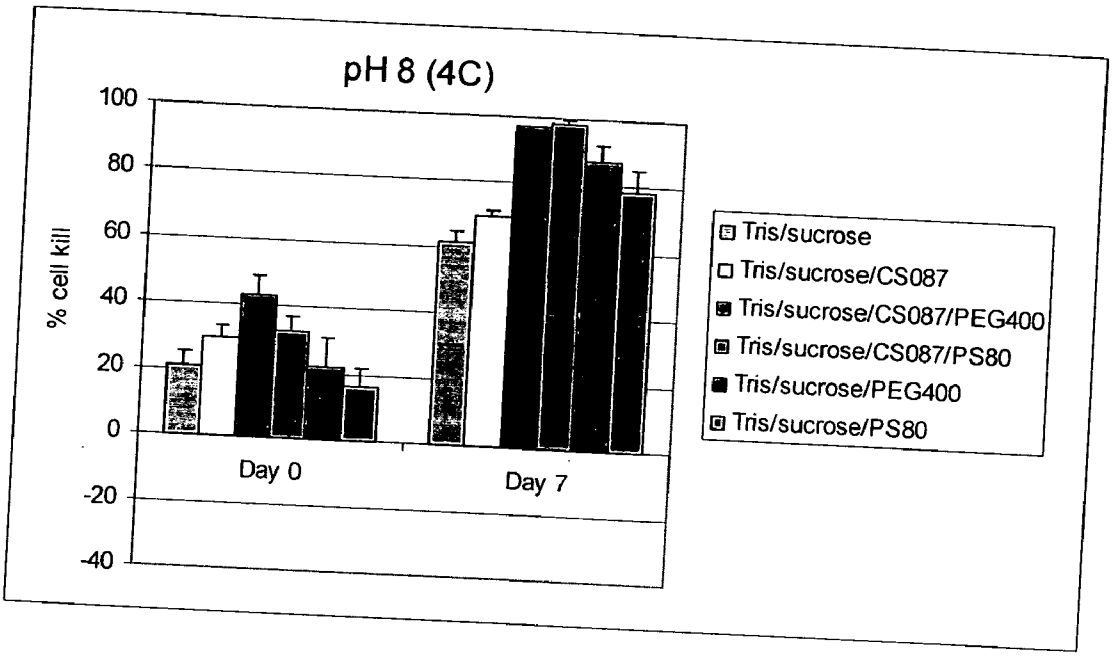

pH 8 (4C)
% cell kill
100
80
60
40
20
0
-20
-40
Day 0
Day 7
Tris/sucrose
Tris/sucrose/CS087
Tris/sucrose/CS087/PEG400
Tris/sucrose/CS087/PS80
Tris/sucrose/PEG400
Tris/sucrose/PS80

Figure 9

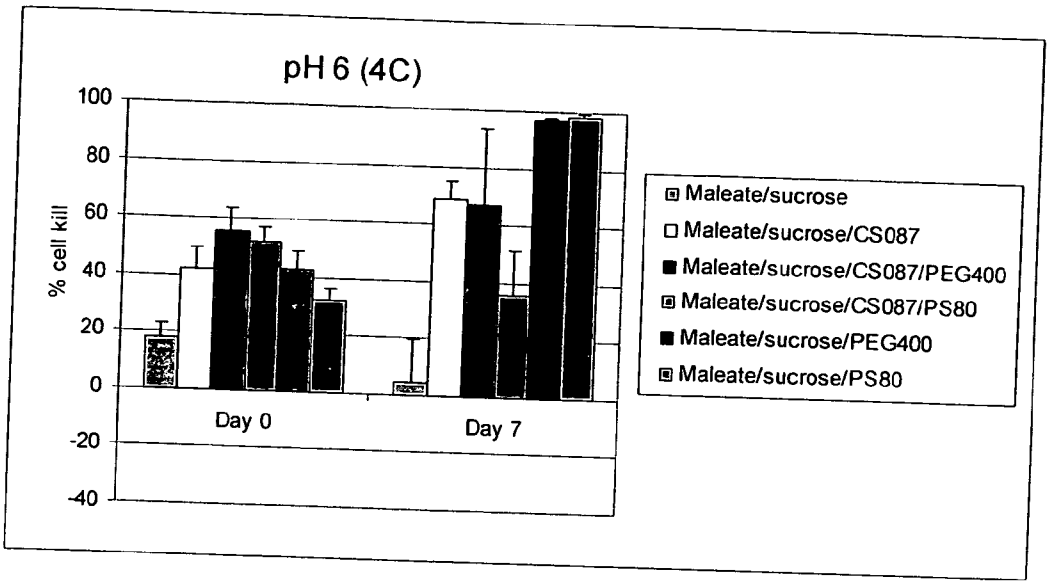
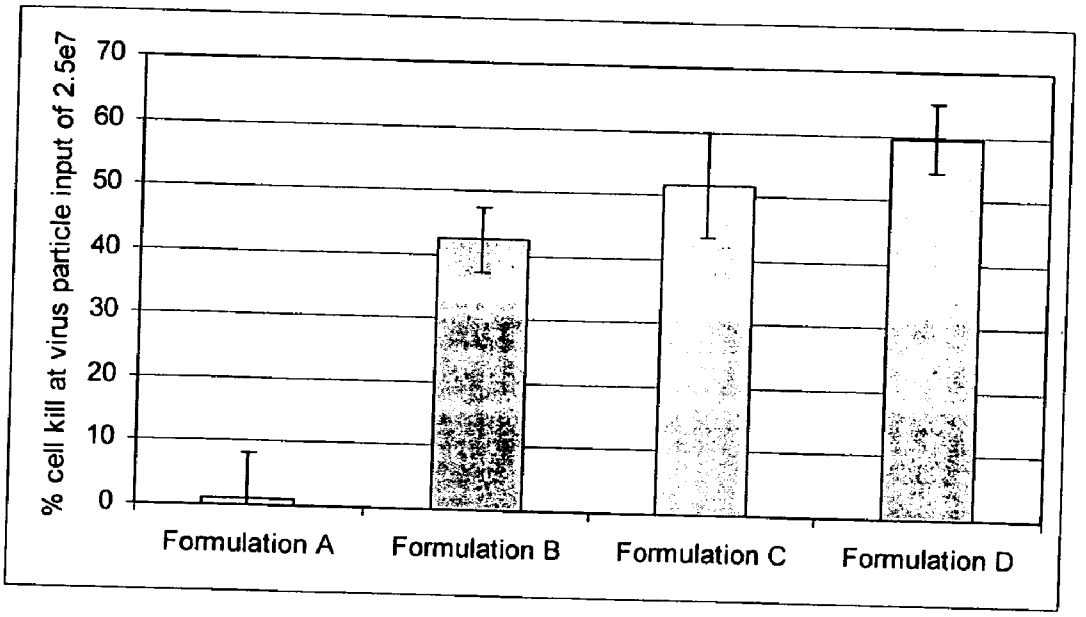

pH 6 (4C)
% cell kill
100
80
60
40
20
0
-20
-40
Day 0
Day 7
Maleate/sucrose
Maleate/sucrose/CS087
Maleate/sucrose/CS087/PEG400
Maleate/sucrose/CS087/PS80
Maleate/sucrose/PEG400
Maleate/sucrose/PS80

Figure 10


% cell kill at virus particle input of 2.5e7
70
60
50
40
30
20
10
0
Formulation A
Formulation B
Formulation C
Formulation D

COMPOSITION FOR THE PRESERVATION OF VIRUSES

[0001] This application is a Continuation-In-Part of co-pending application Ser. No. 10/317,171 filed on Dec. 12, 2002, the entire contents of which are hereby incorporated by reference and for which priority is claimed under 35 U.S.C. § 120.

FIELD OF THE INVENTION

[0002] The present invention relates to compositions for the preservation of viruses. The present invention also relates to methods for preparing compositions for the preservation of viruses.

[0003] It will become apparent from the following description that the viral compositions according to the present invention are most likely to be pharmaceutical compositions for the purposes of vaccination or the delivery of viral particles for gene therapy. However, it must be appreciated that the invention is not to be limited in its application to only pharmaceutical compositions.

BACKGROUND OF THE INVENTION

[0004] The use of viruses for purposes of vaccination is well known. However, viruses are also becoming increasingly important as tools for gene therapy, and for research and diagnosis. The increasing importance of viruses as tools for medical and other purposes has led to a need to develop viral compositions that may be manufactured, stored and used without compromising viral efficacy.

[0005] For example, viral compositions for vaccination must be able to maintain the immunogenicity of a virus, or the immunogenicity of a component of the virus. In the case of compositions of viruses to be used for gene therapy, it is critical that the efficacy of the live viral formulations carrying therapeutic transgenes be maintained.

[0006] The use of viruses for vaccination purposes generally involves the vaccination with live attenuated or altered viruses, vaccination with inactivated viruses, or vaccination with one or more immunogenic components of the virus. Examples of viral infections that are vaccinated against during childhood include varicella virus, poliovirus, measles virus, mumps virus, rubella viruses, and hepatitis viruses.

[0007] Because viruses are also highly efficient at infecting susceptible cells, viruses are now recognised as being useful vehicles for the transfer of therapeutic nucleic acids into cells for the purpose of gene therapy. Gene therapy broadly refers to the transfer of genetic material into cells and the expression of that material in those cells for a therapeutic purpose. The goal is to produce the desired protein in the appropriate quantity and the proper location. Although a variety of methods have been developed to deliver therapeutic nucleic acids to cells, many of these methods are limited by relatively inefficient transfer of the therapeutic nucleic acid to the target cells. Due to their efficiency at infecting cells, viruses are suitable vehicles for the transfer of therapeutic nucleic acids into cells for the purpose of gene therapy.

[0008] In this regard, viruses fall broadly into two distinct groups: those that integrate into the genome of transduced cells and those that do not. An integrating virus inserts its viral genome into host DNA to facilitate long-term gene expression. For a non-integrating virus the viral genome exists extra-chromosomally as an episome in the nucleus of transduced cells. Depending on the ability of the virus to replicate, the viral genome is either passed on faithfully to every daughter cell or is eventually lost during cell division.

