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(54) Title: SUGAR GLASSIFIED VIRUS LIKE PARTICLES (VLPS)

(57) Abstract: The present invention discloses and claims Virus Like Particles (VLPs) associated with sugar glass for enhancing overall stability of VLPs. These VLPs formulations will reduce the cost and increase the distribution and delivery of VLP based vaccines worldwide. The invention also claims methods of making and delivering said formulation to a patient.

SUGAR GLASSIFIED VIRUS LIKE PARTICLES (VLPS)

FIELD OF THE INVENTION

[001] This invention relates to stabilizing virus like particles (VLPs) by associating said VLPs with a sugar glass.

BACKGROUND

[002] The majority of commonly recommended vaccines require storage temperatures of 35°F–46°F (2°C–8°C) and must not be exposed to freezing temperatures nor temperatures greater than room temperature (about 22°C - 25°C). Introduction of varicella vaccine in 1995 and of the live attenuated influenza vaccine (LAIV) more recently increased the complexity of vaccine storage. Both varicella vaccine and LAIV must be stored in a continuously frozen state <5°F (-15°C) with no freeze-thaw cycles.

[003] In recent years, instances of improper vaccine storage have been reported. An estimated 17%–37% of providers expose vaccines to improper storage temperatures and refrigerator temperatures are more commonly kept too cold than too warm. Freezing temperatures can irreversibly reduce the potency of vaccines required to be stored at 35°F–46°F (2°C–8°C). Certain freeze-sensitive vaccines contain an aluminum adjuvant that precipitates when exposed to freezing temperatures. This may result in loss of the adjuvant effect and vaccine potency. Physical changes are not always apparent after exposure to freezing temperatures and visible signs of freezing are not necessary to result in a decrease in vaccine potency. Although the potency of the majority of vaccines can be affected adversely by storage temperatures that are too warm, these effects are usually more gradual, predictable, and smaller in magnitude than losses from temperatures that are too cold. In contrast, varicella vaccine and LAIV are required to be stored in continuously frozen states and lose potency when stored above the recommended temperature range.

[004] Virus like particles (VLPs) are useful as vaccines against diseases (*e.g.* influenza). Virus-like particles (VLPs) closely resemble mature virions, but they do not contain viral genomic material (i.e., viral genomic RNA). Therefore, VLPs are nonreplicative in nature, which make them safe for administration in the form of an immunogenic composition (*e.g.*, vaccine). In addition, VLPs can express envelope glycoproteins on the surface of the VLP, which is the most

physiological configuration. Moreover, since VLPs resemble intact virions and are multivalent particulate structures, VLPs may be more effective in inducing neutralizing antibodies to the envelope glycoprotein than soluble envelope antigens. Further, VLPs can be administered repeatedly to vaccinated hosts, unlike many recombinant vaccine approaches. However, like the varicella vaccine and LAIV, VLPs are sensitive to temperature changes and this must be maintained in a controlled environment. The ideal VLP formulation would resist high (greater that about 25°C) and low temperatures (less than about 2°C), to facilitate distribution.

[005] Current methods for avoiding temperature associated degradation of vaccines are inadequate. For example, live-attenuated vaccines (and some non-live vaccines) are often lyophilized because of their intrinsic instability. The lyophilized products are reconstituted with diluent immediately before administration. Because lyophilization is a time-consuming and capacity-limiting step of vaccine production, lyophilized vaccines are usually presented in multi-dose vials. Some global guidelines require that unused vaccines in a multi-dose vial be discarded within six hours of reconstitution due to the concerns of potential contamination and potency loss. This results in vaccine wastage, which can account for losses of 50% or more of the vaccine doses distributed.

[006] In the public health arena, a common approach for freeze-prevention is to strengthen and optimize the cold chain. Drawbacks of this approach include the expense associated with extending, updating and improving the cold chain equipment, monitoring the equipment, and training those using the equipment. Moreover, although cold chain improvement can minimize freeze-damage, such improvement will not eliminate the occurrence of freeze-damage. Additionally, cold chain improvements will not mitigate the freezing that occurs outside of the cold chain in colder climates.

[007] There remains a need for compositions and methods for stabilizing temperature-sensitive vaccines, and specifically for compositions and methods for stabilizing vaccines.

SUMMARY OF THE INVENTION

[008] The present invention comprises a composition comprising at least one virus like particle (VLP) and a sugar glass. In one embodiment, said sugar glass is composed of a monosaccharide and/or disaccharide. In another embodiment, said monosaccharide is selected from the group consisting of glucose, sorbitol, galactose, mannose, and mannitol. In another embodiment, said

disaccharide is selected from the group consisting of trehalose, maltose, maltotriose, lactose, lactulose, and sucrose. In another embodiment, said VLP and said sugar glass is a powder. In another embodiment, said powder is made by spray drying, freeze drying, milling or a combination thereof. In another embodiment, said powder has a mean particle diameter between about 0.1 nm to about 100 microns. In another embodiment, said powder is suspended in a non-aqueous solvent that will not dissolve said sugar glass. In another embodiment, said non-aqueous solution is selected from the group consisting of triacetin, isopropyl myristate, medium chain triglycerides, short, medium, long-chain mono-, di-, tri-, glycerides, aliphatic and aromatic alcohols or a combination thereof. In another embodiment, said powder is administered to an animal orally, via inhalation, intradermally, intranasally, intramusclarly, intraperitoneally, intravenously, subcutaneously, or mucosally (*e.g.* sublingually or buccally). In another embodiment, said VLP comprises at least one viral protein. In another embodiment, said vial protein is from influenza, RSV and/or VZV. In another embodiment, said VLP has increased stability when compared to VLP that is not associated with a sugar glass.

[009] The present invention also comprises, a composition comprising at least one VLP and a sugar glass, wherein said VLP has increased stability when compared said composition without sugar glass. In another embodiment, said increased stability is increase thermal stability. In another embodiment, said sugar glass is composed of a monosaccharide or disaccharide. In another embodiment, wherein said monosaccharide is selected from the group consisting of glucose, sorbitol, galactose, mannose, and mannitol. In another embodiment, said disaccharide is selected from the group consisting of trehalose, maltose, maltotriose, lactose, lactulose, and sucrose. In another embodiment, said stabilized sugar glass VLP is a powder.

[010] The present invention also comprises, a dry powder formulation comprising a VLP and a sugar glass. In one embodiment, said sugar glass is composed of a monosaccharide or disaccharide. In another embodiment, said monosaccharide is selected from the group consisting of glucose, sorbitol, galactose, mannose, and mannitol. In another embodiment, said disaccharide is selected from the group consisting of trehalose, maltose, maltotriose, lactose, lactulose, and sucrose. In another embodiment, said dry powder is made by spray drying, milling or a combination thereof. In another embodiment, said powder is administered to an animal orally, via inhalation, intradermally, intranasally, intramusclarly, intraperitoneally,

intravenously, or subcutaneously. In another embodiment, said powder is administered to an animal via inhalation using an inhaler and/or subcutaneously using a jet of high-pressure air.

[011] The present invention also comprises, a method of delivering a sugar glass stabilized VLP comprising reconstituting a solid form of sugar glassified VLPs in a solvent and administering said reconstituted VLPs into an animal. In one embodiment, said powder is administered to an animal orally, via inhalation, intradermally, intranasally, intramusclarly, intraperitoneally, intravenously, or subcutaneously.

[012] The present invention also comprises, a method of delivering a sugar glass stabilized VLP comprising administering a solid form of sugar glassified VLPs via inhalation and/or injection.

BRIEF DESCRIPTION OF THE FIGURES

[013] Figure 1A SDS PAGE and western blot of VLPS before the glassification of VLPs

[014] Figure 1B SDS PAGE and western blot of VLPS after the glassification of VLPs

[015] Figure 2 SDS PAGE and western blot of VLPS before and after the glassification of VLPs

DETAILED DESCRIPTION

[016] As used herein, the term "adjuvant" refers to a compound that, when used in combination with a specific immunogen (*e.g.* a VLP) in a formulation, will augment or otherwise alter or modify the resultant immune response. Modification of the immune response includes intensification or broadening the specificity of either or both antibody and cellular immune responses. Modification of the immune response can also mean decreasing or suppressing certain antigen-specific immune responses.

[017] As used herein, the term "ambient" temperatures or conditions are those at any given time in a given environment. Typically, ambient room temperature is approximately 22°C, ambient atmospheric pressure, and ambient humidity are readily measured and will vary depending on the time of year, weather conditions, altitude, etc.

[018] As used herein, the term "buffer" refers to a buffered solution that resists changes in pH by the action of its acid-base conjugate components. The pH of the buffer will generally be chosen to stabilize the active material of choice, and will be ascertainable by those in the art.

Generally, this will be in the range of physiological pH, although some proteins, can be stable at a wider range of pHs, for example acidic pH. Thus, preferred pH ranges are from about 1 to about 10, with from about 3 to about 8, from about 6.0 to about 8.0, from about 7.0 to about 7.4, and from about 7.0 to about 7.2. Suitable buffers include a pH 7.2 phosphate buffer and a pH 7.0 citrate buffer. As will be appreciated by those in the art, there are a large number of suitable buffers that may be used. Suitable buffers include, but are not limited to, amino acids, potassium phosphate, sodium phosphate, sodium acetate, histidine-HCl, sodium citrate, sodium succinate, ammonium bicarbonate and carbonate. Generally, buffers are used at molarities from about 1 mM to about 2 M, with from about 2 mM to about 1 M being preferred, and from about 10 mM to about 0.5 M being especially preferred, and 25 to 50 mM being particularly preferred.

