10 Anexos:

- [x] Comprobante de pago de la tasa de presentación de la solicitud por $ 482.000.oo.
- [ ] Comprobante de pago por reivindicación de prioridad.
- [x] Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.
- [x] Poderes, si fuere el caso.
- [ ] Documento de cesión del inventor al solicitante o a su causante.
- [ ] Declaraciones.
- [x] Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos)
- [x] Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos)
- [ ] Copia del examen preliminar efectuado por la IPEA.
- [ ] Traducción del examen preliminar efectuado por la IPEA.
- [ ] De ser el caso, copia del contrato de acceso.
- [ ] De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.
- [ ] De ser el caso, certificado de depósito del material biológico.
- [ ] Arte final 12x12
- [ ] De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionadas parcial o totalmente con la invención de esta solicitud.
- [x] Otros: VER HOJA DE DOCUMENTOS ANEXOS

11 FIGURA CARACTERÍSTICA

12

Solicito la concesión de la patente,

NOMBRE: EMILIO FERRERO

3

DOCUMENTOS ANEXOS

1. ☒ Publicación Internacional WO 2006/094974 A3
   Descripción:
   Páginas 44 en el idioma original
   Páginas 57 en español
   Reivindicaciones: Número (s) 30
   Figuras: Número (s)
2. ☒ **Forma PCT/ISA/210.** Informe de Búsqueda Internacional.

3. ☒ Traducción al español del nuevo capítulo reivindicatorio modificado de acuerdo al artículo 19 del PCT.

4. ☒Extracto de publicación.

SE REALIZARON MODIFICACIONES EN LA FASE INTERNACIONAL

C. VELIER
ABOGADOS
EDIFICIO SISKI
CARRERA 4ª Nº 72-35
BOGOTA, 8 - COLOMBIA

B

Para patentes, modelos y dibujos; marcas de fábrica y de servicios; nombres comerciales y enseñas.

Für Patente, Modelle und Zeichnungen; Warenzeichen und Servicezeichen; Handelsnamen-und-zeichen.

4

REPRESENTACION Y PODER

VERTRETUNG UND VOLLMACHT

4475
2099

Ich (wir) unten Unterzeichnender (de)/Yo (nosotros), abajo firmado (s)

Intervet International B.V.

wohnhaft in/domiciliado(s) en Wim de Körverstraat 35, 5831 AN Boxmeer, the Netherlands

por el presente nombramos a GERMAN CAVELIER, tpa. 832; GONZALO PERDOMO, tpa. 831; ERNESTO CAVELIER, tpa. 11519; y EMILIO FERRERO, tpa. 31929; domiciliados en la Carrera 4ª Nº 72-35, Bogotá, Colombia, en obedecimiento a lo dispuesto en el parágrafo 1º del Artículo 543 del Código de Comercio, el primero como principal y los demás como suplentes, en su orden, como nuestros representantes en todos los asuntos de propiedad industrial con facultad para recibir notificaciones y nombrar apoderados judiciales o extrajudiciales. El primer representante suplente reemplazará al principal en caso de ausencia o impedimento temporal o definitivo de aquél. La misma regla se aplicará cuando el segundo representante suplente haya de reemplazar al primero, o el tercero al segundo. En tales casos, el representante principal, o el primero, o segundo suplente, en su caso, o ambos, declararán su intención de no ejercer la representación mediante declaración autenticada por Notario Público en Colombia, o por Notario u otro Funcionario Público, o Cónsul de Colombia, en el extranjero; y si se tratare de impedimento, con el certificado respectivo. Si alguno de los nombrados faltare definitivamente, el siguiente tomará su lugar. Cuando cesare la ausencia o impedimento del representante principal, o de su primero o segundo suplente, en su caso, podrán ejercer la representación, en su orden, uno u otros según el caso, asumiéndola por el solo hecho de ejercerla, cesando en ese momento el ejercicio de la representación por el primero, segundo o tercer suplente en su caso.
Además, y bajo las mismas reglas, conferimos poder a los abogados arriba nombrados, para obtener la protección de mi (nuestra) propiedad industrial y contra la competencia desleal, a cuyo efecto autorizo(amos) a los dichos apoderados para que me(nos) representen ante cualesquiera entidades o personas, ejerzan o no jurisdicción, especialmente ante las del orden administrativo, contencioso-administrativo y judicial, en todo lo concerniente a los siguientes asuntos: a) Obtención de patentes de invención, modelos y dibujos industriales; el registro de marcas de fábrica, colectivas, defensivas y de servicios; el depósito de nombres comerciales y enseñas; su renovación, traspaso, cambio de nombre o domicilio, cancelación voluntaria; y el registro de licencias de uso de esos derechos; b) Las acciones de oposición, cancelación, nulidad, medidas cautelares, concesión de licencias y en general los juicios civiles, penales, administrativos o contencioso-administrativos relacionados con estos derechos; acciones y juicios que promovamos o se nos promuevan. Y para que en todos los asuntos arriba determinados, puedan sustituír este mandato, transigir, desistir, cancelar, recibir, renunciar, y ratificar los actos hechos por agentes oficiosos.