[0009] Retroviruses and adeno-associated viruses (AAVs) may integrate into the host DNA to provide a steady level of expression following transduction and incorporation into the host genome. As the target DNA is replicated, so too is the inserted therapeutic gene embedded in the transferred chromosomal DNA. Thus, transduction via these vectors can produce durable gene expression. This can be advantageous in tumour vaccine strategies in which a steady level of gene expression may enhance efficacy.

[0010] In contrast, adenovirus and vaccinia virus vectors do not integrate into the host DNA but exist as episomes. Thus, a transferred gene is expressed without actual integration of the gene into the target cell genome. Generally, non-integrating viruses are used when transient gene expression is desired.

[0011] Examples of viruses that may be used to deliver nucleic acids to cells for gene therapy purposes include adenovirus, adeno-associated virus (AAV), retrovirus, herpes simplex virus, vaccinia virus, poliovirus, sindbis virus, HIV-1, avian leukosis virus, sarcoma virus, Epstein-Barr virus, papillomavirus, foamy virus, influenza virus, Newcastle disease virus, sendai virus, lymphocytic choriomeningitis virus, polyoma virus, reticuloendotheliosis virus, Theiler's virus, and other types of RNA and DNA viruses.

[0012] Because viruses are biological entities consisting of a nucleic acid encapsulated by a protein coat, they are susceptible to the same chemical and physical processes that may degrade or inactivate proteins and nucleic acids. For example, live or attenuated viruses are often very susceptible to damage, as any change in the conformation or integrity of one or more components of the virus coat or the encapsulated nucleic acid may lead to a loss of infectivity. Viruses and viral components for vaccination purposes are often not very stable and readily become degraded or non-immunogenic, particularly at room temperature.

[0013] As such, biopharmaceutical products containing compositions of viruses for vaccination or gene therapy usually require stringent conditions to avoid physicochemical degradation and to maintain biological activity. Degradation of viruses in such compositions may occur during isolation, production, purification, formulation, storage, shipping or delivery of the virus or components of the virus. Accordingly, biopharmaceutical compositions of viruses must be formulated to provide protection of the virus and its components against factors such as temperature, pH, pressure, oxidising agents, ionic content, light, radiation, ultrasound, and changes in phase (for example as occurs during freezing and thawing).

[0014] In addition to the factors already discussed, other factors such as viral concentration, the size and structure of the encapsulated nucleic acid, container composition, headspace gas, and number of freeze-thaw cycles may all affect the activity of viral compositions.

[0015] As a consequence, the utility of many viruses in biopharmaceutical preparations is often limited by the insta-

bility of compositions of the viruses, particularly upon storage. For example, the storage of live viruses at room temperature is particularly problematic, and prevents the use of many viral vaccines in areas where facilities for cold storage are not available or reliable. Indeed, the fact that many viral compositions must be stored at very low temperature, and cannot even be stored at standard freezer temperatures for substantial periods of time represents a serious impediment to the widespread clinical use of many viruses.

[0016] In addition, even when some viral compositions are stored at low temperature in the frozen state, a significant loss of infectivity may still occur over time. A further loss of infectivity may occur upon thawing of the frozen viral composition.

[0017] As will be also appreciated, the storage of products at standard freezer temperatures may also be problematic, because often such freezers undergo temperature cycling that may result in the viral composition being subjected to temperatures above freezing, and as such the compositions may undergo repeated cycles of freezing and thawing. Freeze-thawing may also occur during large scale production, handling or distribution.

[0018] The preparation and storage of viral compositions in the lyophilised, spray-dried or freeze-dried states may also be problematic, as there may be a loss of viral activity upon the change of phase from the liquid to solid state during preparation. A loss of viral activity may also occur upon reconstitution. The use of such forms has the additional disadvantage that upon reconstitution, the viral composition must generally be left for an extended period of time to reconstitute, usually at room temperature.

[0019] It would also be advantageous to develop viral compositions that can maintain the desired pH of the composition for extended periods of time despite being exposed to refrigeration temperatures and/or subjected to conditions such as freeze-thawing, especially the slow rate of freeze-thawing that may occur during large scale production, handling or distribution.

[0020] Finally, increasingly high concentrations of virus are also being required for vaccination and therapeutic purposes. However, the concentration of virus in a composition may present additional problems to the ability to preserve a virus. In particular, a high concentration of virus may contribute significantly to viral instability due to aggregation and/or precipitation.

[0021] Therefore for many viruses a deficiency has been the inability to formulate compositions that acceptably preserve the virus. Such deficiencies with the ability to preserve the activity of viral compositions often preclude their use for vaccination, gene therapy or for other purposes.

[0022] It is therefore an aim of the present invention to provide a composition for the improved preservation of viruses.

[0023] A reference herein to a patent document or other matter which is given as prior art is not to be taken as an admission that that document or matter was known or that the information it contains was part of the common general knowledge as at the priority date of any of the claims.

SUMMARY OF THE INVENTION

[0024] The present invention provides a composition for the preservation of a virus and/or a component of a virus, the composition including a virus and/or a component thereof, a lipid and a cryoprotectant.

[0025] The present invention also provides a method of producing a composition for the preservation of a virus and/or a component of a virus, the method including the step of preparing a liquid composition including a virus and/or a component thereof, a lipid and a cryoprotectant.

[0026] In the context of the present invention, it has been determined that the activity of a virus in a composition may be preserved by including in the composition a lipid and a cryoprotectant.

[0027] The composition according to the present invention provides for improved preservation of a virus and/or a component of a virus. For example, the composition provides for improved preservation of a virus (or a component thereof) upon storage, including storage of the virus (or a component thereof) in a liquid form, in a frozen form, or in a solid form such as lyophilised, spray-dried or freeze-dried states.

[0028] The composition also provides for improved preservation of a virus (or a component thereof) upon freezing and/or thawing. The improved preservation of is also evident over a broad range of storage temperatures and storage periods, and over multiple cycles of freezing and thawing ("freeze-thawing").

[0029] It is to be understood that while the composition according to the present invention may be used for the preservation of viable virus particles, the composition according to the present invention may also be used for the improved preservation of attenuated virus particles, killed virus particles, non-viable viral particles, synthetic viruses, or one or more components of viable, killed, non-viable or synthetic viruses.

[0030] Components of viruses include for example coat and internal proteins, coat and internal glycoproteins, coat or internal antigens, and nucleic acids.

[0031] It will also be appreciated that not only may the present invention be used for pharmaceutical compositions for medical applications, such as the delivery of viruses or viral constituents for vaccination, or the delivery of viruses for the purposes of gene therapy, the present invention may also be used for compositions for the preservation of viable viruses, attenuated viruses, killed viruses, non-viable viruses, synthetic viruses or components of such viruses for non-medical applications, such as the preservation of viral preparations for research and diagnostic applications.

[0032] Various terms that will be used throughout this specification have meanings that will be well understood by a skilled addressee. However, for ease of reference, some of these terms will now be defined.

[0033] The term "preservation" as used throughout the specification is to be understood to mean that a desired activity of a virus (eg immunogenicity, infectivity, or transduction ability), or a desired activity of an activity of a component of a virus (eg immunogenicity) does not decrease substantially over a given period of time, or that a

desired activity of a virus (or a component thereof does not decrease substantially after a particular treatment.

[0034] For example (i) the activity of a virus (or a component thereof) in a composition according to the present invention may not decrease substantially when the virus is stored in the liquid, frozen or solid forms for a given period of time; and/or (ii) the activity of the virus (or a component thereof) in a composition according to the present invention may not substantially decrease when the composition is freeze-thawed, or subjected to repeated cycles of freeze-thawing.

[0035] In the context of the present invention, the ability of a composition to preserve a virus (or a component thereof) is to be understood to be improved over similar compositions that do not contain a lipid, or compositions that do not contain a cryoprotectant. Accordingly, the composition according to the present invention will show an activity of the virus (or a component thereof) over a given period of time, or will show an activity of the virus (or a component thereof) after a particular treatment (eg freeze-thawing), that is higher than a similar composition that does not contain a lipid, or a similar composition that does not contain cryoprotectant.

[0036] In this regard, the demonstration of the preservation of a virus (or a component thereof) in a composition according to the present invention will be achieved by a suitable biological assay. As will be appreciated, given the degree of variability in biological systems, in determining the ability of a composition to preserve a virus, or a component thereof, sufficient repetitions of any biological assay will need to be performed to statistically demonstrate that the composition is able to preserve the virus or the component.

[0037] The term "virus" as used throughout the specification is to be understood to mean any natural, recombinant, in vitro packaged or synthetic virus, being a virus that is live, attenuated or inactivated.

[0038] The term "component of a virus" as used throughout the specification is to be understood to mean any part of a virus, including for example a coat protein, an internal protein, a coat glycoprotein, an internal glycoprotein, a coat antigen, an internal antigen, or a nucleic acid.

[0039] The term "viral composition" as used throughout the specification is to be understood to mean any composition that may be used for the preservation of a virus (or a part of a virus) for vaccination or therapeutic purposes, or for the preservation of virus (or part of a virus) generally. The term encompasses the composition according to the present invention with other additives, such as excipients.

[0040] The term "lipid" as used throughout the specification is to be understood to mean an amphiphilic molecule containing a substantially hydrophilic moiety coupled to a substantially hydrophobic moiety. The hydrophilic moiety will contain one or more substantially hydrophilic groups, and the hydrophobic moiety will contain one or more substantially hydrophobic groups. For example, the lipid may be any fatty acid and derivatives of fatty acids, glycerol-derived lipids including phospholipids, sphingosine-derived lipid (including ceramides, cerebrosides, gangliosides and sphingomyelins) and glycolipids, terpenes and their derivatives, long chain alcohols, lipids based on ethanolamine, and waxes.