[019] As used herein, the term "effective dose" generally refers to that amount of VLPs sufficient to induce immunity, to prevent and/or ameliorate an infection or to reduce at least one symptom of an infection and/or to enhance the efficacy of another dose of a VLP. An effective dose may refer to the amount of VLPs sufficient to delay or minimize the onset of an infection. An effective dose may also refer to the amount of VLPs that provides a therapeutic benefit in the treatment or management of an infection. Further, an effective dose is the amount with respect to VLPs of the invention alone, or in combination with other therapies, that provides a therapeutic benefit in the treatment or management of an infection. An effective dose may also be the amount sufficient to enhance a subject's (*e.g.*, a human's) own immune response against a subsequent exposure to an infectious agent. Levels of immunity can be monitored, *e.g.*, by measuring amounts of neutralizing secretory and/or serum antibodies, *e.g.*, by plaque neutralization, complement fixation, enzyme-linked immunosorbent, or microneutralization assay. In the case of a vaccine, an "effective dose" is one that prevents disease and/or reduces the severity of symptoms.

[020] As used herein, the term "excipients" generally refer to compounds or materials that are added to increase the stability of a therapeutic agent during glassification (by, *e.g.*, the spray freeze dry process) and afterwards, for long-term storage. Suitable excipients can be, *e.g.*, agents that do not thicken or polymerize upon contact with water, are basically innocuous when administered to a patient and does not significantly interact with the therapeutic agent in a manner that alters its biological activity. Suitable excipients are described below and include, but are not limited to, proteins such as human and bovine serum albumin, gelatin, carbohydrates,

sugar alcohols, *e.g.* glycerin, erythritol, glycerol, arabitol, xylitol, sorbitol, and mannitol, propylene glycol, Pluronics, surfactants and combinations thereof. Excipients can be multifunctional constituents of solutions or suspensions of invention.

[021] As used herein, the term "effective amount" refers to an amount of VLPs necessary or sufficient to realize a desired biologic effect. An effective amount of the composition would be the amount that achieves a selected result, and such an amount could be determined as a matter of routine by a person skilled in the art. For example, an effective amount for preventing, treating and/or ameliorating an infection could be that amount necessary to cause activation of the immune system, resulting in the development of an antigen specific immune response upon exposure to VLPs of the invention. The term is also synonymous with "sufficient amount."

[022] As use herein, the term "infectious agent" refers to microorganisms that cause an infection in a vertebrate. Usually, the organisms are viruses, bacteria, parasites and/or fungi.

[023] As used herein, the term "glass" or "glassy state" or "glassy matrix," refers to a liquid that has a markedly reduced ability to flow, *i.e.* it is a liquid with a very high viscosity, wherein the viscosity ranges from 10^{10} to 10^{14} pascal-seconds. It can be viewed as a metastable amorphous system in which the molecules have vibrational motion but have very slow (almost immeasurable) rotational and translational components. As a metastable system, it is stable for long periods of time when stored well below the glass transition temperature. Because glasses are not in a state of thermodynamic equilibrium, glasses stored at temperatures at or near the glass transition temperature relax to equilibrium and lose their high viscosity. The resultant rubbery or syrupy, flowing liquid is often chemically and structurally destabilized. While a glass can be obtained by many different routes, it appears to be physically and structurally the same material by whatever route it was taken. The process used to obtain a glassy matrix for the purposes of this invention is generally a solvent sublimation and/or evaporation technique.

[024] As used herein, the term "glass transition temperature" is represented by the symbol T_g and is the temperature at which a composition changes from a glassy or vitreous state to a syrup or rubbery state. Generally, T_g is determined using differential scanning calorimetry (DSC) and is standardly taken as the temperature at which onset of the change of heat capacity (Cp) of the composition occurs upon scanning through the transition. The definition of T_g is always arbitrary and there is no present international convention. The T_g can be defined as the onset, midpoint or endpoint of the transition; for purposes of this invention we will use the onset of the

changes in Cp when using DSC and DER. See the article entitled "Formation of Glasses from Liquids and Biopolymers" by C. A. Angell: Science, 267, 1924-1935 (Mar. 31, 1995) and the article entitled "Differential Scanning Calorimetry Analysis of Glass Transitions" by Jan P. Wolanczyk: Cryo-Letters, 10, 73-76 (1989). For detailed mathematical treatment see "Nature of the Glass Transition and the Glassy State" by Gibbs and DiMarzio: Journal of Chemical Physics, 28, NO. 3, 373-383 (March, 1958). These articles are incorporated herein by reference.

[025] As used herein, the term "stable" formulation or composition is one in which the biologically active material therein (*e.g.* VLPs) essentially retains its physical stability and/or chemical stability and/or biological activity upon storage. Various analytical techniques for measuring stability are available in the art and are reviewed, *e.g.*, in Peptide and Protein Drug Delivery, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones, A. Adv. Drug Delivery Rev. 10: 29-90 (1993). Stability can be measured at a selected temperature for a selected time period. Trend analysis can be used to estimate an expected shelf life before a material has actually been in storage for that time period.

[026] As used herein, the term "vaccine" refers to a formulation which contains VLPs which is in a form that is capable of being administered to a vertebrate and which induces a protective immune response sufficient to induce immunity to prevent and/or ameliorate an infection and/or to reduce at least one symptom of an infection and/or to enhance the efficacy of another dose of VLPs. Upon introduction into a host, the vaccine is able to provoke an immune response including, but not limited to, the production of antibodies and/or cytokines and/or the activation of cytotoxic T cells, antigen presenting cells, helper T cells, dendritic cells and/or other cellular responses. In one embodiment, VLPs vaccines are associated with sugar glass and/or were stabilized with sugar glass.

[027] As used herein, the term "virus-like particle" (VLP) refers to a structure that in at least one attribute resembles a virus but which has not been demonstrated to be infectious. Virus-like particles in accordance with the invention do not carry genetic information encoding for the proteins of the virus-like particles. In general, virus-like particles lack a viral genome and, therefore, are noninfectious. In addition, virus-like particles can often be produced in large quantities by heterologous expression and can be easily purified.

[028] Vaccines or drugs in solution ready for injections are inherently unstable. Methods to tackle this instability are known in the art (*e.g.* freeze drying). However, this is an inconvenient

and inherently dangerous, since incorrect reconstitution or dried vaccines or drugs can result in wrong doses or contaminated solution and freezing and/or thawing of a vaccine or drug can also result in wrong dosing. Thus, there is a need for new improved vaccine formulations that makes vaccine delivery easier and safer, decrease dependency on the cold chain and/or increase thermal stability of vaccines and/or reduce the number of immunizations interventions. Such vaccine formulations would make distribution of vaccines worldwide more efficient and economical.

[029] One of the keystones in the improvement of vaccine formulations is obtaining stable antigens in the dry state. The most commonly used method for preparing solid proteinaceous drug is freeze-drying (lyophilization). However, during freeze-drying the proteinaceous drug is subjected to freezing and drying stress by which activity can be lost. Therefore, a protective agent is required to prevent damaging effects of lyophilization.

[030] It is known that carbohydrates can protect various types of drug substances like proteins and vaccines during freezing, drying and storage. If dried properly, a proteinaceous drug can be incorporated in a matrix consisting of carbohydrate in the amorphous glassy state (sugar glass). The stabilizing effect of these sugar glasses has been explained by the formation of a matrix that strongly reduces diffusion and molecular mobility and acts as a physical barrier between particles or molecules. Both the lack of mobility and physical barrier provided by the glass matrix, prevent aggregation and degradation of the dried material (Amorij, JP *et al.* (2007) Vaccine, 25, 6447-6457).

[031] To make sugar glass, the sugar must be dried below the glass transition temperature of a carbohydrate, *e.g.* a sugar. The glass transition temperature is the temperature below which the physical properties of amorphous materials vary in a manner similar to those of a solid phase (glassy state), and above which amorphous materials behave like liquids (rubbery state). A material's glass transition temperature, T_g, is the temperature below which molecules have little relative mobility. T_g is usually applicable to wholly or partially amorphous phases such as glasses and plastics. Above T_g, the secondary, non-covalent bonds between the polymer chains become weak in comparison to thermal motion, and the polymer becomes rubbery and capable of elastic or deformation without fracture.

[032] Sugar glass is made when drying a sugar mixture below the T_g temperature. As the sugar solution containing an active molecule is dried, it can either crystallize when the solubility limit of the sugar is reached or can become a supersaturated syrup. The ability of the sugar to resist

crystallization is a crucial property of a good stabilizer. Trehalose is good is known to make glass (Green J L. & Angel C A. Phase relations and vitrification in saccharide water solutions and the trehalose anomaly J. Phys. Chem. 93 2880-2882 (1989)) but is not unique. Further drying progressively solidifies the syrup, which turns into a glass at low residual water content. Imperceptibly, the active molecules change from liquid solution in the water to solid solution in the dry sugar glass. Chemical diffusion is negligible in a glass and therefore chemical reactions virtually cease. Since denaturation is a chemical change it cannot occur in the glass and the molecules are stabilized.

[033] Thus, the invention comprises a composition comprising at least one virus like particle (VLP) associated and a sugar glass. In one embodiment, said VLP is encased by said sugar glass. In one embodiment, said VLP associates with said sugar glass. In one embodiment, said sugar glass is composed of a monosaccharide and/or disaccharide. In another embodiment, said monosaccharide is selected from the group consisting of glucose, sorbitol, galactose, mannose, and mannitol. In another embodiment, said disaccharide is selected from the group consisting of trehalose, maltose, maltotriose, lactose, lactulose, and sucrose. Other sugars include galactose, mannose, xylose, sorbose, lactose, palactose, raffinose, maltodextrins, melezitose, maltose, fructose, arabinose, xylose, ribose, rhamnose, xylitol, erythritol, threitol, gluconate, and/or the like. The suspension or solution can also include, *e.g.*, a polymer, such as starch, starch derivatives, carboxymethyl starch, hydroxyethyl starch (HES), and/or dextran.