verleihen durch dieses Schreiben an GERMAN CAVELIER, tpa. 832; GONZALO PERDOMO, tpa. 831; ERNESTO CAVELIER, tpa. 11519; und EMILIO FERRERO, tpa. 31929; wohnhaft in der Carrera 4ª Nº 72-35, Bogotá, Kolumbien, um die Verfügung des Absatzes 1 im Artikel 543 des Handelsgesetzbuch zu beachten, der erste als Hauptvertreter und die übrigen als Stellvertreter, beziehungsweise, als unsere Vertreter in allen was sich auf das industrielle Eigentum bezieht, berechtigt Meldungen zu empfangen und gerichtilche und aussergerichtilche Bevollmägtigte zu ernennen. Der erste Stellvertreter wird den Hauptvertreter ersetzen wegen zeitweiligen oder definitiven Abwesenheit oder Hindernisses des ersten. Dieselbe Regel wird angewandt werden wenn der zweite Stellvertreter den ersten ersetzen muss, oder der dritte den zweiten. In solchen Fälle der. Hauptvertreter, oder der erste oder der zweite Stellvertreter, beziehungsweise, oder beide, werden ihre Absicht aussprechen um nicht die Vertretung auszuüben mittelst Aussage notariell beglaubigt in Kolumbien, oder vor Notar oder vor einem anderen öffentlichen Beamter, oder Kolumbianischen Konsul im Auslande; und wenn es ein Hinderniss sei, mittels der betreffenden Beglaubigung. Wenn einer der Gennanten immerfort abwesend wäre, der Folgende wird ihn ersetzen. Im Falle dass die Abwesenheit oder das Hindernis des Hauptvertreters, oder diejenigen seines ersten, zweiten oder dritten Vertreters, beziehungsweise, beendeten, einer oder anderen, ordnungsweise werden die Vertretung ausüben und sie zu sich nehmen nur auf Grund sie auszuüben, mit Aufhören zur Zeit der Ausübung der Vertretung durch den ersten, den zweiten oder den dritten Stellvertreter beziehungsweise.
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COPIA FIEL DEL ORIGINAL QUE TUVO A LA VISTA
30 AGO 2007
NOTARIO 61

Unterzeichnet heute am/Dado y firmado hoy

Arnhem May 28 2002

TRADUCCIÓN OFICIAL No. 13.08/02 preparada por María Elvira Martelo, traductora oficial aprobada por el Ministerio de Justicia mediante Resolución No. 2879 de 1995 y reconocida por el Ministerio de Relaciones Exteriores.

A continuación se traducen los sellos y legalizaciones de un documento por el cual **INTERVET INTERNATIONAL B.V.** otorga poder a **GERMÁN CAVELIER, GONZALO PERDOMO, ERNESTO CAVELIER Y EMILIO FERRERO.**

Dado y firmado el 28 de mayo de 2002.

(Firmas ilegibles), J.B.H. Aarntzen y Lentjes-Sissing (Apoderados).