[0041] The term "cryoprotectant" as used throughout the specification is to be understood to mean any molecule that has the function of substantially inhibiting the formation of ice crystals upon freezing of a liquid composition. In this regard, it will be understood that a molecule with cryoprotective function may also perform one or more additional functions in any particular composition (for example being a tonicity modifier or lyoprotectant). Accordingly, the demonstration that a molecule has a cryoprotectant capacity will be achieved by a suitable method known in the art to test whether the molecule has the ability to inhibit the formation of crystals upon the freezing of a liquid composition.

[0042] The term "surfactant" as used throughout the specification is to be understood to mean any compound that can reduce the interfacial tension between two immiscible phases. In this regard, it will be understood that a molecule with surfactant function may also perform one or more additional functions in any particular composition. Accordingly, the demonstration that a molecule has a surfactant capacity will be achieved by a suitable method known in the art to test whether the molecule has the ability to reduce the interfacial tension between two immiscible phases.

BRIEF DESCRIPTION OF THE DRAWINGS

[0043] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the office upon request and payment of the necessary fee.

[0044] FIGS. **1-10** are graphs which illustrate various data contained in the Examples.

GENERAL DESCRIPTION OF THE INVENTION

[0045] As mentioned above, the composition of the present invention provides for the improved preservation of a virus, and/or the preservation of a component of a virus.

[0046] Examples of viruses include Adenoviridae, including Mastadenovirus such as Human Adenovirus, and Atadenovirus such as Ovine Adenovirus; Herpesviridae; Poxyiridae including vaccinia, fowlpox, swinepox and sheeppox; Papovaviridae; Orthohepadnavirus; Parvoviridae including adeno-associated virus; Birnaviridae; Reoviridae; Flaviviridae; Picornaviridae including poliovirus; Togaviridae including Sindbis virus and Semliki Forest virus; Filoviridae; Paramyxoviridae; Rhabdoviridae; Arenaviridae; Bunyaviridae; Orthomyxoviridae; and Retroviridae including Lentivirus.

[0047] In one embodiment, the virus is from the Paramyxovirinae family including viruses of the genera Avulavirus (for example Newcastle disease virus), Henipavirus (for example Hendravirus and Nipahvirus), Morbillivirus (Measles virus, Rinderpest virus, Canine distemper virus, porcine distemper virus), Respirovirus (Sendai virus, Human parainfluenza viruses 1 and 3, viruses of the common cold) Rubulavirus (Mumps virus, Simian parainfluenza virus 5, Menangle virus, Tioman virus), and TPMV-like viruses (Tupaia paramyxovirus), a natural or recombinant derivative of any of these viruses, or a component of these viruses.

[0048] In another embodiment, the virus is from the Pneumovirinae family, including viruses of the genera Pneumovi-

rus (for example Human respiratory syncytial virus, Bovine respiratory syncytial virus) Metapneumovirus (for example Avian pneumovirus), Fer-de-Lance virus, Nariva virus Tupaia paramyxovirus, Salem virus, J virus, Mossman virus, Beilong virus, a natural or recombinant derivative of any of these viruses, or a component of these viruses.

[0049] In another embodiment, the virus is from the Togaviridae family, including viruses of the genera Alphavirus (for example Sindbis virus, Eastern equine encephalitis virus, Western equine encephalitis virus, Ross River virus, O'nyong'nyong virus), Rubivirus (for example Rubella virus), a natural or recombinant derivative of any of these viruses, or a component of these viruses.

[0050] In another embodiment, the virus is from the Herpesviridiae family, including viruses of the subfamily Alphaherpesvirinae, (Genus Simplexvirus; for example Human herpesvirus 1 or Herpes simplex; Genus Varicellovirus; for example Human herpesvirus 3 or Varicella-zoster virus; Genus Mardivirus; for example Gallid herpesvirus 2, Genus Iltovirus; for example Gallid herpesvirus 1; Subfamily Betaherpesvirinae, Genus Cytomegalovirus; for example Human herpesvirus 5, Genus Muromegalovirus; for example Murid herpesvirus 1, Genus Roseolovirus; for example Human herpesvirus 6); Subfamily Gammaherpesvirinae (Genus Lymphocryptovirus; for example Human herpesvirus 4 or Epstein-Barr virus; Genus Rhadinovirus; for example Human Herpesvirus 8, Saimiriine herpesvirus 2); Unassigned, (Genus Cercopithecine; for example Cercopithecine herpesvirus 1, Genus Ictalurivirus; for example Ictalurid herpesvirus 1), a natural or recombinant derivative of any of these viruses, or a component of these viruses.

[0051] Human herpesviridae (HHV) include HSV-1 (herpes simplex virus 1), HSV-2 (herpes simplex virus 2), VZV (varicella zoster virus), EBV (Epstein-Barr virus), CMV (cytomegalovirus), Human B-cell lymphotrophic virus or roseolovirus, HHV-7, and HHV-8.

[0052] In another embodiment, the virus is from the Hepadnaviridae family, including viruses of the genera Orthohepadnavirus (for example Hepatitis B virus) and Avihepadnavirus (for example Duck hepatitis B virus), a natural or recombinant derivative of any of these viruses, or a component of these viruses.

[0053] In another embodiment, the virus is from the Picornaviridae family, including viruses of the genera Enterovirus (for example Poliovirus), Rhinovirus (for example Human rhinovirus A), Hepatovirus (for example Hepatitis A virus), Cardiovirus (for example Encephalomyocarditis virus), Aphthovirus (for example Foot-and-mouth disease virus), Parechovirus (for example Human parechovirus), Erbovirus (for example Equine rhinitis B virus), Kobuvirus (for example Aichi virus), Teschovirus (for example Porcine teschovirus), a natural or recombinant derivative of any of these viruses, or a component of these viruses.

[0054] In another embodiment, the virus is from the Orthomyxoviridae family, including viruses of the genera Influenzavirus A, Influenzavirus B, Influenzavirus C, Isavirus, Thogotovirus, a natural or recombinant derivative of any of these viruses, or a component of these viruses.

[0055] In another embodiment, the virus is from the Flaviviridaefamily, including viruses of the Flavivirus (for example Yellow fever virus, West Nile virus, Dengue Fever), Hepacivirus (Hepatitis C virus), Pestivirus (Bovine viral diarrhea virus, classical swine fever, hog cholera), a natural or recombinant derivative of any of these viruses, or a component of these viruses.

[0056] In another embodiment, the virus particle is derived from the Adenoviridae family of viruses. In another embodiment, the virus is an Atadenovirus, such an ovine atadenovirus.

[0057] As discussed above, the virus may be for example a natural virus, a derivative of a natural virus, or a recombinant virus.

[0058] In one embodiment, the virus is a virus and/or a component of a virus, that is used for vaccination, such as measles virus.

[0059] Accordingly, in one embodiment the compositions according to the invention are suitable for use in a vaccine.

[0060] In another embodiment, the present invention also provides a vaccine formulation including a virus and/or a component of a virus, a lipid and a cryoprotectant.

[0061] In another embodiment, the virus is a virus, and/or a component of a virus, that has utility for the purposes of gene therapy. For example, the virus may be a recombinant ovine adenovirus, such as the adenoviral vector OAdV623 or derivatives of this vector. OAdV623 encodes the purine nucleoside phosphorylase (PNP) gene which catalyses the conversion of the immunosuppressive prodrug Fludarabine to the toxic 2-fluoro-adenine product.

[0062] Adenoviral vector OAdV623 is as described in Lockett L. J. and Both G. W. (2002) *Virology* 294:333-341.

[0063] As discussed previously, the composition according to the present invention may be used for the preservation of viruses that retain the ability to infect or transduce cells, or for the preservation of viral particles that have been attenuated, killed, are non-viable, have been produced by in vitro packaging or are of synthetic origin.

[0064] The composition according to the present invention may also be used for the preservation of part of a virus, such as the preservation of one or more constituents of the virus coat.

[0065] In this regard, an attenuated virus is to be understood to mean a virus whose virulence has been lowered by a biological, physical or chemical process. For example, the virulence of a virus may be attenuated by passaging through a semi-permissive host.

[0066] A killed virus is to be understood to mean a viral particle that has been inactivated by a treatment so that the viral particle no longer retains the ability to infect a permissive host. Examples of treatments that may kill a viral particle are heat or chemical modification.

[0067] A non-viable virus is to be understood to mean a viral particle that is not able to infect or transduce permissive host cells.

[0068] A synthetic virus is to be understood to mean any nucleic acid packaged with a protein and/or lipid coat.

[0069] In one embodiment, the composition according to the present invention may be used for the preservation of viruses that are to be used for the purposes of eliciting an immunogenic response, such as for vaccination. It will be understood in this regard that the composition may be used for the preservation of whole viruses, or for the preservation of one or more immunogenic constituents of a virus, such as the preservation of one or more protein, polypeptides or other antigens that make up part of the virus coat or internal constituents of the virus.

[0070] In another embodiment, the composition according to the present invention may be used for the preservation of viruses that are to be used for therapeutic applications. In one embodiment, the composition is for the preservation of viruses that are to be used for the purposes of gene therapy. In a further embodiment, the composition is for the preservation of viruses that are to be used for the delivery of therapeutic nucleic acids, such as the delivery of therapeutic nucleic acids to prostatic cells for gene therapy.

[0071] When the composition according to the present invention is used for the preservation of a virus to be used for medical applications, the composition may also include one or more pharmaceutically acceptable additives, such as pharmaceutically acceptable salts, amino acids, polypeptides, polymers, solvents, buffers and bulking agents.

[0072] The composition according to the present invention may be a liquid or solid composition. In the case of a liquid composition, the composition may be a substantially aqueous composition, or a composition composed of one or more other solvents.

[0073] The composition may be stored in a container suitable for the preservation of the virus, such as borosilicate glass. The composition may also be stored under a gaseous atmosphere that is suitable for the preservation of the virus including air, argon or nitrogen.