[034] In another embodiment of the invention, VLPs are stable in ambient temperatures, when said VLPs are associated with a sugar glass. Said sugar is preferably one which does not crystallize at freezing temperatures such that it destabilizes said VLPs in a glassy formulation. The amount of sugar used in the suspension or solution can vary depending on the nature of the VLP, the type of sugar, and the intended use. However, generally, the final concentration of the sugar is between about 1% and 40%; more preferably, between about 1% and 20% by weight. In one embodiment, the suspension or solution comprises about 60 mg/ml Trehalose. In another embodiment, the suspension or solution comprises about 50 mg/ml of Mannitol. In another embodiment, the suspension or solution comprises about 30 mg/ml of Trehalose and about 25 mg/ml of Mannitol. In another embodiment, the suspension or solution comprises about 30 mg/ml of Trehalose and about 30 mg/ml of Mannitol.

[035] In one embodiment, said VLP and sugar glass composition may be a powder. In another embodiment, said powder is made by spray drying, freeze-drying, milling or a combination thereof. In other embodiment, said powder has a mean particle diameter between about 0.1 nm to about 100 microns. Spray drying and freeze drying is well known in the art (see 6,372,258, 7,258,873, and 5,230,162, all of which are herein incorporated by reference in their entireties.)

[036] The formulation of the invention comprise virus-like particles (VLPs) and a sugar glass. Said VLPs comprise at least a viral core protein (*e.g.* Influenza M1, retrovirus gag, RSV M, Newcastle disease M etc.) and at least one viral surface envelope protein (*e.g.* influenza HA and/or NA, HIV gp120, RSV F, Newcastle disease F). Chimeric VLPs are VLPs having at least two proteins in the VLP, wherein one protein can drive VLP formation (*e.g.* matrix protein) and the other protein is from a heterologous infectious agent. In one embodiment, said infectious agent proteins may have antigenic variations of the same protein. In another embodiment, said infectious agent protein is from an unrelated agent. In another embodiment, said chimeric VLPs comprise a chimeric protein (fusion protein) comprising the antigenic portion of one protein fused to the transmembrane and/or cytoplasmic region of a different (heterologous) protein (See U.S. applications 60/902,337, filed February 21, 2007, and 60/970,592, filed September 7, 2007, both of which are incorporated herein by reference in their entireties for all purposes.

[037] Infectious agents can be viruses, bacteria and/or parasites. A protein that may be expressed on the surface of VLPs can be derived from viruses, bacteria and/or parasites. The proteins derived from viruses, bacteria and/or parasites can induce an immune response (cellular and/or humoral) in a vertebrate that which will prevent, treat, manage and/or ameliorate an infectious disease in said vertebrate.

[038] Non-limiting examples of viruses from which said infectious agent proteins can be derived from are the following: influenza (A and B, *e.g.* HA and/or NA), coronavirus (*e.g.* SARS), hepatitis viruses A, B, C, D & E3, human immunodeficiency virus (HIV), herpes viruses 1, 2, 6 & 7, cytomegalovirus, varicella zoster, papilloma virus, Epstein Barr virus, parainfluenza viruses, adenoviruses, bunya viruses (*e.g.* hanta virus), coxsakie viruses, picoma viruses, rotaviruses, rhinoviruses, rubella virus, mumps virus, measles virus, Rubella virus, polio virus (multiple types), adeno virus (multiple types), parainfluenza virus (multiple types), avian influenza (various types), shipping fever virus, Western and Eastern equine encephalomyelitis, Japanese encephalomyelitis, fowl pox, rabies virus, slow brain viruses, rous sarcoma virus,

Papovaviridae, Parvoviridae, Picomaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (*e.g.*, Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), Togaviridae (*e.g.*, Rubivirus), Newcastle disease virus, West Nile fever virus, Tick borne encephalitis, yellow fever, chikungunya virus, and dengue virus (all serotypes).

[039] In another embodiment, the specific proteins from viruses may comprise: HA and/or NA from influenza virus (including avian), S protein from coronavirus, gp160, gp140 and/or gp41 from HIV, gp I to IV and Vp from varicella zoster, E and preM/M from yellow fever virus, Dengue (all serotypes) or any flavivirus. Also included are any protein from a virus that can induce an immune response (cellular and/or humoral) in a vertebrate that can prevent, treat, manage and/or ameliorate an infectious disease in said vertebrate. In one embodiment, said VLP comprises at least one influenza protein. In another embodiment, said influenza protein is HA or NA. In another embodiment, said VLP comprises at least one RSV protein. In another embodiment, said RSV protein is RSV M, F and/or G. In another embodiment, said VLP comprises a VZV protein. In another embodiment, said VZV protein is gE and/or at least one tegument protein.

[040] Non-limiting examples of bacteria from which said infectious agent proteins can be derived from are the following: *B. pertussis*, *Leptospira pomona*, *S. paratyphi* A and B, *C. diphtheriae*, *C. tetani*, *C. botulinum*, *C. perfringens*, *C. feseri* and other gas gangrene bacteria, *B. anthracis*, *P. pestis*, *P. multocida*, *Neisseria meningitidis*, *N. gonorrheae*, *Hemophilus influenzae*, Actinomyces (*e.g.*, Norcardia), Acinetobacter, Bacillaceae (*e.g.*, *Bacillus anthrasis*), Bacteroides (*e.g.*, *Bacteroides fragilis*), Blastomycosis, Bordetella, Borrelia (*e.g.*, *Borrelia burgdorferi*), Brucella, Campylobacter, Chlamydia, Coccidioides, Corynebacterium (*e.g.*, *Corynebacterium diptheriae*), *E. coli* (*e.g.*, Enterotoxigenic *E. coli* and Enterohemorrhagic *E. coli*), Enterobacter (*e.g. Enterobacter aerogenes*), Enterobacteriaceae (Klebsiella, Salmonella (*e.g.*, *Salmonella typhi*, *Salmonella enteritidis*, Serratia, Yersinia, Shigella), Erysipelothrix, Haemophilus (*e.g.*, *Haemophilus influenza* type B), Helicobacter, Legionella (*e.g.*, *Legionella pneumophila*), Leptospira, Listeria (*e.g.*, *Listeria monocytogenes*), Mycoplasma, Mycobacterium (*e.g.*, *Mycobacterium leprae* and *Mycobacterium tuberculosis*), Vibrio (*e.g.*, *Vibrio cholerae*), Pasteurellacea, Proteus, Pseudomonas (*e.g.*, *Pseudomonas aeruginosa*), Rickettsiaceae, Spirochetes (*e.g.*, Treponema spp., Leptospira spp., Borrelia spp.), Shigella spp., Meningiococcus, Pneumococcus and Streptococcus (*e.g.*, *Streptococcus pneumoniae* and Groups

A, B, and C Streptococci), Ureaplasmas. *Treponema pollidum*, *Staphylococcus aureus*, *Pasteurella haemolytica*, *Corynebacterium diptheriae* toxoid, Meningococcal polysaccharide, *Bordetella pertusis*, *Streptococcus pneumoniae*, *Clostridium tetani* toxoid, and *Mycobacterium bovis.*

[041] Non-limiting examples of parasites from which said infectious agent proteins can be derived which are the causative agent for following: leishmaniasis (*Leishmania tropica mexicana, Leishmania tropica, Leishmania major, Leishmania aethiopica, Leishmania braziliensis, Leishmania donovani, Leishmania infantum, Leishmania chagasi)*, trypanosomiasis (*Trypanosoma brucei gambiense, Trypanosoma brucei rhodesiense)*, toxoplasmosis (*Toxoplasma gondii*) , schistosomiasis (*Schistosoma haematobium*, *Schistosoma japonicum*, *Schistosoma mansoni*, *Schistosoma mekongi*, *Schistosoma intercalatum)*, malaria (*Plasmodium virax*, *Plasmodium falciparium*, *Plasmodium malariae* and *Plasmodium ovale*) Amebiasis (*Entamoeba histolytica*), Babesiosis (Babesiosis *microti)*, Cryptosporidiosis (*Cryptosporidium parvum*), Dientamoebiasis (Dientamoeba fragilis), Giardiasis (*Giardia lamblia*), Helminthiasis and Trichomonas (*Trichomonas vaginalis*). The above lists are meant to be illustrative and by no means are meant to limit the invention to those particular bacterial, viral or parasitic organisms.

[042] Another embodiment of the invention comprises a composition comprising at least one VLP in a sugar glass, wherein said VLP has increased stability when compared to VLP that is not in a sugar glass. In one embodiment, said increased stability is increase thermal stability and increased stability in ambient temperature. VLPs are "stable" in a pharmaceutical composition if, *e.g.*, said VLP shows no significant increase in aggregation, precipitation and/or denaturation upon visual examination of color and/or clarity, as measured by UV light scattering or by size exclusion chromatography, or any biological assay. A "stable" formulation or composition is one in which the biologically active material therein essentially retains its physical stability and/or chemical stability and/or biological activity upon storage. Various analytical techniques for measuring stability are described below and available in the art and are reviewed, *e.g.*, in Peptide and Protein Drug Delivery, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones, A. Adv. Drug Delivery Rev. 10: 29-90 (1993). Stability can be measured at a selected temperature for a selected time period. Trend analysis can be used to estimate an expected shelf life before a material has actually been in storage for that time period. For example, the composition can be stable at room temperature (about 25°C) for at least 3

months, and/or stable at about 2-8°C for at least 1 year. Furthermore, the composition can be stable following freezing (to, *e.g.*, -70°C) and thawing of the composition. In another embodiment, said sugar glass is composed of a monosaccharide or disaccharide. In another embodiment, said monosaccharide is selected from the group consisting of glucose, sorbitol, galactose, mannose, and mannitol. In another embodiment, said disaccharide is selected from the group consisting of trehalose, maltose, maltotriose, lactose, lactulose, and sucrose. In another embodiment, said stabilized sugar glass VLP is a powder.