REINO DE LOS PAÍSES BAJOS

Legalización, entre otras, ex Sección 69 del Código de Comercio

Yo, Petrus Charles HENDRIKS, "Makelaar" (sic) en Marcas y Diseños de la Corte de Distrito (Publicado en la Gaceta de 10 de marzo de 1976, No. 49) por el presente certifico:

1. que el Sr. Joseph Bernardus Hendrikus AARNTZEN y la Sra. Linda LENTJES, née SISSING, quienes firmaron este documento a nombre de la Intervet International B.V. son competentes conjuntamente para ejecutar dicho documento en virtud de un Poder General legalmente expedido por la Junta Directiva de Intervet International B.V. el 20 de febrero de 2002;
2. Que INTERVET INTERNATIONAL B.V. está domiciliada en 5831 AN Boxmeer at Wim de Köverstraat No. 35, Países Bajos, es una

NOTARÍA SEXTA DEL CÍRCULO
DE BOGOTÁ HAGO CONSTAR QUE LA
FOTOCOPIA COINCIDE CON EL ORIGINAL
QUE SE TUVO A LA VISTA
30 AGO 2007

leyes de los Países Bajos, como lo evidencia el extracto expedido por la Cámara de Industria y Comercio de Eastern Brabant (No. 16028015 de fecha 28 de enero de 2002), el cual he visto.

Dado en Bussum hoy 4 de junio de 2002. (Firma ilegible). Sello de P.CH HENDRIKS.

APOSTILLA

Apostilla de los Países Bajos otorgada en español con fecha **07 de junio de 2002** bajo el **número 010107.** Firmada por L.G. der Horst.

Es traducción fiel y completa de la cual se deja copia en esta oficina para su confrontación.

COLO 04975-704-001-6

WPCL 4975 ID 17

AGO 22/02

María E. Martelo.
TRADUCCION No.: 13.08.02
MARIA ELVIRA MARTELO
Traductora e Interprete Oficial
Inglés - Español - Inglés
Res. No. 2879 Min. Justicia

CONSULADO NOTARIO SEXTO (2) DEL CIRCULO DE BOGOTA, HAGO CONSTAR QUE LA FOTOCOPIA COINCIDE CON EL ORIGINAL QUE HE TENIDO A LA VISTA
30 AGO 2007
PABLO MENDEZ BARAJAS
NOTARIO (E)

CAVELIER
ABOGADOS

EDIFICIO SISKI
CARRERA 4 No. 72 - 35
BOGOTA 8, COLOMBIA

WEB SITE: www.cavelier.com
E-MAIL: cavelier@cavelier.com

TELEFONO: (57) (1) 347-3611
TELEFAX: (57) (1) 217-9211 (57) (1) 310-4995

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Señora Jefe de la
DIVISION DE SIGNOS DISTINTIVOS
Superintendencia de Industria y Comercio
E. S. D.

Poderdante: INTERVET INTERNATIONAL B.V.

Poder conferido el: 28-05-2002

Yo, GERMÁN CAVELIER, obrando en mi calidad de apoderado principal del poderdante arriba mencionado, atentamente manifiesto a Usted que sustituyo el poder que me ha sido conferido en la persona del doctor EMILIO FERRERO, Abogado con Tarjeta Profesional N° 31.929, con todas las facultades que me fueron conferidas.

Hago la anterior sustitución conforme al artículo 66 del Código de Procedimiento Civil ya que los otros sustitutos faltan en este momento.

Señora Jefe,

EL SUSCRITO NOTARIO SEXTO (E) DEL CIRCULO DE BOGOTA, HACE CONSTAR QUE LA FOTOCOPIA COINCIDE CON EL ORIGINAL QUE HE TENIDO A LA VISTA
30 AGO 2007
PABLO MENDEZ BARAJAS
NOTARIO (E)

GERMÁN CAVELIER
T.P.A. No. 832
C.C. No. 2.877.924 de Bogotá

Acepto:

EMILIO FERRERO
T.P.A. N° 31.929
C.C. No. 438.019 de Usaquén

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(71) Applicant *(for all designated States except US)*: **INTERVET INTERNATIONAL B.V.** [NL/NL]; Wim de Körverstraat 35, NL-5831 AN Boxmeer (NL).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **GELDER VAN, Petrus** [NL/NL]; P.O. Box 31, NL-5830 AA Boxmeer (NL). **LOERMANS, Arnoldus** [NL/NL]; P.O. Box 31, NL-5830 AA Boxmeer (NL). **MAASSEN, Mathias** [NL/NL]; P.O. Box 31, NL-5830 AA Boxmeer (NL).