[0074] As discussed previously, the composition according to the present invention may also be used for the preservation of viable, attenuated, killed, non-viable or synthetic viruses for research applications.

[0075] For example, the composition may be used for the preservation of viral particles that have use in research applications, such as the use of viral preparations for immunological research. The composition may also be used for the preservation of viral preparations for use in molecular biological research, such as the use of viral preparations for the infection or transduction of cells in culture.

[0076] In a similar fashion, the composition according to the present invention may also be used for the preservation of viral particles that have use in diagnostic applications, such as the use of viral preparations (or a component thereof) as positive and negative test standards for diagnostic applications.

[0077] With regard to viral activity, the activity of the virus may be measured by any suitable assay that is known in the art. Such assays include both direct and indirect biological and physicochemical assays of viral activity. Examples of assays include the measurement of the number of infectious viral particles in the product, the expression of a reporter gene or other transgene carried by the virus, the cell killing or cell viability following viral infection or transduction of a suitable cell line, or the quantity of components produced following administration of the viral particles or constituents to a suitable model (eg. immune response in case of vaccination). Examples of other assays include the measurement of the number of intact and non-aggregated viral particles or the size of the viral particles (as an indication of viral aggregation) in the product

[0078] In the case of the measurement of the activity of a virus or a component of the virus to produce an immune response, methods are known in the art for determining the ability to elicit an immune response.

[0079] In the case of the measurement of the activity of a viable virus, the number of permissive cells killed following infection or transduction with a defined amount of virus may be determined by any suitable assay. Alternatively, as an indirect measure of viral activity, the number of intact and non-aggregated viral particles in the product may be determined by anion-exchange HPLC and the particle size determined by light scattering analysis.

[0080] The concentration of virus in the composition of the present invention may also affect the ability of the composition to preserve the virus. In one embodiment, the concentration of virus in the composition is in the range from 1×10^{6} to 1×10^{14} virus particles/ml. In a further embodiment, the concentration of virus is in the range from 1×10^{8} to 5×10^{12} virus particles/ml.

[0081] The lipid is an amphiphilic molecule that contains a substantially hydrophilic moiety coupled (directly or by way of a spacer) to a substantially hydrophobic moiety. The hydrophilic moiety will contain one or more substantially hydrophilic groups and the hydrophobic moiety will contain one or more substantially hydrophobic groups.

[0082] The lipid may be for example any fatty acid or derivative of a fatty acid, glycerol-derived lipid including a phospholipid, sphingosine-derived lipid (including ceramides, cerebrosides, gangliosides and sphingomyelins) and glycolipid, terpene and their derivatives, long chain alcohol, a lipid based on ethanolamine, and a wax.

[0083] The lipid present in the composition according to the various embodiments of the present invention may be a cationic lipid, anionic lipid, zwitterionic lipid, non-ionic lipid or any combination of such lipids.

[0084] Examples of cationic lipids include 2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA), dioctadecylaminoglycyl spermine (DOGS), dipalmitoyl phosphatidylethanolamyl spermine (DPPES), 1,3-dioleoyoxy-2-(6-carboxy-spermyl)-propylamide (DOSPER), dioleyldimethylammonium chloride (DODAC), N-[1-(2,3-dioleyloxy) propyl]-N,N,N-trimetylammonium chloride (DOTMA), 1,2-dioleoyl-sn-

glycero-3-trimethylammonium-propane (DOTAP), 1,2-dimyristyloxypropyl-3-dimethyl-hydroxy ethyl ammonium bromide (DMRIE), 3β-(N-((N',N'-dimethylamino)ethane)carbamoyl)-cholesterol (DC-Chol), dimethyldioctadecyl ammonium bromide (DDAB), 1-[2-(oleoyoxy)-ethyl]-2-oleyl-3-(2-hydroxyethyl)imidazolinium chloride (DOTIM), bis(oleoyl)-trimethylaminomethylphosphonate, 1,2-dimyristoylglycerolpentalysine salt, N,N',N'',N'''-tetramethyl-N,N',N'',N'''-tetrapalmitylspermine (TMTPS), cetyltrimethylammonium bromide (CTAB) and the following proprietary cationic lipids: Upofectamine (DOSPA:DOPE 3:1 w/w), Upofectin (DOTMA:DOPE 1:1 w/w), Lipofectace (DDAB:DOPE 1:1.25 w/w), Transfectam, Cellfectin (TMTPS:DOPE 1:1.5 M/M), Superfect, LipoTaxi, DMRIE-C (DMRIE/cholesterol: 1:1) and trilysine-carpryloyl-tris-trilaurate (T-shape; CS087).

[0085] Examples of anionic lipids include 1,2-dioleoyl-sn-glycero-3-[phospho-L-serine] (DOPS), 1,2-dimyristoyl-sn-glycero-3-phosphoglycerol (DMPG), and PEG-PE lipids such as 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[poly(ethyleneglycol) 2000] (PEG2000 DMPE), 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-[poly(ethyleneglycol) 2000] (PEG2000 DPPE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[poly(ethyleneglycoo) 2000] (PEG2000 DSPE).

[0086] Examples of zwitterionic/neutral lipids include 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dioleoyl-sn-glycero-3-phosphcholine (DOPC), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine (DMPE).

[0087] In one embodiment, the lipid is a cationic lipid. In another embodiment, the lipid is an anionic lipid.

[0088] In a further embodiment, the lipid is a cationic lipid that has a hydrophilic moiety that includes one or more amino residues. In another embodiment, the lipid is a cationic lipid that has a hydrophilic moiety that includes one or more groups derived from amino acids. In one embodiment, the lipid is a cationic lipid that has a hydrophilic moiety that includes one or more groups derived from a positively charged amino acid, such as lysine, arginine or histidine. In one embodiment, the lipid is a cationic lipid that has a hydrophilic moiety including one or more lysine groups.

[0089] In another embodiment, the lipid is a poly-cationic lipid. In one embodiment, the lipid is a poly-cationic lipid that has a hydrophilic moiety that includes two or more amino residues. In a further embodiment, the lipid is a poly-cationic lipid that has a hydrophilic moiety that includes two or more groups derived from amino acids. In a further embodiment, the lipid is a poly-cationic lipid that has a hydrophilic moiety that includes two or more groups derived from positively charged amino acids, such as lysine, arginine or histidine. In one embodiment, the lipid is a poly-cationic lipid that has a hydrophilic moiety that includes three lysine groups.

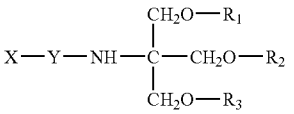
[0090] In another embodiment, the lipid is a lipid based on linking amino acids or peptides to acyl derivatives of fatty acids via a tromethamine or ethanolamine derivative, such as trilysine alanine tris tripalmitate. Examples of such lipids are as described in Australian patent application No. 649242.

[0091] The hydrophobic moiety of the lipid in the composition according to the present invention includes one or more hydrophobic groups. Hydrophobic groups include, for example, acyl, alkyl, or alkoxy chains. In one embodiment, the one or more hydrophobic groups are derived from an acyl group of a fatty acid. In a further embodiment, the one or more acyl groups have a carbon chain length of 3 to 24 carbon atoms. In one embodiment, the one or more acyl groups is a laurate group.

[0092] In one embodiment, the lipid in the composition according to the present invention has a hydrophobic moiety that includes two or more hydrophobic groups. In a further embodiment the lipid has a hydrophobic moiety that includes three hydrophobic groups. In one embodiment, the lipid has a hydrophobic moiety that includes three laurate groups.

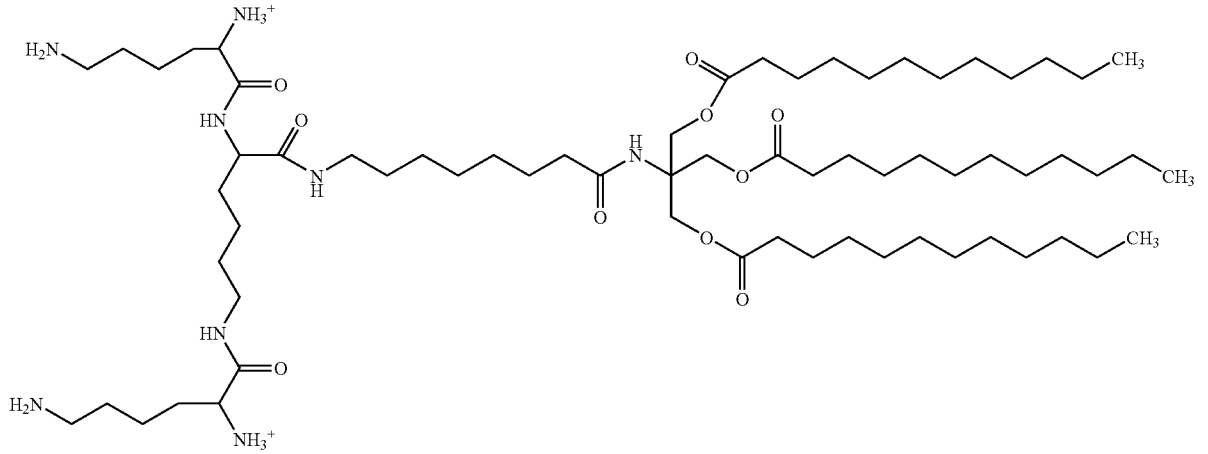
[0093] The lipid in the composition according to the present invention may also include a spacer group between the hydrophilic moiety and the hydrophobic moiety. The spacer group may include any combination or series of atoms that covalently join the hydrophilic and hydrophobic moieties. In one embodiment, the spacer region has a chain length equivalent to 1 to 30 carbon-carbon single covalent bonds.

[0094] In one embodiment, the lipid in the composition is derived from a tris-conjugated cationic lipid (or a salt thereof according to the following general formula:



[0095] In this general formula, X represents the hydrophilic moiety, Y represents a spacer group (which may or may not be present), and R_1, R_2 and R_3 are acyl groups of fatty acids. In a further embodiment, a spacer group Y is present in the molecule. In one embodiment the spacer group has a chain length equivalent to 1 to 30 carbon-carbon single covalent bonds.