[043] The VLP associated sugar glass composition may have additional exceptions that help stabilize said VLPs. Thus, the present invention includes compositions, such as suspensions or solutions of VLPs, a polymer additive, an amino acid additive, and/or a surfactant, to help improved stability. The suspensions or solutions can include other ingredients (i.e. excipients) such as buffers, carriers, preservatives, colloidal stabilizers, fillers, diluents, lubricants, amphiphiles, and/or stabilizers. For example, polymers can be included in the suspensions or solutions of the method, *e.g.*, to provide protective and structural benefits. The linear or branching strands of polymers can provide, *e.g.*, increased structural strength to the particle compositions of the invention. Polymers can be applied as a protective and/or time release coat to the outside or powder particles of the invention. Many polymers such as polyvinyl pyrrolidone, polyethylene glycol, poly amino acids, such poly L-lysines, can significantly enhance reconstitution rates in aqueous solutions. Polymer protective agents, in the methods of the invention can include, *e.g.*, starch and starch derivatives, such as oxidized starch, carboxymethyl starch and hydroxyethyl starch (HES). Others include hydrolyzed gelatin, unhydrolyzed gelatin, ovalbumin, collagen, chondroitin sulfate, a sialated polysaccharide, actin, myosin, microtubules, dynein, kinetin, human serum albumin, and/or the like. Other excipients can be included in the formulation. For example, amino acids, such as arginine and methionine can be constituents of the formulation and compositions. The amino acids can, *e.g.*, act as zwitterions that block charged groups on processing surfaces and storage containers preventing nonspecific binding of bioactive materials. The amino acids can increase the stability of compositions by, *e.g.*, scavenging oxidation agents, scavenging deamidation agents, and stabilizing the conformations of proteins. In another example, glycerol can be included in the formulations of the invention, *e.g.*, to act as a plasticizer in the powder particle compositions.

EDTA can be included in the composition, *e.g.*, to reduce aggregation of formulation constituents and/or to scavenge metal ions that can initiate destructive free radical chemistries.

[044] VLP and sugar glass composition of the invention may also include, *e.g.*, a surfactant compatible with the particular bioactive material involved. A surfactant can enhance solubility of other formulation components to avoid aggregation or precipitation at higher concentrations. Surface active agents can, *e.g.*, lower the surface tension of the suspension or solution so that bioactive materials are not denatured at gas-liquid interfaces, and/or so that finer droplets can be formed during spraying. The suspensions or solutions according to the invention comprise between about 0.001 and 5%; and preferably, between about 0.05 and 1%, or about 0.2%, of a nonionic surfactant, an ionic surfactant, or a combination thereof.

[045] Buffers can be added to the formulations of the invention, *e.g.*, to provide a suitable stable pH to the formulations of the method and compositions of the invention. Typical buffers of the invention include, *e.g.*, amino acids, potassium phosphate, sodium phosphate, sodium acetate, sodium citrate, histidine, glycine, sodium succinate, ammonium bicarbonate, and/or a carbonate. The buffers can be adjusted to the appropriate acid and salt forms to provide, *e.g.*, pH stability in the range from about pH 3.0 to about pH 10.0, from about pH 4.0 to about pH 8.0. A pH near neutral, such as, *e.g.*, pH 7.2, is preferred for many compositions. In one embodiment, said VLP associated sugar glass comprises a phosphate buffer at pH 7.2 with 0.5 M NaCl.

[046] In another embodiment, said VLP and sugar glass composition is a powder suspended in a non-aqueous solvent that will not dissolve said sugar glass. In another embodiment, said non-aqueous solution is selected from the group consisting of triacetin, isoprppyl myristate, medium chain triglycerides, short, medium, and/or long-chain monoglycerides, dimonoglycerides, trimonoglycerides, aliphatic and aromatic alcohols, hydrofluoroether, perfluoroether, hyrofluoroamine, perfluoroamine, hydrofluorothioether, perfluorothioether, and hydrofluoropolyether or a combination thereof (see WO 2005/099669, herein incorporated by reference in its entirety). In another embodiment, said powder is administered to an animal orally, *via* inhalation, intradermally, intranasally, intramusclarly, intraperitoneally, intravenously, or subcutaneously.

[047] In another embodiment, said powder is made by spray drying, milling or a combination thereof. In another embodiment, said powder has a mean particle diameter between about 0.1 nm to about 100 microns. In another embodiment, said powder is suspended in a non-aqueous

solvent that will not dissolve said sugar glass. In another embodiment, said powder is administered to an animal orally, *via* inhalation, intradermally, intranasally, intramusclarly, intraperitoneally, intravenously, or subcutaneously. VLPs can have the protein from a bacteria, virus, fungal, parasite, as described above. In one embodiment, said VLP comprises at least one influenza protein. In another embodiment, said influenza protein is HA or NA. In another embodiment, said VLP comprises at least one RSV protein. In another embodiment, said VLP comprises a VZV protein. In another embodiment, said VZV protein is gE. In another embodiment, said composition further comprises an adjuvant.

[048] Another embodiment of the invention comprises a dry powder formulation comprising a VLP in a sugar glass. In one embodiment, said sugar glass is composed of a monosaccharide or disaccharide. In another embodiment, said monosaccharide is selected from the group consisting of glucose, sorbitol, galactose, mannose, and mannitol. In another embodiment, said disaccharide is selected from the group consisting of trehalose, maltose, maltotriose, lactose, lactulose, and sucrose. In another embodiment, said dry powder is made by spray drying, freeze drying, milling or a combination thereof. In another embodiment, said powder has a mean particle diameter between about 0.1 nm to about 100 microns. In another embodiment, said powder is administered to an animal orally, via inhalation, intradermally, intranasally, intramusclarly, intraperitoneally, intravenously, or subcutaneously. In another embodiment, said powder is reconstituted in a solvent before administration. In another embodiment, said powder is administered to an animal *via* inhalation using an inhaler and/or subcutaneously using a jet of high-pressure air. The composition may comprise any of the excipients described above.

[049] Another embodiment of the invention comprises a method of delivering a sugar glass stabilized VLP comprising reconstituting a solid form of sugar glassified VLPs in a solvent and administering said reconstituted VLPs into an animal. In one embodiment, said VLPs are administered to an animal orally, *via* inhalation, intradermally, intranasally, intramusclarly, intraperitoneally, intravenously, or subcutaneously.

[050] Another embodiment of the invention comprises a method of delivering a sugar glass stabilized VLP comprising administering a solid form of sugar glassified VLPs *via* inhalation and/or injection. In one embodiment, said injection is administered *via* a jet of high-pressure air.

[051] Another embodiment of the invention comprises a method of enhancing thermal stability of a VLP, comprising formulating said VLP into a sugar glass. In one embodiment, said sugar

glass is composed of a monosaccharide and/or disaccharide. In another embodiment, said monosaccharide is selected from the group consisting of glucose, sorbitol, galactose, mannose, and mannitol. In another embodiment, said disaccharide is selected from the group consisting of trehalose, maltose, maltotriose, lactose, lactulose, and sucrose. In another embodiment, said sugar glass comprising the VLP is a powder. In another embodiment, said powder is made by spray drying, freeze drying, milling or a combination thereof. In another embodiment, said powder has a mean particle diameter between about 0.1 nm to about 100 microns. In another embodiment, said powder is suspended in an organic solvent that will not dissolve said sugar glass. In one embodiment, said VLP comprises at least one influenza protein. In another embodiment, said influenza protein is HA or NA. In another embodiment, said VLP comprises at least one RSV protein. In another embodiment, said VLP comprises a VZV protein.

[052] As also well known in the art, the immunogenicity of a particular composition can be enhanced by the use of non-specific stimulators of the immune response, known as adjuvants. Adjuvants have been used experimentally to promote a generalized increase in immunity against unknown antigens (*e.g.*, U.S. Pat. No. 4,877,611). Immunization protocols have used adjuvants to stimulate responses for many years, and as such, adjuvants are well known to one of ordinary skill in the art. Some adjuvants affect the way in which antigens are presented. For example, the immune response is increased when protein antigens are precipitated by alum. Emulsification of antigens also prolongs the duration of antigen presentation. The inclusion of any adjuvant described in Vogel *et al.*, "A Compendium of Vaccine Adjuvants and Excipients (2nd Edition)," herein incorporated by reference in its entirety for all purposes, is envisioned within the scope of this invention. In one embodiment said VLP complexed with a sugar glass formulation comprises at least one adjuvant.

[053] Exemplary, adjuvants include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed Mycobacterium tuberculosis), incomplete Freund's adjuvants and aluminum hydroxide adjuvant. Other adjuvants comprise GMCSP, BCG, aluminum hydroxide, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, and monophosphoryl lipid A (MPL). RIBI, which contains three components extracted from bacteria, MPL, trehalose dimycolate (TDM) and cell wall skeleton (CWS) in a 2% squalene/Tween 80 emulsion also is contemplated. MF-59, Novasomes®, MHC antigens may also be used.

[054] In one embodiment of the invention the adjuvant is a paucilamellar lipid vesicle having about two to ten bilayers arranged in the form of substantially spherical shells separated by aqueous layers surrounding a large amorphous central cavity free of lipid bilayers. Paucilamellar lipid vesicles may act to stimulate the immune response several ways, as non-specific stimulators, as carriers for the antigen, as carriers of additional adjuvants, and combinations thereof. Paucilamellar lipid vesicles act as non-specific immune stimulators when, for example, a vaccine is prepared by intermixing the antigen with the preformed vesicles such that the antigen remains extracellular to the vesicles. By encapsulating an antigen within the central cavity of the vesicle, the vesicle acts both as an immune stimulator and a carrier for the antigen. In another embodiment, the vesicles are primarily made of nonphospholipid vesicles. In other embodiment, the vesicles are Novasomes. Novasomes® are paucilamellar nonphospholipid vesicles ranging from about 100 nm to about 500 nm. They comprise Brij 72, cholesterol, oleic acid and squalene. Novasomes have been shown to be an effective adjuvant for influenza antigens (*see*, U.S. Patents 5,629,021, 6,387,373, and 4,911,928, herein incorporated by reference in their entireties for all purposes).