(74) Agents: **KEUS, J.** et al.; Intervet International B.V., P.O. Box 31, NL-5830 AN Boxmeer (NL).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

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— *of inventorship (Rule 4.17(iv))*

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— *with international search report*
— *with amended claims*

(88) Date of publication of the international search report:
10 May 2007

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: CHEMICALLY DEFINED STABILISER COMPOSITION

(57) Abstract: The present invention relates to a stabiliser composition comprising an amino acid, and a sugar wherein all compounds are chemically defined; to a vaccine composition comprising such a stabiliser composition and a biological molecule and/or a micro-organism; to a method for preparing a pharmaceutical composition comprising admixing such a stabiliser composition with a biological molecule and/or a micro-organism; to the use of such a stabiliser composition, and of vaccines prepared therewith.

WO 2006/094974 A3

Chemically defined stabiliser

The present invention relates to a stabiliser composition comprising an amino acid, and a sugar wherein all compounds are chemically defined; to a vaccine composition comprising such a stabiliser composition and a biological molecule and/or a micro-organism; to a method for preparing a pharmaceutical composition comprising admixing such a stabiliser composition with a biological molecule and/or a micro-organism; to the use of such a stabiliser composition, and of vaccines prepared therewith.

In the bio-pharmaceutical industry involved in producing micro-organisms and biological molecules as products, the stability of these products is a major issue. Almost always, these products need to be formulated, stored and transported. Inevitably by influences experienced over time from temperature-changes and other physical or chemical influences, the materials produced lose their original effective quantity or desired qualitative properties. Consequently, conditions and additives for preventing such a loss of quality and/or of effective quantity are applied.

Much used stabilizing conditions are storage at reduced temperature, above or below zero °C, and reduction of water content; especially useful is freeze-drying.

In freeze-drying a sample containing a biologically or pharmaceutically active molecule, a micro-organism, a cell, or a tissue is first frozen, and than dried under vacuum. Freeze-dried samples can then be stored at e.g. 4°C and often remain stable for years. See for reviews: M. Pikal, 1990 (BioPharm, vol. 3, no. 8, p. 18-27 and no. 9, p. 26-30); L. Gatlin, et al., 1994 (Bioprocess Techn., vol. 18, p. 317-367); J. Carpenter et al., 1997 (Pharm. Research, vol. 14, p. 969-975); F. Bedu-Addo, 2004 (Pharm. Techn., 1 February, p. 10-18).

Ideally, a freeze-dried product has an almost unchanged quality and quantity of the freeze dried component(s) over a certain period of time, as well as

the physical influences of transportation, and that dissolves rapidly upon reconstitution in a diluent.

To achieve all these requirements for a freeze dried sample, and to make the sample 'survive' the various unfavourable conditions that occur during the freeze-drying process itself (freezing, drying, heating, cooling), the prolonged storage in freeze-dried state and the reconstitution, the sample to be freeze-dried is usually mixed with a stabilising composition prior to freeze-drying.

Compounds of a stabiliser composition are commonly termed: bulking agent, cake-former, lyoprotectant, tonicity modifier, surfactant, cryo(genic) protectant, freeze protectant, desiccant, lyophilisation agent, etc., wherein a specific compound can have several functions.

The term "compatible solute" is also used to indicate a compound that stabilizes molecules and organisms in conditions of a low water environment (M.S. da Costa et al. 1998, Adv. Bioch. Eng and Techn., vol. 61, p. 117 - 153).

Stabilising compositions, either for freeze-drying or for other stabilising uses, are complex mixtures of proteins, carbohydrates, lipids and salts; the composition of which can be adapted for each molecule or micro-organism to be stabilised, or for a specific purpose.

Although it is seldom known how a stabiliser functions exactly, the common finding is that large compounds of high molecular weight generally provide good stabilising effects. Therefore, of old the major ingredients used in stabilisers were such bulky compounds. To keep material-costs down they were taken from readily available products of animal origin, e.g.: milk powder, tryptose, gelatine, serum-albumin, collagen, casein hydrolysate, chondroitin sulphate, etc.

Unfortunately, the use of such compounds of animal origin potentially introduces the risk of contamination with extraneous agents of an otherwise sterile or controlled product. This has been a major concern ever since the discovery of intra-species zoonotic diseases and the newly discovered prion-related diseases. As a result, regulatory authorities have required extensive safety-testing of (stabilised) biological products for absence of extraneous agents before release onto the market.