[0096] In a further embodiment, the lipid in the composition is the molecule trilysine-carpryloyl-tris-trilaurate (T-shape; CS087), or a salt thereof, the structure of which is as follows:



[0097] In another embodiment, the lipid is an ethanolamine derivative.

[0098] In one embodiment, the ethanolamine derived lipid has the following formula, or is a salt thereof:

$$X—Y—NH—CH_2—CH_2O—R_4;$$

[0099] In this general formula, X represents a hydrophilic moiety, Y represents a spacer group (which may or may not be present), and R_4 is a hydrophobic moiety.

[0100] In one embodiment, R_4 is an acyl group of a fatty acid, such as being a fatty acid having a carbon chain of 3 to 18 carbon atoms. In one embodiment, the fatty acid has 10 to 18 carbon atoms, such as having 16 carbon atoms.

[0101] In this regard, it has been previously been recognised that tromethamine derived lipids, such as CS087, share many functional characteristics (such as their interaction with other lipids) with ethanolamine derived lipids, as described for example in U.S. Pat. No. 5,583,198.

[0102] In one embodiment, X in the ethanolamine derived lipid is an amino acid, peptide or derivative thereof linked to the ethanolamine derivative, to which is optionally linked a fatty acid.

[0103] In one embodiment, the ethanolamine derived lipid may be an anionic lipid.

[0104] To preserve virus, a suitable concentration of the lipid in the composition is a concentration in the range from 0.1 μM to 1 mM. In one embodiment, the concentration of the lipid is in the range from 1 μM to 500 μM. In a further embodiment, the concentration of the lipid is in the range from 5 μM to 100 μM.

[0105] For example, in the case where the lipid in the composition is trilysine-carpryloyl-tris-trilaurate (CS087), a suitable concentration of the lipid is in the range from 10 to 50 μM. In one embodiment, the concentration of trilysine-carpryloyl-tris-trilaurate in the composition is 10 μM.

[0106] The cryoprotectant in the composition according to the various embodiments of the present invention is any molecule that has the function of substantially inhibiting the formation of ice crystals upon freezing of a liquid. The cryoprotectant may be for example a sugar, sugar alcohol, glycerol, amino acid including glycine, histidine or arginine, peptide, polypeptide, protein including albumin and gelatine, or polymer including dextran, polyvinyl pyrrolidone, polyvinyl alcohol or polyethylene glycol, or any combination of such molecules.

[0107] The determination of whether a molecule may function as a cryoprotectant may be by a suitable method known in the art in which the function of a molecule to substantially inhibiting the formation of ice crystals upon freezing of a liquid may be tested.

[0108] In one embodiment, the cryprotectant is a polyhydroxy compound, such as a sugar. In a further embodiment, the sugar is sucrose, trehalose, dextrose, lactose, maltose or glucose.

[0109] To preserve virus, a suitable concentration of the cryoprotectant in the composition is in the range of 0.1 to 20% weight/volume. In one embodiment, the concentration is in the range of 1 to 10% weight/volume.

[0110] For example, when the cryoprotectant present in the composition is sucrose, a suitable concentration of sucrose is 8.5%.

[0111] It has also been found that the presence of a surfactant in the composition may further improve the ability of the composition to preserve a virus. The surfactant is any molecule that can reduce the interfacial tension between two immiscible phases. In one embodiment the surfactant is a non-ionic surfactant.

[0112] The determination of whether a molecule may function as a surfactant may be by a suitable method known in the art in which the function of a molecule to reduce the interfacial tension between two immiscible phases may be tested.

[0113] In one embodiment, the surfactant is present in the composition at a concentration in the range from 0.0001% to 50% volume/volume. In a further embodiment, the surfactant is present in the composition at a concentration in the range from 0.001% to 10% volume/volume.

[0114] In one embodiment, the non-ionic surfactant is a molecule that includes an oxyethylene group and a hydroxy

group, such as polysorbate 80 or polyethylene glycol 400, or any combination of these non-ionic surfactants.

[0115] For example, when polysorbate 80 is used in the composition, a suitable concentration of the polysorbate 80 is in the range from 0.0001 to 1% volume/volume. In one embodiment, the concentration of polysorbate 80 in the composition is 0.001 to 0.1% volume/volume. In a further embodiment, the concentration of polysorbate 80 in the composition is 0.005% volume/volume.

[0116] In another example, when polyethylene glycol 400 is used in the composition, a suitable concentration may be for example in the range from 0.001 to 50% volume/volume. In one embodiment, the concentration of polyethylene glycol 400 is 0.01 to 10% volume/volume. In a further embodiment, the concentration of polyethylene glycol 400 is 0.01 to 5% volume/volume. In one embodiment, the concentration of polyethylene glycol 400 in the composition is 0.5% volume/volume.

[0117] The pH of the composition may also be selected to improve viral preservation. The pH may also be selected to be compatible with the administration of the composition to a subject. In one embodiment, the pH of the composition is in the range of 4 to 10. In a further embodiment, the pH is in the range of 5 to 9. In one embodiment, the pH of the composition is in the range of 6 to 8.5.

[0118] In one embodiment, the pH of the composition according to the various embodiments of the present invention may be obtained by buffering with a pharmaceutically acceptable buffer.

[0119] In one embodiment, the buffer is selected from one or more buffers selected from the group consisting of monobasic acids including acetic, benzoic, gluconic, glyceric and lactic acids, dibasic acids including aconitic, adipic, ascorbic, carbonic, glutamic, maleic, malic, succinic, tartaric acids, polybasic acids including citric and phosphoric acids. The buffer may also be selected from one or more buffers selected from the group consisting of bases including ammonia or ammonium chloride, diethanolamine, glycine, tromethamine (also known as Tris and Tham).

[0120] In one embodiment, the buffer is selected from one or more buffers selected from the group consisting of a tris-based buffer, a sodium hydrogen maleate buffer, succinate buffer, or phosphate buffer.

[0121] The composition according to the present invention provides a composition for the improved preservation of a virus, and/or a component of a virus.

[0122] In one embodiment, the present invention provides a composition for the preservation of a virus and/or a component of a virus, the composition including a virus (or a component thereof), a lipid and a cryoprotectant, wherein the virus is storage stable.

[0123] In one embodiment, the composition is in a liquid form.

[0124] In another embodiment, the composition is in a solid form such as lyophilised, spray-dried or freeze-dried forms.

[0125] In another embodiment, the composition is in a frozen form.

[0126] In one embodiment, the temperature of storage of the composition of the present invention is from −200° C. to 37° C.

[0127] Depending upon the requirements, suitable storage temperatures for frozen compositions (0° C. or less) are; −200° C. to 0° C.; 80° C. to 0° C.; and −20° C. to 0° C.

[0128] Depending upon the requirements, suitable storage temperatures for non-frozen compositions are >0° C. to 30° C.; 2° C. to 25° C.; 2° C. to 8° C., 22° C. to 25° C.; and 22° C. to 35° C.

[0129] In one embodiment, the storage temperature is room temperature.

[0130] With respect to the period of time over which the composition according to the present invention shows improved storage stability, the composition according to the present invention may be stored for a period of greater than 24 months. In one embodiment, the period of storage is 12 months or greater. In another embodiment, the period of storage is 6 months or greater. In another embodiment, the period of storage is 3 months or greater. In another embodiment, the period of storage is 1 week or greater. In another embodiment, the period of storage is 1 day or greater.

[0131] In another embodiment, the present invention provides a composition wherein the virus (or a component of the virus) is stable when the composition is subjected to one or more of agitation, shearing forces or mechanical action.

[0132] In this regard, as has been discussed previously, the improved preservation will be as compared to a composition that does not contain lipid, or a composition that does not contain cryoprotectant. That is, the activity of the virus (and/or a component of a virus) will not decrease substantially with time when the composition is stored at the abovementioned temperatures or for the abovementioned periods of time, as compared to a composition not containing lipid, or a composition not containing a cryoprotectant. The activity of the virus or the component may be a desired activity, such as infectivity, ability to transduce or immunogenicity.

[0133] As will be appreciated, in the case of comparing the preservation of a virus under frozen conditions, generally the activity of the virus (and/or a component of a virus) will not decrease substantially with time when the composition is stored, as compared to a composition not containing lipid.

[0134] The composition also shows improved preservation of a virus (and/or a component of a virus) when the composition is thawed, or when the composition is subjected to one or more cycles of freezing and thawing. In one embodiment, the present invention provides a composition for the preservation of a virus, the composition including a virus (and/or a component of a virus), a lipid and a cryoprotectant, wherein the virus (or the component) is stable to freeze-thawing.

[0135] The composition according to the present invention may also be in a dosage form suitable for administration to a human or an animal subject. The dosage form includes the composition according to the present invention and may further include other pharmaceutically acceptable additives.

[0136] The addition of such pharmaceutically acceptable additives to the dosage form may be for example to improve

the immunogenicity of a virus and/or a component of a virus (eg the inclusion of an adjuvant compound in a vaccine to enhance immune response), to improve the ability of the virus to infect or transduce target cells, or to improve the activity elicited by the administration of virus.

[0137] For example, local bystander killing can be enhanced by co-administration of a pharmaceutical or genetic agent which enhances cell-cell communications. Another example is the co-administration of a DNA encoding a cytokine to increase the immunogenicity of tumour cells.

[0138] The present invention also provides a method of producing a composition for the preservation of a virus, the method including the step of preparing a liquid composition including a virus, a lipid and a cryoprotectant.

[0139] As will be appreciated, the methods according to the present invention will embody the same features as those for the composition as discussed in detail above.

[0140] With regard to the preparation of virus, the virus may be purified by any suitable means.