[055] In one aspect, an adjuvant effect is achieved by use of an agent, such as alum, used in about 0.05 to about 0.1% solution in phosphate buffered saline. Alternatively, the VLPs can be made as an admixture with synthetic polymers of sugars (Carbopol®) used as an about 0.25% solution. Some adjuvants, for example, certain organic molecules obtained from bacteria; act on the host rather than on the antigen. An example is muramyl dipeptide (N-acetylmuramyl-L-alanyl-D-isoglutamine [MDP]), a bacterial peptidoglycan. In other embodiments, hemocyanins and hemoerythrins may also be used with VLPs of the invention. The use of hemocyanin from keyhole limpet (KLH) is preferred in certain embodiments, although other molluscan and arthropod hemocyanins and hemoerythrins may be employed.

[056] Various polysaccharide adjuvants may also be used. For example, the use of various pneumococcal polysaccharide adjuvants on the antibody responses of mice has been described (Yin *et al.*, 1989). The doses that produce optimal responses, or that otherwise do not produce suppression, should be employed as indicated (Yin *et al.*, 1989). Polyamine varieties of polysaccharides are particularly preferred, such as chitin and chitosan, including deacetylated chitin. In another embodiment, a lipophilic disaccharide-tripeptide derivative of muramyl

dipeptide which is described for use in artificial liposomes formed from phosphatidyl choline and phosphatidyl glycerol.

[057] Amphipathic and surface active agents, *e.g.*, saponin and derivatives such as QS21 (Cambridge Biotech), form yet another group of adjuvants for use with the VLPs of the invention. Nonionic block copolymer surfactants (Rabinovich *et al.*, 1994) may also be employed. Oligonucleotides are another useful group of adjuvants (Yamamoto *et al.*, 1988). Quil A and lentinen are other adjuvants that may be used in certain embodiments of the present invention.

[058] Another group of adjuvants are the detoxified endotoxins, such as the refined detoxified endotoxin of U.S. Pat. No. 4,866,034. These refined detoxified endotoxins are effective in producing adjuvant responses in vertebrates. Of course, the detoxified endotoxins may be combined with other adjuvants to prepare multi-adjuvant formulation. For example, combination of detoxified endotoxins with trehalose dimycolate is particularly contemplated, as described in U.S. Pat. No. 4,435,386. Combinations of detoxified endotoxins with trehalose dimycolate and endotoxic glycolipids is also contemplated (U.S. Pat. No. 4,505,899), as is combination of detoxified endotoxins with cell wall skeleton (CWS) or CWS and trehalose dimycolate, as described in U.S. Pat. Nos. 4,436,727, 4,436,728 and 4,505,900. Combinations of just CWS and trehalose dimycolate, without detoxified endotoxins, is also envisioned to be useful, as described in U.S. Pat. No. 4,520,019.

[059] Those of skill in the art will know the different kinds of adjuvants that can be conjugated to vaccines in accordance with this invention and these include alkyl lysophosphilipids (ALP); BCG; and biotin (including biotinylated derivatives) among others. Certain adjuvants particularly contemplated for use are the teichoic acids from Gram-cells. These include the lipoteichoic acids (LTA), ribitol teichoic acids (RTA) and glycerol teichoic acid (GTA). Active forms of their synthetic counterparts may also be employed in connection with the invention (Takada *et al.*, 1995).

[060] Various adjuvants, even those that are not commonly used in humans, may still be employed in other vertebrates, where, for example, one desires to raise antibodies or to subsequently obtain activated T cells. The toxicity or other adverse effects that may result from either the adjuvant or the cells, *e.g.*, as may occur using non-irradiated tumor cells, is irrelevant in such circumstances.

[061] Another method of inducing an immune response can be accomplished by formulating the VLPs of the invention with "immune stimulators." These are the body's own chemical messengers (cytokines) to increase the immune system's response. Immune stimulators include, but not limited to, various cytokines, lymphokines and chemokines with immunostimulatory, immunopotentiating, and pro-inflammatory activities, such as interleukins (*e.g.*, IL-1, IL-2, IL-3, IL-4, IL-12, IL-13); growth factors (*e.g.*, granulocyte-macrophage (GM)-colony stimulating factor (CSF)); and other immunostimulatory molecules, such as macrophage inflammatory factor, Flt3 ligand, B7.1; B7.2, etc. The immunostimulatory molecules can be administered in the same formulation as the influenza VLPs, or can be administered separately. Either the protein or an expression vector encoding the protein can be administered to produce an immunostimulatory effect.

[062] Where it is appropriate, the VLP associated with sugar glass of the invention can be administered, *e.g.*, to a mammal. Said VLP and sugar glass composition of the invention can include, influenza, HIV, RSV, VZV VLPs.

[063] Said VLP and sugar glass composition can be administered to a patient by topical application. For example, the powder particles can be mixed directly into a salve, carrier ointment, pressurized liquid, gaseous propellants, and/or penetrant, for application to the skin of a patient. Alternately, the powder particles can, e.g., be reconstituted in an aqueous solvent before admixture with other ingredients before application.

[064] Said VLPs and sugar glass composition can be administered by inhalation. Dry powder particles less than about 10 um in aerodynamic diameter can be inhaled into the lungs for pulmonary administration. Optionally, powder particles of about 20 um, or greater, in aerodynamic diameter can be administered intranasally, or to the upper respiratory tract, where they are removed from the air stream by inertial impact onto the mucus membranes of the patient. The powder particles can alternately be reconstituted to a suspension or solution for inhalation administration as an aqueous mist.

[065] Said VLP and sugar glass composition can be administered by injection. The powder particles can be administered directly under the skin of a patient using, *e.g.*, a jet of high pressure air. More commonly, the powder particles can be, *e.g.*, reconstituted with a sterile aqueous buffer for injection through a hollow syringe needle. Such injections can be, e.g., intramuscular, intra venous, subcutaneous, intrathecal, intraperitoneal, and the like, as appropriate. Powder

particles of the invention can be reconstituted to a solution or suspension with a bioactive material concentration, *e.g.*, from less than about 0.1 ng/ml to from less than about 1 mg/ml to about 500 mg/ml, or from about 5 mg/ml to about 400 mg/ml, as appropriate to the dosage and handling considerations. Reconstituted powder particles can be further diluted, e.g., for multiple vaccinations, administration through IV infusion, and the like.

[066] The appropriate dosage ("therapeutically effective amount") of the VLPs material will depend, for example, on the condition to be treated, the severity and course of the condition, whether the biologically active material is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the biologically active material, the type of biologically active material used, and the discretion of the attending physician. The VLPs are suitably administered to the patent once, or over a series of administrations, and may be administered to the patient at any time. The VLPs may be administered as the sole treatment or in conjunction with other drugs or therapies useful in treating the condition in question.

[067] As a general proposition, the therapeutically effective amount of VLPs administered will be in the range of about 0.00001 to about 50 mg/kg of patent body weight whether by one or more administrations, with the typical range of protein used being from less than about 0.01 ng/kg to about 20 mg/kg, more preferably about 0.1 mg/kg to about 15 mg/kg, administered daily, for example (as measured by the antigenic protein on said VLP). However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques.

[068] The invention also encompasses methods of increasing the "shelf-life" or storage stability of VLPs stored at elevated temperatures. Increased storage stability can be determined by recovery of biological activity in accelerated aging trials. The dry particle compositions produced by methods of the invention can be stored at any suitable temperature. Preferably, the compositions are stored at about 0°C. to about 80°C. More preferably, the compositions are stored at about 20°C. to about 60°C. Most preferably, the compositions are stored at ambient temperatures.

EXAMPLES

Example 1 Freeze-drying of VLPs

[069] H5N1 Clade 2 Influenza VLPs were made and purified according to the methods described in co-pending application 11/582,540, filed October 18, 2006, herein incorporated by reference in its entirety. The VLPs were purified and suspending in phosphate buffered saline, pH 7.2 with the desired concentration of NaCl in a PETG bottle with the desired concentration of sugar. The formulations are described in Table 1.

Table 1 Formulations

Formulation code	Vehicle	Sugar concentration (mg/mL)
Control (C)	PB, pH 7.2 with 0.5M NaCl	None
Cryo-I	PB, pH 7.2 with 0.5M NaCl	Trehalose (60 mg/mL)
Cryo-II	PB, pH 7.2 with 0.15M NaCl	Trehalose (60 mg/mL)
Cryo-III	Water with 0.01% w/v PS80	Trehalose (60 mg/mL)
Cryo-IV	PB, pH 7.2 with 0.15M NaCl	Mannitol (50 mg/mL)
Cryo-V	PB, pH 7.2 with 0.15M NaCl	Trehalose (30 mg/mL) + Mannitol (25 mg/mL)
Cryo-VI	PB, pH 7.2 with 0.15M NaCl	Sucrose (30 mg/mL) + Mannitol (30 mg/mL)

[070] Next, 1.2 mL of the formulation (aqueous suspension) was aliquoted into 2 mL USP Type I glass vials. The potency of the formulations was in the range of 40-80 μg HA/mL. The potency was measured using the quantitative single radial immunodiffusion (SRID) assay. Lyo-stoppers (13 mm, grey chlorobutyl) were placed on the vials and all the formulations were subjected to glassification *via* freeze-drying using a 2 sq.ft. Hull model 2FS48C freeze dryer according to the parameters outlined in the Table 2.

Table 2 Freeze Drying Parameters

Step	Shelf Temperature Setpoint (°C)	Soak Time (hours)	Ramping Rate (°C/hour)	Pressure Set Point (mHg)
Product Loading	5	2		5-10 inHg vac
			30	5-10 inHg vac
Freeze/Evacuation	-50	3		5-10 inHg vac
			30	80
Primary Drying, Step 1	-30	1		80
			30	80
Primary Drying, Step 2	-25	45		80
			15	80
Secondary Drying	25	8		80

Stoppering Pressure	14.7 psi
Total Processing Time	**66 hrs**

[071] After glassification the material in each vial was reconstituted with 1.2 mL of sterile water for injections (WFI) before analyzing VLP potency and stability (post-lyophilization).