This has led to abandonment of the use of such animal components, initially only in the culture media used in the production of the biological molecules or micro-organisms. For instance, B. Makoschey et al. (2002, Cytotechnology vol. 39, p. 139-145), describe a freeze-drying stabiliser for use in stabilising viruses that had been produced serum free; the stabiliser comprised sugars, amino acids, gelatin, and peptides from a hydrolysate of protein of animal origin.

Later, also the stabilisers that are used subsequently after production, were modified in their composition; as a result, stabiliser compositions were developed that are: serum free (without animal serum); protein free (without animal protein, but may contain other animal derived components) or even animal compound free (ACF) (not containing any component derived from an animal).

However, in the stabiliser compositions, the need remained for high molecular weight, large molecules for their stabilising effect. Therefore bulky compounds for ACF stabilisers were obtained for instance from plant or microbial origin, such as: yeastolate, soybean peptone, recombinant gelatine, (hydroxy-ethyl-) starch, alginate, etc..

Alternatively, large polymeric compounds of natural or synthetic origin are employed such as: polysaccharides, polyvinyl pyrrolidone, ficol, poly-lysine, poly-ethylene glycol, (carboxy-) methyl-cellulose, dextran, poly-sorbates (e.g. Tween® 80), etc.. For instance T. Osterberg et al. (1997, Pharm. Res., vol. 14, p. 892-898), replaced serum albumin by Tween® 80 as stabilising compound for Factor VIII protein.

There are however several problems even with these ACF stabiliser compounds. For instance, several of these stabilising compounds are not resistant to heat sterilisation, and because sterility of the stabiliser composition is often an absolute requirement, the only other way to achieve this is then by the more tedious process of filter-sterilisation.

Also, in case the stabilised product is to be administered to a target human or animal, some of the ACF stabiliser compounds can not be used as they are toxic, or may cause allergic reactions.

Most prominent problem however in all these cases, is that the stabiliser-compositions, even when ACF, still share the common disadvantage that they contain or consist of unknown, heterogenous and ill-defined constituents. For instance the composition of the hydrolysates of natural vegetable products is not known at all. Similarly, for the polymer structures mentioned, their chemical structure is only known in general terms; the compounds used are only defined in that they have an average molecular size or an average length of the side chains. These molecules therefore actually comprise a family of chemical structures, with a wide variety of molecules.

Such compounds therefore still are of ill-defined nature, and have lot-to-lot variation. This requires careful selection of the starting compounds for suitable batches for production of a stabiliser composition. The batches of compounds that have the desired quality then have limited availability.

Use of such ill-defined compounds in stabilising compositions causes unknown and uncontrollable variations in composition, quality and yield of the products that are stabilised by these compositions.

Such an uncontrollable product quality is highly undesirable in an industry that is trying to produce consistent, safe, high quality products, and can only be overcome by costly and time-consuming procedures for selection of raw materials, verification of incoming goods, product release and final product quality control.

Object of the invention is to provide an alternative and improved stabiliser composition that does not suffer these disadvantages of the prior art.

Surprisingly it was found that a stabiliser composition comprising at least one amino acid, and at least one sugar, wherein all compounds are chemically defined, can provide efficient stabilisation of the quality and the quantity of biological molecules and of micro-organisms, without the need for high molecular weight, large, or ill-defined compounds. This provides many advantages over the prior art.

The stabilising composition of the invention is serum free, protein free and animal compound free, and therefore does not introduce any extraneous agents into the controlled product that is to be stabilised. Also the stabilising composition

obviates the need for incorporation of ill-defined, heterogenous or unknown compounds, this results in product stabilisation leading to predictable and controllable end-products. Both aspects reduce the requirements for control on the end-products for quality and extraneous agents.

The stabilising composition comprises only exactly known compounds, not suffering from lot-to-lot variation, or limited availability of batches with required favourable characteristics. This reduces the requirements for the intake control and selection of starting materials. Further, the stabilising composition according to the present invention is non-toxic to target humans or animals, and is heat steriliseable.

The stabilising composition provides good stabilisation of biological molecules and of micro-organisms against physical and chemical influences, in particular it stabilises the quality and effective quantity of such samples against the negative influences experienced over time and through temperature-changes.

When used in freeze-drying, the stabilising composition stabilises biological molecules and micro-organisms against the process conditions of freezing, drying, heating, cooling, as well as against any negative influences during prolonged storage in freeze dried state and in reconstitution.