[0141] In one embodiment, the virus is purified by a chromatographic method including ion-exchange chromatography or HPLC, or centrifugation including CsCl centrifugation, after the virus has been recovered from infected permissive cells and/or the supernatant thereof. When purified by CsCl centrifugation, the virus may be prepared after recovery from infected permissive cells by centrifugation through a CsCl step gradient and centrifugation to equilibrium on a CsCl gradient. When virus is purified in this manner, the CsCl is generally removed by column chromatography.

[0142] In one embodiment, the concentrated virus so formed is diluted in a solution that includes a suitable buffer and a cryoprotectant. In a further embodiment, the solution further includes a non-ionic surfactant. In one embodiment, the concentrated virus is diluted in a solution including a Tris buffer, sucrose and polyethylene glycol 400 and/or polysorbate 80. In another embodiment, the concentrated virus is diluted in a solution (at pH 8.0) including 10 mM Tris buffer, 8.5% sucrose and 2% polyethylene glycol 400. In another embodiment, the solution (which may exist as a suspension) containing virus is then filtered to remove unwanted micro-organisms. In one embodiment, the solution is filtered through a 0.2 micron membrane filter.

[0143] A component of a virus may be prepared according to a method known in the art, including the use of recombinant DNA technology to produce the component. See for example, Sambrook et al. Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) and Ausubel, F. M. et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y.

[0144] For the preparation of a composition according to the present invention, the lipid may be first dispersed in a solution identical to that used for the dilution of virus (or the component of the virus). In one embodiment, the solution (which may exist as a suspension) containing lipid is filtered to remove unwanted micro-organisms, such as the solution being filtered through a 0.2 micron membrane filter.

[0145] To prepare a composition for preservation, the diluted solution of virus or a component thereof (which may exist as a suspension) may then be combined with a solution containing lipid (which may also exist as a suspension), the relative proportions of each selected so as to achieve the desired final concentrations of virus (or component) and lipid. Accordingly, the present invention also provides a method for producing a composition for the preservation of a virus and/or a component of a virus, wherein the composition is formed by combining a solution including a virus (or component thereof) and a cryoprotectant with a solution including lipid.

[0146] The composition so formed may be stored in a suitable closed container. In one embodiment, the composition is stored in borosilicate glass vials. In addition, the composition may be stored under a suitable gas or mixture of gases.

[0147] Finally, standard techniques may be used for recombinant DNA technology, oligonucleotide synthesis, and tissue culture and transfection (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See e.g., Sambrook et al. Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) and Ausubel, F. M. et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y.

DESCRIPTION OF SPECIFIC EMBODIMENTS

[0148] Reference will now be made to examples that embody the above general principles of the present invention. However, it is to be understood that the following description is not to limit the generality of the above description.

Example 1

Preparation of a Composition for the Preservation of Adenovirus Virus

[0149] CsCl purified OAdV623 virus was suspended in a pH 8.0 buffer containing 10 mM Tris, 8.5% sucrose, 2% PEG buffer, in a polypropylene tube, at two-times the final concentration. CS087 was supplied as a freeze-dried solid that was first dissolved in ethanol and the ethanol then removed to produce a film. The film was dispersed in a pH 8.0 buffer containing 10 mM Tris, 8.5% sucrose, 2% polyethylene glycol 400, in a polystyrene tube, at two-times the final concentration.

[0150] The suspensions of OAdV623 and CS087 were filtered separately through a 0.2 μm membrane filter. An equal volume of OAdV623 and CS087 were combined aseptically. The suspension was then gently agitated continuously at approximately 40 rpm for 60 to 90 minutes at 18° C.-20° C., to ensure viral mixing. The final product was then aseptically dispensed into washed and autoclaved Type I borosilicate glass vials and stored at the appropriate temperature.

Example 2

Preparation of a Composition for the Preservation of Measles Virus

[0151] Measles virus may be purified according to a method known in the art, such as Calain and Roux (1988) *J. Virol.* 62(8):2859-2866.

[0152] The measles virus may be suspended at physiological pH buffer containing 10 mM Tris, 8.5% sucrose, 2% PEG buffer, in a polypropylene tube, at two-times the final concentration. Lipid (for example ethanolamine) may be supplied at two-times the final concentration in the same buffer.

[0153] The suspensions of measles virus and lipid may be filtered separately through a 0.2 μm membrane filter. An equal volume of virus and lipid may be combined aseptically. The suspension may then be gently agitated continuously at approximately 40 rpm for 60 to 90 minutes at 18° C.-20° C., to ensure viral mixing. The final product may then be aseptically dispensed into washed and autoclaved Type I borosilicate glass vials and stored at the appropriate temperature.

Example 3

Storage Stability of Various Viral Compositions at −80° C.

[0154] The stability of various OAdV623 compositions (approximately 6×10^8 VP/ml) stored at −80° C. was assessed by determining the extent of cell killing after storage for 14 days, 1 month or 3 months. Cell killing was determined for compositions stored at pH 8 and pH 6.

[0155] OAdV623 encodes the PNP gene which catalyses the conversion of the immunosuppressive prodrug Fludarabine to the toxic 2-fluoro-adenine product. This results in the death of cells producing PNP and to a limited extent, cells in the vicinity with a near neighbour bystander effect. The death of susceptible cells, such as the PC3 cell line, following transduction with OAdV623 and treatment with Fludarabine phosphate, is a direct indicator of the potency of the OAdV623 preparation.

[0156] To determine the extent of cell killing, an aliquot of virus in the relevant composition was thawed and approximately 6×10^6 virus particles were used to transduce 1×10^4 PC3 cells in culture. The ability of the virus to kill PC3 cells by converting the prodrug fludarabine, supplied to the cells as fludarabine phosphate, to active 2-fluoroadenine, was then determined quantitatively. Cell killing was determined by an MTS assay (Promega) to measure the number of viable cells in treated wells compared to a standard curve of cells not treated with the virus.

[0157] The concentration of the various components used was as follows:

10 mM Tris

10 mM sodium hydrogen maleate

8.5% sucrose

50 μM CS087

2% (vN) polyethylene glycol 400 (PEG400)

0.005% (v/v) polysorbate 80 (PS80)

[0158] The whole composition was buffered to the desired pH with Tris or maleate buffer.

[0159] (a) Stability at pH 8.

[0160] The results are shown in Table 1 and in FIG. **1**.

TABLE 1

	% Cell Killing		
Composition	14 days	1 month	3 months
Tris/sucrose	41.4 ± 13.9	−13.3 ± 30.3	−14.2 ± 16.0
Tris/sucrose/CS087	34.6 ± 19.3	52.6 ± 14.6	0.1 ± 11.0
Tris/sucrose/CS087/PEG400	62.1 ± 1.3	68.9 ± 7.9	53.5 ± 5.0
Tris/sucrose/CS087/PS80	63.3 ± 0.7	74.2 ± 0.8	48.2 ± 4.9
Tris/sucrose/PEG400	52.8 ± 1.5	3.0 ± 40.2	4.0 ± 4.4
Tris/sucrose/PS80	40.3 ± 7.3	15.1 ± 9.3	5.3 ± 17.4

[0161] As can be seen, OAdV623 stored in tris/sucrose at −80° C. was stable for 2 weeks only. The addition of lipid to the tris/sucrose composition enhanced the preservation of the virus, when the virus was stored at −80° C. for periods of at least 1 month and then subsequently thawed. The addition of a non-ionic surfactant to the tris/sucrose/lipid composition further enhanced the preservation of virus, such that the virus remained active even after a storage period of 3 months and a subsequent freeze-thaw cycle. Thus the preservation of the virus after storage and subsequent thawing was improved by the addition of lipid and, in particular, lipid plus a non-ionic surfactant.

[0162] (b) Stability at pH 6.

[0163] The results are shown in Table 2 and in FIG. **2**.

TABLE 2

	% Cell Killing		
Composition	14 days	1 month	3 months
Maleate/sucrose	−15.4 ± 1.8	7.9 ± 16.9	−12.8 ± 19.8
Maleate/sucrose/CS087	−0.2 ± 4.1	66.8 ± 5.7	18.9 ± 15.2
Maleate/sucrose/CS087/PEG400	9.1 ± 3.5	71.7 ± 6.2	28.5 ± 4.6
Maleate/sucrose/CS087/PS80	23.4 ± 1.1	75.2 ± 6.5	48.6 ± 3.5
Maleate/sucrose/PEG400	−15.2 ± 1.8	20.9 ± 8.7	2.7 ± 6.3
Maleate/sucrose/PS80	−15.4 ± 1.0	24.1 ± 6.5	−4.0 ± 7.9

[0164] As can be seen, the addition of lipid to the tris/sucrose composition enhanced the preservation of the virus, when the virus was stored at −80° C. such that the virus remained active after a storage period of 3 months and a subsequent freeze-thaw cycle. The addition of a non-ionic surfactant to the tris/sucrose/lipid composition further enhanced the preservation of virus. Thus the preservation of the virus after storage and subsequent thawing was improved by the addition of lipid and, in particular, lipid plus a non-ionic surfactant.

Example 4

Storage Stability of Various Viral Compositions at −20° C.

[0165] The stability of various OAdV623 compositions (approximately 6×10^8 VP/ml) stored at −20° C. was assessed by determining the extent of cell killing after

storage for 14 days, 1 month or 3 months. Cell killing was determined for formulations stored at pH 8 and pH 6.

[0166] To determine the extent of cell killing, an aliquot of virus in the relevant composition was thawed and approximately 6×10^6 virus particles were used to transduce 1×10^4 PC3 cells in culture. The ability of the virus to kill PC3 cells by converting the prodrug fludarabine, supplied to the cells as fludarabine phosphate, to active 2-fluoroadenine, was then determined quantitatively. Cell killing was determined by an MTS assay (Promega) to measure the number of viable cells in treated wells compared to a standard curve of cells not treated with the virus.

[0167] The concentration of the various components used was as follows:

10 mM Tris

10 mM sodium hydrogen maleate

8.5% sucrose

50 μM CS087

2% (v/v) polyethylene glycol 400 (PEG400)

0.005% (v/v) polysorbate 80 (PS80)

[0168] The whole composition was buffered to the desired pH with Tris or maleate buffer.