Example 2 VLP analysis after glassification

[072] After resuspention, Single Radial Immunodiffusion (SRID), total protein analysis, Particle size analysis, HA and NA activity were checked. Total protein was measured by the BCA method (using a commercial kit that employs bicinchoninic acid). Particle size was measured using the Malvern zeta sizer (dynamic light scattering principle). HA activity was measured using haemagglutination assay employing Turkey RBCs and NA activity was measured by fluorimetry using Munana reagent). In addition, SDS PAGE and western blot analysis were conducted. Tables 3 to Table 7 and Figure 1 summarize the results of these analysis.

TABLE 3 SRID (µg HA/mL)

	Before FD	After FD	% change
Control	71.72	59.19	-17.47
Cryo-I	71.98	56.88	-20.98
Cryo-II	68.94	70.01	1.55
Cryo-III	41.29	36.06	-12.67
Cryo-IV	66.34	54.71	-17.53
Cryo-V	67.01	69.58	3.84
Cryo-VI	66.99	64.78	-3.30

TABLE 4 Total proteins by BCA (mg/mL)

	Before FD	After FD	% change
Control	0.13	0.133	-0.58
Cryo-I	0.15	0.143	-4.67
Cryo-II	0.13	0.134	0.59
Cryo-III	0.12	0.12	-1.66
Cryo-IV	0.12	0.122	-0.48
Cryo-V	0.14	0.131	-6.38
Cryo-VI	0.13	0.126	-6.59

TABLE 5 Particle size (nm)

	Before FD	After FD	% change
Control	156	274	75.64
Cryo-I	161	163	1.24
Cryo-II	155	158	1.94
Cryo-III	1320	1190	-9.85
Cryo-IV	155	176	13.55
Cryo-V	154	158	2.60
Cryo-VI	155	159	2.58

TABLE 6 HA titer

	Before FD	After FD	% change
Control	2048	8192	300.00
Cryo-I	4096	4096	0.00
Cryo-II	4096	8192	100.00
Cryo-III	128	32	-75.00
Cryo-IV	256	8192	3100.00
Cryo-V	2048	8192	300.00
Cryo-VI	2048	2048	0.00

TABLE 7 NA activity (mU/mL)

	Before FD	After FD	% change
Control	7	5.30	-24.29
Cryo-I	7.3	6.50	-10.96
Cryo-II	6.5	6.30	-3.08
Cryo-III	0.3	0.45	50.00
Cryo-IV	5.8	5.00	-13.79
Cryo-V	6	5.70	-5.00
Cryo-VI	6.4	5.80	-9.38

[073] Figures 1 A and B compares VLPs comprising HA and NA *via* SDS and western blots before glassification and after glassification. As shown, after glassification of the VLPs there is no difference in the amount HA and NA as shown in the gels. Thus, glassification does not reduce or chew up the VLPs or antigen expressed on the VLPs.

[074] The these results show that the formulations Control, Cryo-II and Cryo-V in particular retained the physical, chemical and biological properties of the VLPs most effectively upon

freeze-drying. It was surprising to see a protection effect without any sugar (control). But the benefit of sugar-glassification can be shown from the stability study at higher temperatures.

Example 3 VLP stability at high temperatures

[075] The recommended storage temperature for an aqueous suspension containing VLPs is 4-8°C. The freeze-dried vials (control, cryo-II and cryo-V for 5 or 12 weeks) where stored at 25°C and 50°C to investigate the protection effect of sugars on VLP stability. The VLPs were analyzed using the same methods as described above. The results are summarized in the following tables (FD = freeze drying).

Table 8 SRID (µg HA/mL)

	Before FD	After FD	5 weeks @ 25°C	5 weeks @ 50°C	12 weeks @ 25°C	12 weeks @ 50°C
Control	71.72	59.19	78.20	0.00	41.59	0.00
Cryo-II	68.94	70.01	80.60	33.38	83.27	59.99
Cryo-V	67.01	69.58	78.80	52.41	67.30	36.88

Table 9 Particle size (nm)

	Before FD	After FD	5 weeks @ 25°C	5 weeks @ 50°C	12 weeks @ 25°C	12 weeks @ 50°C
Control	156	274	364.00	414.00	359.9	386.4
Cryo-II	155	158	160.00	178.00	160.4	166.1
Cryo-V	154	158	160.00	182.00	164.2	175.2

Table 10 HA titer

	Before FD	After FD	5 weeks @ 25°C	5 weeks @ 50°C	12 weeks @ 25°C	12 weeks @ 50°C
Control	2048	8192	1024	256	512	64
Cryo-II	4096	8192	2048	64	2048	2048
Cryo-V	2048	8192	2048	2048	2048	2048

[076] Even though there seem to be no significant differences in the data between control and sugar-glassified products at 25°C up to 5 weeks, there was a significant drop in potency (SRID value) at 12 weeks for control. At 50°C, the control formulation lost its potency completely within 5 weeks and showed aggregation and loss in bioactivity (as indicated by HA titer).

[077] The sugar-glassified formulations (cryo-II and V) preserved the potency, particle size and HA titer at 25°C up to 3 months. At 50°C a potency loss of approx. 13% and 45% was observed

for cryo-II and cryo-V respectively while the particle size and HA titer remained unaffected. Trehalose appeared to preserve the integrity of the VLPs better than the trehalose + mannitol combination.

Example 4 Spray-drying of VLPs

[078] H5N1 Clade 2 Influenza VLPs were made and purified according to the methods described in co-pending application 11/582,540, filed October 18, 2006, herein incorporated by reference in its entirety.

[079] Placebo was prepare in 6% w/v solution of Trehalose by dissolving 24 grams of Trehalose in 400 mL of phosphate buffered saline, pH 7.2 with 0.15 M NaCl. The solution was sterilized by passing it through a 0.22µ membrane filter.

[080] VLPs were collect in Phosphate buffered saline, pH 7.2 with 0.15 M NaCl (potency of about 45 µg HA/mL) in a PETG bottle. Trehalose was then added to constitute 6% w/v solution. The solution was mixed gently (hand shaking) until all Trehalose is dissolved.

[081] Next, the Spray-dryer was set-up and run under the following conditions: SD-Micro (Niro) with glass chambers, 2.4/3/4 mm Morprene tubing with "Y". Compressed air was used as the drying air. The conditions for each of the runs are in Table 11.

Table 11 Spray Dry Conditions

Run	Inlet temp(°C)	Outlet temp(°C)	Atom pressure (bar)	Atom rate (kg/h)	Airflow (kg/h)	Spray rate (g/min)
1	160	90	1.6	1.6	29.9	6.69
2	160	100	1.1	1.2	29.9	4.37

Run #1 = Placebo formulation
Run #2 = VLP-sugar formulation

[082] After resuspention, Single Radial Immunodiffusion (SRID), HA titer particle size and NAA were checked (as described above). In addition, SDS PAGE blot analysis was conducted. The results are summarized on Table 12 and on the gels in Figure 2.

Table 12 Summary of Spay Dried VLPs Assays

Sample ID	NAA (mU/ml)	HA Titer	SRID (µg/ml)	Particle Size (nm)
Before spray drying	48.0	1024	40.8	173.4
200mg dried powder in 2ml WFI after spray drying	25.1	1024	35.4 (354 µg HA/gm dried product)	203.8
500mg dried powder in 2ml WFI after spray drying	64.0	2048	64.8 (259 µg HA/gm dried product	221.9

Note: Theoretical potency (assuming 5% w/w residual moisture): 45 µg HA per 115 mg dried product.
Practical potency: 45 µg HA per 127 to173 mg of dried product

[083] These data show that the practical potency (the potency achieved *after* spray drying in terms of µg HA/mg of dried powder) was comparable to theoretical potency (the potency that is estimated by a calculation based on material inputs e.g. quantity of VLPs, trehalose and an assumption of approx. 5-10% moisture upon drying and greater than 95% recovery), thus indicating a preservation of original properties of the VLPs after spray-drying. In addition, the SDS-PAGE and Western blot gels (Figures 1A) indicate the integrity of the three principal proteins (HA, NA and M1) with no band shifting or broadening for any of the formulations, before and after freeze-drying.

[084] All publications, patents and patent applications herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

[085] The foregoing detailed description has been given for clearness of understanding only and no unnecessary limitations should be understood therefrom as modifications will be obvious to those skilled in the art. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed inventions, or that any publication specifically or implicitly referenced is prior art.

[086] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[087] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or

customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

Claims:

1. A composition comprising at least one virus like particle (VLP) and a sugar glass.

2. The composition of claim 1, wherein said sugar glass is composed of a monosaccharide and/or disaccharide.

3. The composition of claim 2, wherein said monosaccharide is selected from the group consisting of glucose, sorbitol, galactose, mannose, and mannitol.

4. The composition of claim 2, wherein said disaccharide is selected from the group consisting of trehalose, maltose, maltotriose, lactose, lactulose, and sucrose.

5. The composition of claim 1, wherein said VLP and sugar glass is a powder.

6. The composition of claim 5, wherein said powder is made by spray drying, freeze drying, milling or a combination thereof.

7. The composition of claim 6, wherein said powder has a mean particle diameter between about 0.1 nm to about 100 microns.

8. The composition of claim 5, wherein said powder is suspended in a non-aqueous solvent that will not dissolve said sugar glass.

9. The composition of claim 8, wherein said non-aqueous solution is selected from the group consisting of triacetin, isoprppyl myristate, medium chain triglycerides, short/medium/long-chain mono-/di-/tri-glycerides, aliphatic and aromatic alcohols or a combination thereof.

10. The composition of claim 5, wherein said powder is administered to an animal orally, via inhalation, intradermally, intranasally, intramusclarly, intraperitoneally, intravenously, subcutaneously or mucosally (*e.g.* sublingually or buccally).