Also the stabilising composition provides a freeze-dried body or cake that is of attractive and elegant appearance, that can resist the physical influences of transportation, and that dissolves rapidly upon reconstitution in a diluent.

Therefore, the invention relates to a stabiliser composition comprising at least one amino acid and at least one sugar, characterised in that all compounds are chemically defined.

A "stabiliser composition" is defined as a composition that when admixed with biological molecules or micro-organisms has stabilising effect; i.e. the stabiliser composition can prevent to a large degree the loss of quality or effective quantity of samples containing biological molecules and/or micro-organisms.

The "effective quantity" is for instance the quantity of biologically or pharmaceutically active biological molecules, or the number of live or infectious

The "loss of quality" might otherwise have occurred by physical and/or chemical influences, such as over time by variation in temperature, mechanical influences (e.g. transport) and/or changes in physical form (e.g. freezing, drying).

The stabiliser composition may be in the form of an aqueous solution, an aqueous concentrate, or may be provided as a dry mixture of chemicals suitable for preparing an aqueous solution or an aqueous concentrate of the stabiliser composition according to the invention.

The biological molecules and/or the micro-organisms that are to be stabilised can themselves be in liquid, dried, frozen, or freeze-dried form, before the admixing with the stabiliser composition.

The micro-organisms may be alive or dead, e.g. as result of deliberate inactivation.

A "sugar" is generally known to be a compound giving a sweet or sweetish taste, and more specifically is a carbohydrate such as a mono- or di-saccharide, a sugar-alcohol, or a polyol, and derivatives thereof.

For the invention, a compound is "chemically defined" when it consists exclusively of identical molecules.

Such a chemically defined compound therefore has a specifically known, unambiguous, and uniform chemical structure, and it is used in a specifically known quantity.

A chemically defined compound of the stabiliser according to the invention therefore is not an ill-defined, heterogenous or unknown compound such as a proteinaceous lysate, extract, hydrolysate, or peptide mixture. Also, no polymeric compound is comprised in the stabiliser composition according to the invention, of which only an average molecular weight, or an average length of the side chains is known.

A chemically defined compound of the stabiliser according to the invention is readily available from commercial suppliers of fine chemicals; preferably the highest purity available is employed. However, the requirement of being chemically defined does not rule out the presence of trace amounts of impurities in such a compound. Such impurities are for instance heavy metals, residues,

solvents or the like. Preferably such impurities are present in an amount of less than 1 % by weight of the chemically defined compound, more preferably less than 0.1, 0.01, 0.001, or 0.0001 % by weight.

Preferably, the requirement of being chemically defined relates to the molecules of a compound of the stabiliser according to the invention, when in ionised form in an aqueous solution. Consequently, in a preferred embodiment such a chemically defined compound may in its solid or non-ionised form be present in different salt forms, or in forms having a different number of bound molecules of crystal water (hydrate forms).

Preferably the stabiliser composition according to the invention provides stabilisation of biological molecules and/or micro-organisms to at least the same level as prior art stabilisers comprising ill-defined compounds.

Preferably the loss of titre when using the stabiliser composition according to the invention for stabilisation of a product containing a live micro-organism, is less than 1 Log_{10}. More preferably less than 0.5 Log_{10}.

However this is not always required: for some applications, not having ill-defined compounds in the stabiliser and consequently in the final product is of overwhelming importance; a slightly lower yield in quality and/or quantity resulting from the use of the stabiliser composition according to the invention is in that case perfectly acceptable in exchange for the many advantages this offers over the prior art stabilisers.

This is for instance the case when the stabiliser according to the invention is to be used as stabiliser for long term storage of micro-organism seed material, such as in glycerol stocks. In that case ACF stabilisation by a fully defined stabiliser may be more important, and a loss in titre of up to 2 Log_{10} may be acceptable, as such seed material will normally be cultured to the right quantity for inoculation of a new culture anyway.

Further, a skilled person is perfectly capable of further optimisations to for instance the composition of the stabiliser composition according to the invention, or the conditions for its use, by routine experimentation. Therefore

To assess the level of stabilisation, corresponding to the remaining effective quantity and quality of the stabilised product, any suitable technique can be used. For instance, the quantity and virulence (hence the quality) of viral and bacterial compounds can be quantified by titration or limiting dilution; biological molecules can be detected biochemically (e.g. enzymatically), immunologically (immuno-fluorescence test), or physically (e.g. electrophoresis, chromatography, or mass-spectrometry). All these techniques are well known and available in the art.