[0169] (a) Stability at pH 8.

[0170] The results are shown in Table 3 and in FIG. **3**.

TABLE 3

	% Cell Killing		
Composition	14 days	1 month	3 months
Tris/sucrose	56.0 ± 1.3	−7.6 ± 21.7	−14.2 ± 14.7
Tris/sucrose/CS087	57.3 ± 1.8	6.9 ± 12.9	13.5 ± 15.5
Tris/sucrose/CS087/PEG400	67.2 ± 1.6	49.0 ± 7.5	66.8 ± 2.1
Tris/sucrose/CS087/PS80	65.5 ± 1.9	61.2 ± 5.0	57.2 ± 4.2
Tris/sucrose/PEG400	60.5 ± 1.1	−11.2 ± 24.0	4.8 ± 11.7
Tris/sucrose/PS80	55.9 ± 0.6	9.2 ± 15.8	9.4 ± 8.1

[0171] As can be seen, the addition of lipid to the tris/sucrose composition enhanced the preservation of the virus, when the virus was stored at −20° C. such that the virus remained active after a storage period of 3 months and a subsequent freeze-thaw cycle. The addition of a non-ionic surfactant to the tris/sucrose/lipid composition further enhanced the preservation of virus, such that greater activity (% cell kill) was observed compared to the tris/sucrose/lipid composition without a non-ionic surfactant. Thus the preservation of the virus after storage and subsequent thawing was improved by the addition of lipid and, in particular, lipid plus a non-ionic surfactant.

[0172] (b) Stability at pH 6.

[0173] The results are shown in Table 4 and in FIG. **4**.

TABLE 4

	% Cell Killing		
Composition	14 days	1 month	3 months
Maleate/sucrose	−7.1 ± 4.8	3.0 ± 10.8	−20.2 ± 14.2
Maleate/sucrose/CS087	16.4 ± 5.7	35.0 ± 8.7	−2.6 ± 10.2
Maleate/sucrose/CS087/PEG400	29.4 ± 10.0	29.9 ± 18.0	−31.3 ± 10.8
Maleate/sucrose/CS087/PS80	53.6 ± 1.5	66.1 ± 5.2	44.7 ± 7.6
Maleate/sucrose/PEG400	12.4 ± 8.7	19.7 ± 17.7	1.9 ± 6.1
Maleate/sucrose/PS80	3.1 ± 8.1	21.6 ± 9.7	−1.1 ± 4.6

[0174] The addition of lipid to the tris/sucrose composition enhanced the preservation of the virus, when the virus was stored at −20° C. such that the virus remained active after a storage period of 1 month and a subsequent freeze-thaw cycle. The addition of a non-ionic surfactant to the tris/sucrose/lipid composition further enhanced the preservation of virus. Thus the preservation of the virus after storage and subsequent thawing was improved by the addition of lipid and, in particular, lipid plus a non-ionic surfactant.

Example 5

Preservation of Various Viral Compositions Upon Multiple Freeze-Thaw Cycles (−80° C. to 25° C.)

[0175] The ability of various OAdV623 compositions (1×10^{10} VP/ml) to be preserved after multiple freeze-thaw cycles was assessed by determining the extent of cell killing after exposure to 1, 2 or 3 freeze-thaw cycles. For each cycle, virus was frozen at −80° C. for no less than 1 hour and thawed at 25° C. for 30 minutes. Cell killing was determined for virus formulated in 10 mM Tris, 8.5% sucrose, 50 μM CS087 and either 2% or 4% polyethylene glycol 400 (pH 8).

[0176] To determine the extent of cell killing, virus particles in the range of 4×10^5 to 3×10^7 were used to transduce 1×10^4 PC3 cells in culture. The ability of the virus to kill PC3 cells by converting the prodrug fludarabine, supplied to the cells as fludarabine phosphate, to active 2-fluoroadenine, was then determined quantitatively. Cell killing was determined by an MTS assay (Promega) to measure the number of viable cells in treated wells compared to a standard curve of cells not treated with the virus.

[0177] The concentration of the various components used was as follows:

10 mM Tris

8.5% sucrose

50 μM CS087

2% or 4% (v/v) polyethylene glycol 400 (PEG400)

[0178] The whole composition was buffered to the desired pH with Tris or maleate buffer.

[0179] The results are as shown FIG. **5**. As can be seen, the composition containing either 2% or 4% polyethylene glycol 400 provides substantial protection to the virus against the

effects of freeze-thawing of the composition. In particular, protection against the effect of repeated freeze-thawing is most significant for the composition containing 4% polyethylene glycol.

Example 6

Preservation of Various Viral Compositions Upon Multiple Freeze-Thaw Cycles (−80° C. to 20° C.)

[0180] The ability of various OAdV623 compositions (approximately 9.6×10^{11} VP/ml) to be preserved after multiple freeze-thaw cycles was assessed by determining the extent of cell killing after exposure to 1 or 3 freeze-thaw cycles. For each cycle, virus was frozen at −80° C. for at least 1 hour and thawed at 20° C. for 40 minutes. Cell killing was determined for virus formulated at pH 8 in 10 mM Tris, 8.5% sucrose and 0.5% polyethylene glycol 400 with or without 10 μM CS087.

[0181] To determine the extent of cell killing, virus particles in the range of 8×10^5 to 1×10^8 were used to transduce 5×10^3 PC3 cells in culture. The ability of the virus to kill PC3 cells by converting the prodrug fludarabine, supplied to the cells as fludarabine phosphate, to active 2-fluoroadenine, was then determined quantitatively. Cell killing was determined by an MTS assay (Promega) to measure the number of viable cells in treated wells compared to control cells not treated with the virus.

[0182] The concentration of the various components used was as follows:

10 mM Tris

8.5% sucrose

10 μM CS087

0.5% (v/v) polyethylene glycol 400 (PEG400)

[0183] The whole composition was buffered to the desired pH with Tris or maleate buffer.

[0184] The results are as shown in FIG. **6** (OAdV623 in tris/sucrose/PEG400) and in FIG. **7** (OAdV623 in tris/sucrose/lipid/PEG400).

[0185] As can be seen, the composition containing 10 μM lipid provides substantial protection to the virus against the effects of repeated freeze-thawing compared to the composition containing tris/sucrose/PEG400 without any lipid.

Example 7

Storage Stability of Various Viral Compositions at Refrigeration Temperature.

[0186] The stability of various OAdV623 compositions (approximately 6×10^8 VP/ml) stored at refrigeration temperature (approximately 4° C.) was assessed by determining the extent of cell killing at Day 0 and after storage for 7 days. Cell killing was determined for compositions stored at pH 8 and pH 6.

[0187] To determine the extent of cell killing, an aliquot of virus in the relevant composition containing approximately 6×10^6 virus particles were used to transduce 1×10^4 PC3 cells in culture. The ability of the virus to kill PC3 cells by converting the prodrug fludarabine, supplied to the cells as fludarabine phosphate, to active 2-fluoroadenine, was then determined quantitatively. Cell killing was determined by an MTS assay (Promega) to measure the number of viable cells in treated wells compared to a standard curve of cells not treated with the virus.

[0188] The concentration of the various components used was as follows:

10 mM Tris

10 mM sodium hydrogen maleate

8.5% sucrose

50 μM CS087

2% (v/v) polyethylene glycol 400 (PEG400)

0.005% (v/v) polysorbate 80 (PS80)

[0189] The whole composition was buffered to the desired pH with Tris or maleate buffer.

[0190] (a) Stability at pH 8.

[0191] The results are shown in FIG. **8**. As can be seen, the addition of lipid to the tris/sucrose composition enhanced the preservation of the virus, when the virus was stored at 4° C. for 7 days. The addition of a non-ionic surfactant to the tris/sucrose/lipid composition further enhanced the preservation of virus. Thus the preservation of the virus after storage was improved by the addition of lipid and, in particular, lipid plus a non-ionic surfactant.

[0192] (b) Stability at pH 6.

[0193] The results are shown in FIG. **9**. As can be seen, the addition of lipid to the maleate/sucrose composition enhanced the preservation of the virus, when the virus was stored at 4° C. for 7 days. The addition of a non-ionic surfactant to the maleate/sucrose/lipid composition also enhanced the preservation of virus compared to a composition without any lipid or surfactant. Thus the preservation of the virus after storage was improved by the addition of lipid with or without a non-ionic surfactant.

Example 8

Preservation of Various Viral Compositions Upon Storage and when Subjected to Gentle Agitation, Transportation and Multiple Freeze-Thaw Cycles

[0194] Preservation of various viral compositions (pH 8) upon storage (−80° C.) and when subjected to gentle agitation, transportation and multiple freeze-thaw cycles (−80° C. to wet ice temperature of approximately 0° C. and −80° C. to 20° C.)

[0195] The ability of various OAdV623 compositions to be preserved upon storage and when subjected to gentle agitation, transportation and multiple freeze-thaw cycles was assessed by determining the extent of cell killing after the formulations had been subjected to the following:

[0196] a storage period of 11 weeks,

[0197] packed in dry ice and transported by road for approximately 1 hour,

[0198] a freeze-thaw cycle,

[0199] mixing by gentle agitation (eg. pipetting up and down),

[0200] frozen, packed in dry ice and again transported by road for approximately 1 hour,

[0201] an additional storage period of 2 weeks and

[0202] a second freeze-thaw cycle.

[0203] The storage temperature was −80° C. For the first freeze-thaw cycle, virus was thawed on wet ice (approximately 0° C.) for a total of 6 hours. For the second freeze-thaw cycle, virus was thawed at 20° C. for 30 minutes.