11. The composition of claim 1, wherein said VLP comprises at least one influenza protein.

12. The composition of claim 11, wherein said influenza protein is HA or NA.

13. The composition of claim 1, wherein said VLP comprises at least one RSV protein.

14. The composition of claim 1, wherein said VLP comprises at least one VZV protein.

15. A composition comprising at least one VLP and a sugar glass, wherein said VLP has increased stability when compared to a composition without sugar glass.

16. The composition of claim 15, wherein said increased stability is increased thermal stability.

17. The composition of claim 15, wherein said sugar glass is composed of a monosaccharide or disaccharide.

18. The composition of claim 17, wherein said monosaccharide is selected from the group consisting of glucose, sorbitol, galactose, mannose, and mannitol.

19. The composition of claim 17, wherein said disaccharide is selected from the group consisting of trehalose, maltose, maltotriose, lactose, lactulose, and sucrose.

20. The composition of claim 15, wherein said stabilized sugar glass VLP is a powder.

21. The composition of claim 20, wherein said powder is made by spray drying, milling or a combination thereof.

22. The composition of claim 21, wherein said powder has a mean particle diameter between about 0.1 nm to about 100 microns.

23. The composition of claim 20, wherein said powder is suspended in a non-aqueous solvent that will not dissolve said sugar glass.

24. The composition of claim 20, wherein said powder is administered to an animal orally, via inhalation, intradermally, intranasally, intramusclarly, intraperitoneally, intravenously, or subcutaneously.

25. The composition of claim 15, wherein said VLP comprises at least one influenza protein.

26. The composition of claim 25, wherein said influenza protein is HA or NA.

27. The composition of claim 15, wherein said VLP comprises at least one RSV protein.

28. The composition of claim 15, wherein said VLP comprises at least one VZV protein.

29. The composition of claim 15, where said composition further comprises an adjuvant.

30. A dry powder formulation comprising a VLP and a sugar glass.

31. The dry powder formulation of claim 30, wherein said sugar glass is composed of a monosaccharide or disaccharide.

32. The dry powder formulation of claim 30, wherein said monosaccharide is selected from the group consisting of glucose, sorbitol, galactose, mannose, and mannitol.

33. The dry powder formulation of claim 31, wherein said disaccharide is selected from the group consisting of trehalose, maltose, maltotriose, lactose, lactulose, and sucrose.

34. The dry powder formulation of claim 30, wherein said dry powder is made by spray drying, milling or a combination thereof.

35. The dry powder formulation of claim 34, wherein said powder has a mean particle diameter between about 0.1 nm to about 100 microns.

36. The dry powder formulation of claim 30, wherein said powder is administered to an animal orally, via inhalation, intradermally, intranasally, intramusclarly, intraperitoneally, intravenously, or subcutaneously.

37. The dry powder formulation of claim 36, wherein said powder is reconstituted in a solvent before administration.

38. The dry powder formulation of claim 36, wherein said powder is administered to an animal via inhalation using an inhaler and/or subcutaneously using a jet of high-pressure air.

39. A method of delivering a sugar glass stabilized VLP comprising reconstituting a solid form of sugar glassified VLPs in a solvent and administering said reconstituted VLPs into an animal.

40. The method of claim 39, wherein said powder is administered to an animal orally, via inhalation, intradermally, intranasally, intramusclarly, intraperitoneally, intravenously, or subcutaneously.

41. A method of delivering a sugar glass stabilized VLP comprising administering a solid form of sugar glassified VLPs via inhalation and/or injection.

42. The method of claim 41, wherein said injection is administered via a jet of high-pressure air.

43. A method of enhancing thermal stability of a VLP, comprising formulating said VLP into a sugar glass.

44. The method of claim 43, wherein said sugar glass is composed of a monosaccharide and/or disaccharide.

45. The method of claim 44, wherein said monosaccharide is selected from the group consisting of glucose, sorbitol, galactose, mannose, and mannitol.

46. The sugar glass stabilized VLP of claim 42, wherein said disaccharide is selected from the group consisting of trehalose, maltose, maltotriose, lactose, lactulose, and sucrose.

47. The method of claim 43, wherein said sugar glass comprising the VLP is a powder.

48. The method of claim 47 wherein said powder is made by spray drying, milling or a combination thereof.

49. The method of claim 48, wherein said powder has a mean particle diameter between about 0.1 nm to about 100 microns.

50. The method of claim 47, wherein said powder is suspended in a non-aqueous solvent that will not dissolve said sugar glass.

51. The method of claim 43, wherein said VLP comprises at least one influenza protein.

52. The method of claim 51, wherein said influenza protein is HA or NA.

53. The method of claim 43, wherein said VLP comprises at least one RSV protein.

54. The method of claim 43, wherein said VLP comprises a VZV protein.

55. The composition of claim 1, wherein said composition comprises at least one excipient selected from the group consisting of buffers, carriers, preservatives, colloidal stabilizers, fillers, diluents, lubricants, amphiphiles and stabilizers.