[0204] A batch of virus was first harvested in tris/sucrose and the potency determined using a cell-killing assay. Four formulations were then prepared from this batch of virus as follows:

[0205] Formulation A: OAdV623 (1×10^{12} VP/ml) was stored in tris/sucrose at −80° C. for 11 weeks. This formulation was subsequently packed in dry ice and transported, thawed, mixed by gentle agitation and held on wet ice (approximately 0° C.) for 6 hours before being re-frozen at −80° C., packed in dry ice and again transported and then stored for a further 2 weeks. This formulation containing OAdV623 at 1×10^{12} VP/ml was then thawed again at 20° C. for 30 minutes immediately prior to the cell killing assay.

[0206] Formulation B: OAdV623 (1×10^{12} VP/ml) was stored in tris/sucrose at −80° C. for 11 weeks. This formulation was subsequently packed in dry ice and transported, thawed on wet ice (approximately 0° C.) for 2 hours, mixed with lipid with gentle agitation, held at room temperature (approximately 20° C.) for 30 minutes and then left to stand on wet ice for 3.5 hours before being re-frozen at −80° C., packed in dry ice and again transported and then stored for a further 2 weeks. This formulation containing OAdV623 at 5×10^{11} VP/ml was then thawed again at 20° C. for 30 minutes immediately prior to the cell killing assay.

[0207] Formulation C: OAdV623 (1×10^{12} VP/ml) was stored in tris/sucrose at −80° C. for 11 weeks. This formulation was subsequently packed in dry ice and transported, thawed on wet ice (approximately 0° C.) for 2 hours, mixed with lipid/PEG400 with gentle agitation, held at room temperature (approximately 20° C.) for 30 minutes and then left to stand on wet ice for 3.5 hours before being re-frozen at −80° C., packed in dry ice and again transported and then stored for a further 2 weeks. This formulation containing OAdV623 at 5×10^{11} VP/ml was then thawed again at 20° C. for 30 minutes immediately prior to the cell killing assay.

[0208] Formulation D: OAdV623 (1×10^{12} VP/ml) in tris/sucrose was mixed with lipid/PEG400 at room temperature (approximately 20° C.) for 30 minutes. This OAdV623 formulation in tris/sucrose/lipid/PEG400 (5×10^{11} VP/ml) was stored at −80° C. for 11 weeks. This formulation was subsequently packed in dry ice and transported, thawed, mixed by gentle agitation and held on wet ice (approximately 0° C.) for 6 hours before being re-frozen at −80° C., packed in dry ice and again transported and then stored for a further 2 weeks. This formulation containing OAdV623 at 5×10^{11} VP/ml was then thawed again at 20° C. for 30 minutes immediately prior to the cell killing assay.

[0209] To determine the extent of cell killing, virus particles in the range of 2×10^{6} to 2×10^{8} were used to transduce 5×10^{3} PC3 cells in culture. The ability of the virus to kill PC3 cells by converting the prodrug fludarabine, supplied to the cells as fludarabine phosphate, to active 2-fluoroadenine, was then determined quantitatively. Cell killing was determined by an MTS assay (Promega) to measure the number of viable cells in treated wells compared to a standard curve of cells not treated with the virus.

[0210] The concentration of the various components in the final formulations were as follows:

10 mM Tris

8.5% sucrose

10 μM CS087

0.5% (v/v) polyethylene glycol 400 (PEG400)

[0211] The whole composition was buffered to the desired pH with Tris or maleate buffer.

[0212] The results are as shown in FIG. **10**. Note: % Cell kill at virus particle input of 2×10^{-7} upon harvest and prior to preparation of formulations was 55%

[0213] As can be seen, the composition containing tris/sucrose/lipid/PEG400 (Formulation D) provided the most protection to the virus against the effects of storage, agitation, transportation and freeze-thawing. In contrast, the composition containing tris/sucrose without any lipid or PEG400 (Formulation A) provided the least protection to the virus against the effects of storage, agitation, transportation and freeze-thawing.

Example 9

Preservation of Measles Virus

[0214] A batch of measles virus stored at −80° C. in cell culture medium, as physiological conditions can be formulated as outlined in the aforementioned examples, specifically looking at the following variables.

[0215] Formulation I: Measles virus unformulated

[0216] Formulation II: Measles virus and sucrose (eg 8.5%) and PEG 400.

[0217] Formulation III: Measles virus, sucrose (eg 8.5%), CS087 (eg 50 μM) and PEG 400 (eg 0.5% v/v).

[0218] Formulation IV: Measles virus, sucrose (eg 8.5%), ethanolamine (eg at 50 μM) and/or ethanolamine based lipid, and PEG 400 (eg 0.5% v/v).

[0219] The stability of the above compositions at −80° C., 4° C. and 25° C. for various periods of time can then be determined by a plaque assay, for example as exemplified in J Virol Methods. 1981 December; 3(5):251-60.

[0220] Finally, it will be appreciated that various modifications and variations of the described compositions and methods of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention, which are apparent to those skilled in the field of virology, molecular biology, cryobiology or related fields are intended to be within the scope of the present invention.

1. A composition for the preservation of a virus and/or a component of a virus, the composition including a virus and/or a component thereof, a lipid and a cryoprotectant.

2. A composition according to claim 1, wherein the lipid is a cationic or an anionic lipid.

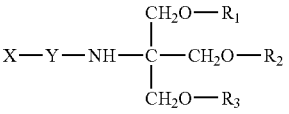
3. A composition according to claim 2, wherein the cationic lipid has a hydrophilic moiety including one or more amino residues derived from an amino acid.

4. A composition according to claim 3, wherein the one or more amino acids is lysine, arginine or histidine.

5. A composition according to claim 1, wherein the lipid has a hydrophobic moiety including one or more acyl groups with a carbon chain length of 3 to 24 carbon atoms.

6. A composition according to claim 1, wherein the lipid is a tris-conjugated cationic lipid.

7. A composition according to claim 6, wherein the tris-conjugated cationic lipid has the following chemical formula:



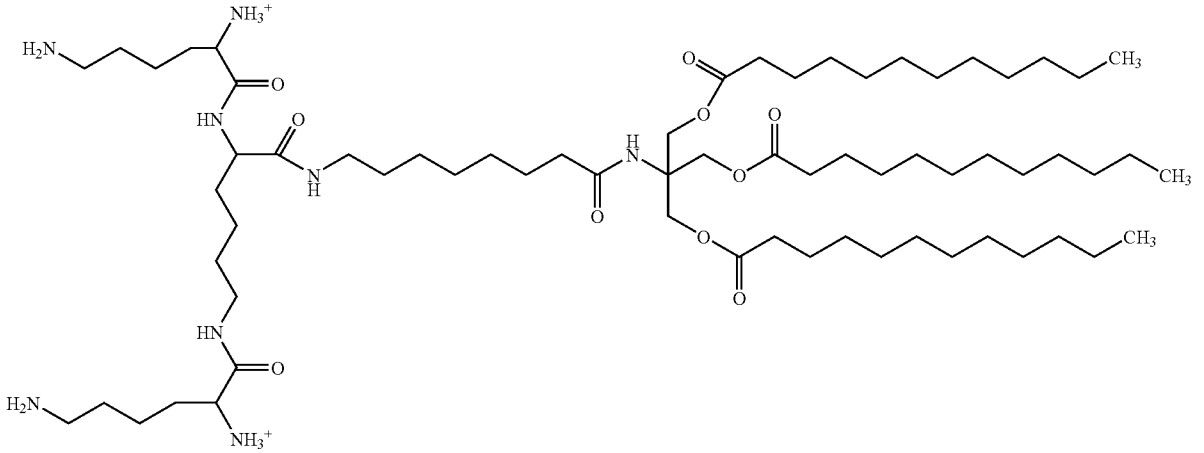
or a salt thereof, wherein:

X is a positively charged hydrophilic moiety;

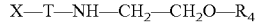
Y is spacer having a chain length equivalent to 1 to 30 carbon-carbon single covalent bonds or is absent; and

R_1, R_2, and R_3 are the same or different and are acyl groups derived from a fatty acid.

8. A composition according to claim 7, wherein the tris-conjugated lipid has the following chemical formula:



9. A composition according to claim 1, wherein the lipid is an ethanolamine derived lipid with the following chemical formula:



or a salt thereof; wherein X is a hydrophilic moiety, Y is a spacer group or is absent, and R_4 is a hydrophobic moiety.

10. A composition according to claim 9, wherein the lipid is an anionic lipid.

11. A composition according to claim 1, wherein the cryoprotectant is a sugar selected from one or more of sucrose, trehalose, dextrose, lactose, maltose, and glucose.

12. A composition according to claim 1, wherein the composition further includes a surfactant.

13. A composition according to claim 12, wherein the surfactant is a non-ionic surfactant.

14. A composition according to claim 13, wherein the non-ionic surfactant is polysorbate 80 or polyethylene glycol 400, or a combination of these surfactants.

15. A composition according to claim 1, wherein the virus or the component of the virus is selected from the group consisting of a Paramyxovirinae virus, including Measles virus; a Pneumovirinae virus family, including human respiratory syncytial virus; a Togaviridae virus, including Rubella virus; a Herpesviridiae virus, including Human herpesvirus, Herpes simplex 1 or 2; Varicella-zoster virus, Cytomegalovirus, Roseolovirus; Epstein-Barr virus; a Hepadnaviridae virus, including Hepatitis B virus; a Picornaviridae virus, including Poliovirus, Rhinovirus, Hepatitis A virus; an Orthomyxoviridae virus, including Influenzavirus A, Influenzavirus B, Influenzavirus C; a Flaviviridae virus, Yellow fever virus, West Nile virus, Dengue Fever, Hepatitis C virus; a natural or recombinant derivative of any of these viruses, or a component of these viruses.

16. A composition according to claim 1, wherein the virus and/or the component of the virus is storage stable.

17. A composition according to claim 1, wherein the composition is suitable for use in a vaccine.

18. A vaccine including the composition according to claim 1.

19. A method of producing a composition for the preservation of a virus and/or a component of a virus, the method including the step of preparing a liquid composition including a virus and/or a component thereof, a lipid and a cryoprotectant.

* * * * *