FIG.1A

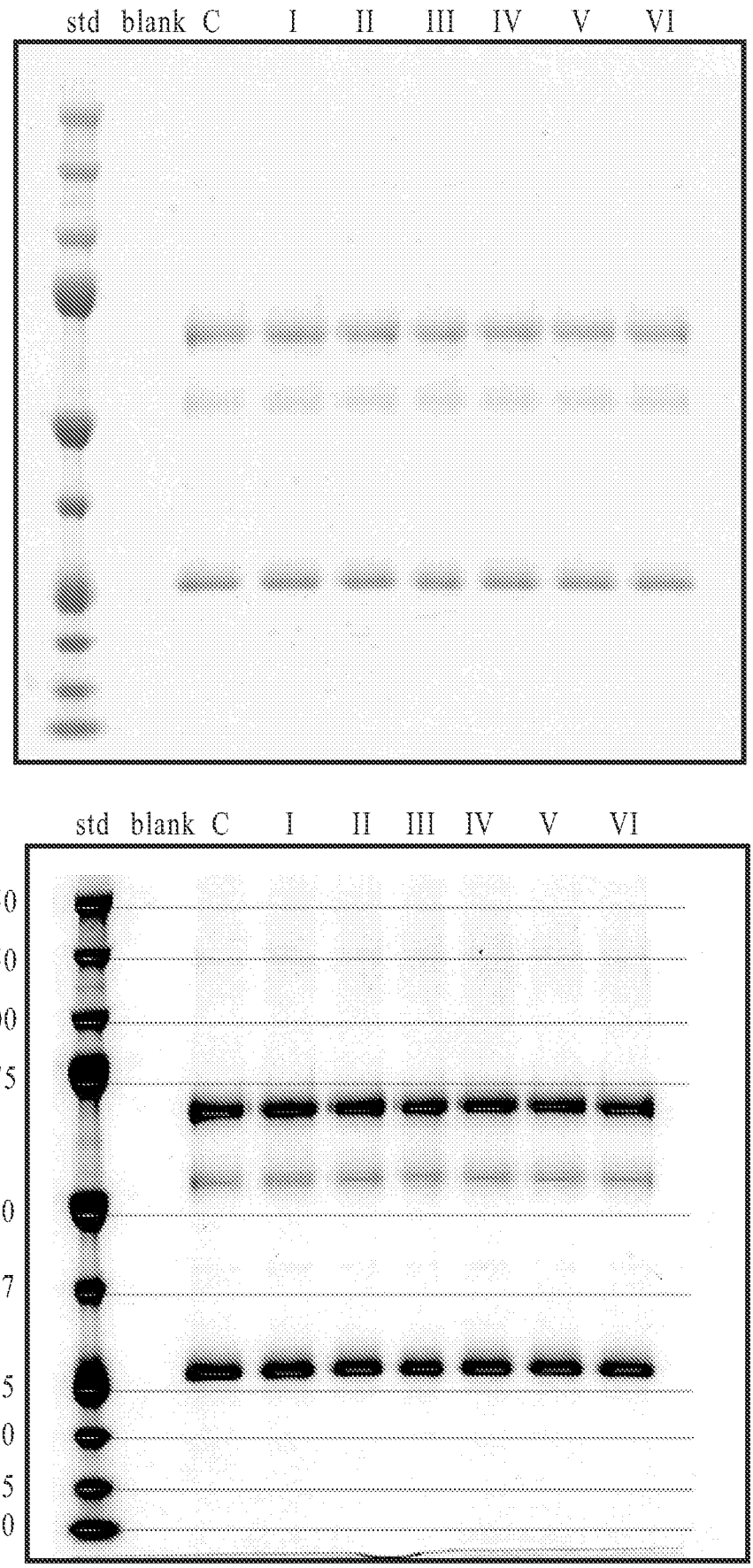
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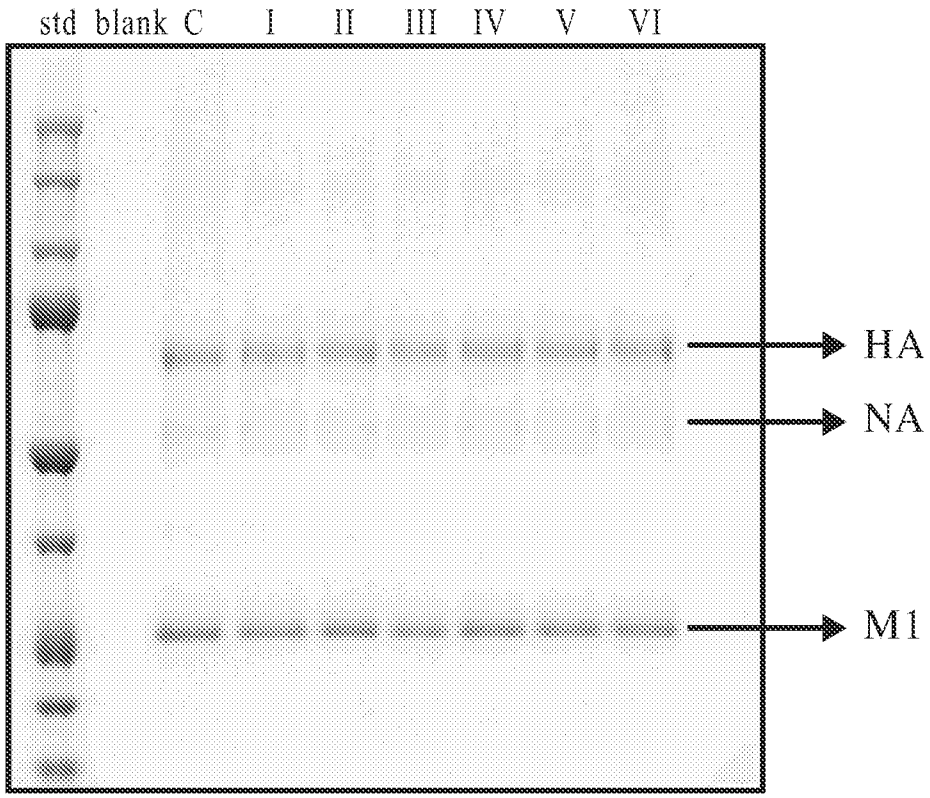
FIG.1B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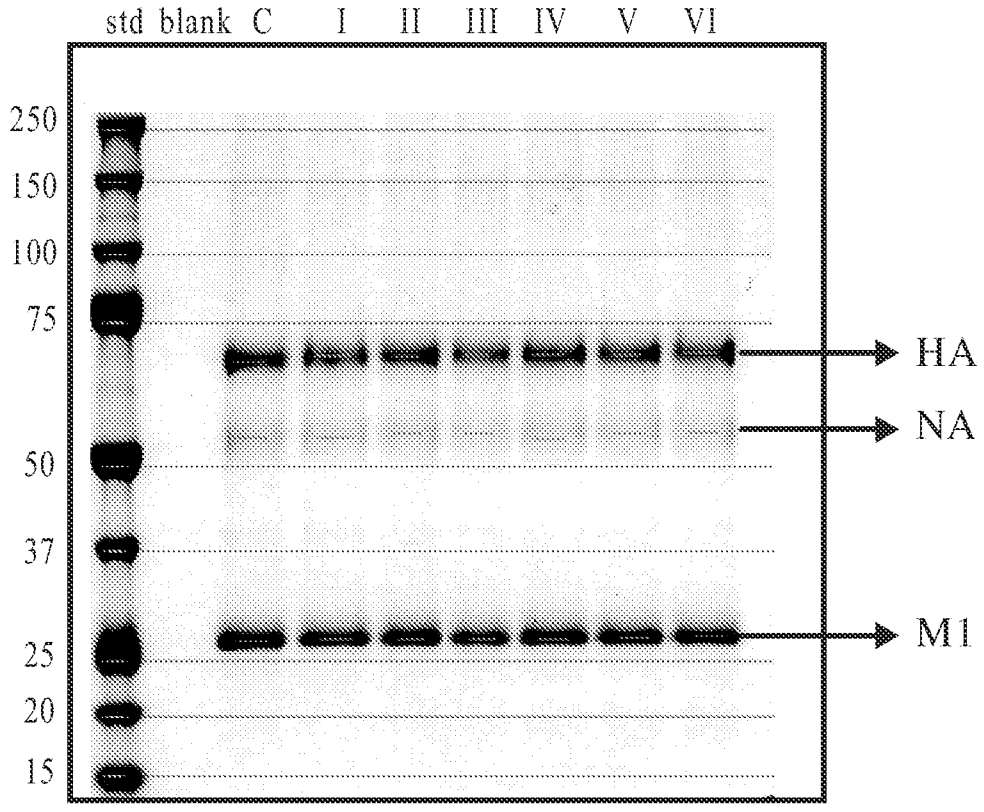
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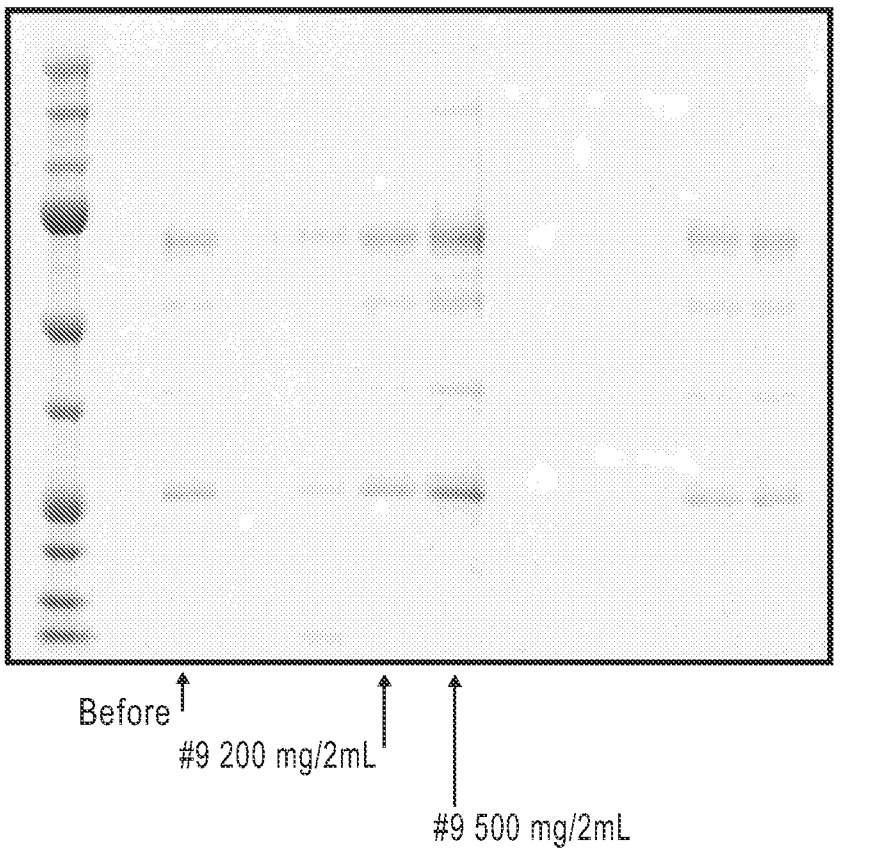
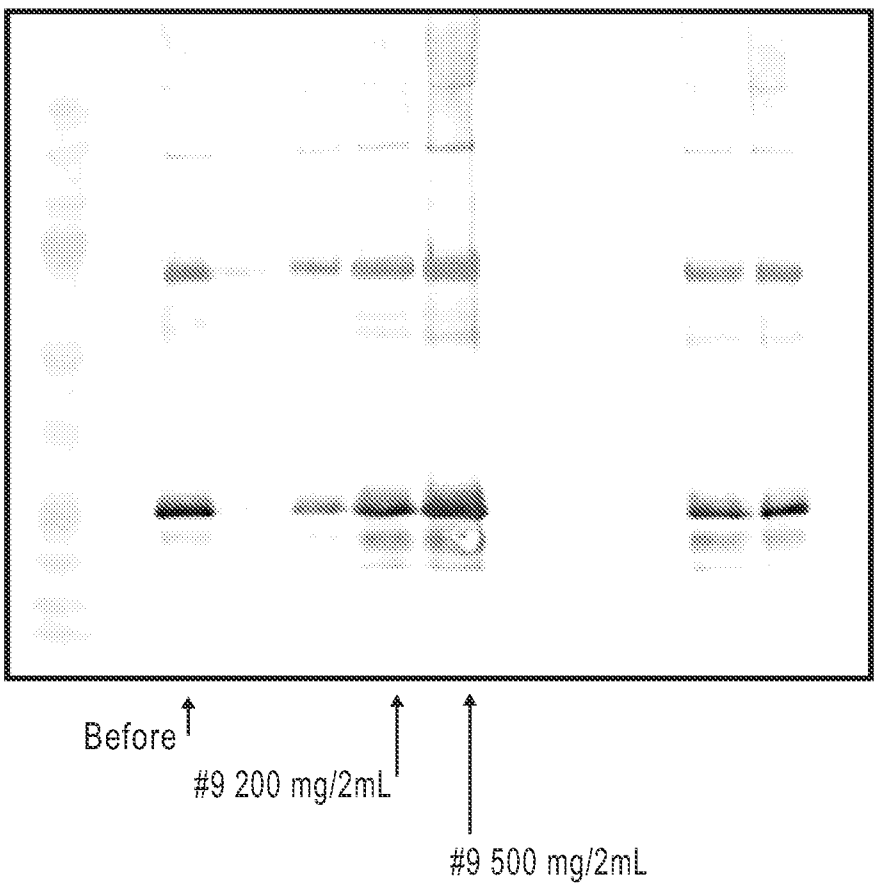
FIG.2





INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 09/35122

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 9/14; C12N 15/00 (2009.01)
USPC - 424/488; 435/320.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 424/488; 435/320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 424/192.1, 199.1; 435/69.1; 514/54 (text search-see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST (PGPB,USPT,EPAB,JPAB), Patentscope (worldwide), Google Scholar, Dialogweb
sugar glass, virus-like particles, glucose, galactose, mannose, trehalose, influenza vaccine, protein, antigen

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/085082 A1 (DREW) 17 August 2006 (17.08.2006) pg 4, ln 15-32; pg 7, ln 20-23; pg 9, ln 1-4; ln 20-22; pg 12, ln 20-22; pg 13, ln 1-2	1-2, 4-6, 10-17, 19-21, 24-31, 33-34, 36-44, 46-48, 51-55
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Y		3, 7-9, 18, 22-23, 32, 35, 45, 49-50
Y	US 2005/0276846 A1 (ROSER et al.) 15 December 2005 (15.12.2005) para [0029]-[0030], [0033], [0037], [0081], [0083]; Fig. 1, Fig. 3	3, 7, 18, 22, 32, 35, 45, 49
Y	US 6,669,963 B1 (KAMPINGA) 30 December 2003 (30.12.2003) col 6, ln 55-67	8-9, 23, 50

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
03 April 2009 (03.04.2009)	13 APR 2009
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (April 2007)