In a preferred embodiment, the stabiliser composition according to the invention additionally comprises at least one polyamine.

A "polyamine" is defined as a compound with two or more amino groups.

In more preferred embodiments, the invention relates to a stabiliser composition according to the invention:

- wherein the sugar is at least one selected from the group consisting of glucose, lactose, sucrose, maltose, trehalose, sorbitol and mannitol;
- wherein the polyamine is at least one selected from the group consisting of ethylene-diamine, cadaverine, putrescine, spermidine and spermine;
- wherein the amino acid is glutamate (glutamic acid) or glycine, or wherein both these amino acids are comprised.

In a further more preferred embodiment, the stabiliser composition according to the invention is a buffered aqueous solution; preferably the aqueous solution is buffered between pH 6 - 8, more preferably at about pH 6.9

The buffer can for instance be Tris / citrate, preferably at a concentration of about 50 mM / 10 mM.

Preferably the buffer is phosphate based, more preferably di-sodium-phosphate di-hydrate.

Preferably the buffer is present in an amount of between 0.1 and 100 g/l, more preferably about 1 g/l.

In an alternate more preferred embodiment, the aqueous solution of the stabiliser composition according to the invention is a concentrated solution, comprising the compounds of the stabiliser composition in a concentration that is higher than the concentration at which these compounds will be in the final pharmaceutical composition that is formed upon admixing the stabiliser composition according to the invention with the biological molecules or the micro-organisms that are to be stabilised.

Preferably the stabiliser composition is 2x, 3x, 4x, 5x, 10, or 20x concentrated in comparison to the concentration or the amount in the final pharmaceutical composition; more preferably the stabiliser composition is 4x concentrated.

In a more preferred embodiment, a dry composition of chemicals is provided that is suitable for dissolving in an appropriate solvent, to prepare an aqueous solution or a concentrate of the stabiliser composition of the invention.

Providing a dry mixture of chemicals for later dissolution has the advantages of storage in a smaller volume. This saves storage space, and facilitates transportation and sales.

Even more preferably, in the stabiliser composition according to the invention:

- the sugar is sucrose, sorbitol, or mannitol; preferably sorbitol; more preferably D-sorbitol;
- the amino acid is glutamate or glycine; more preferably L-glutamate; even more preferably both L-glutamate and glycine are comprised;
- a combination of polyamines is used; more preferably spermine and putrescine, or spermine and ethylene diamine.

In a further more preferred embodiment of the stabiliser composition according to the invention, the 4x concentrate comprises:

- the sugar in an amount of between 10 and 200 g/l, preferably at about 75 g/l;
- the amino acid in an amount of between 0.1 and 400 g/l; preferably L-glutamate is comprised at about 20 g/l or glycine is comprised at about 120 g/l

- the polyamine in an amount of between 0.1 and 100 g/l; preferably spermine is comprised at about 10 g/l or spermidine is comprised at about 2.5 g/l.

In a further preferred embodiment, the stabiliser composition according to the invention, comprises a sugar and glutamate as amino acid and additionally comprises at least one compatible solute. The compatible solute preferably is at least one selected from the group consisting of sarcosine, betaine, di-glycine, and choline. The compatible solute in the 4x concentrate is preferably present in an amount of between 0.1 and 400 g/l; preferably between 50 - 200 g/l, more preferably at about 160 g/l.

As outlined above, it is irrelevant in which form of salt or hydrate a compound of the stabiliser composition is used; for instance spermine can be used in the forms of: spermine, spermine-dyhydrate, or spermine tetra-hydrochloride; similarly, spermidine may be employed as spermidine, or spermidine tri-hydrochloride.

As the stabiliser according to the invention is intended to be admixed with biological molecules and/or micro-organisms, in some uses, the compounds of the stabiliser may be part of a product that is administered to a target human or animals. Therefore, the compounds of the stabiliser composition according to the invention preferably are non-toxic, at least: non-toxic in the concentrations present in the final product to be administered.

Preferably the compounds of the stabiliser composition according to the invention do not induce an unwanted allergic reaction.

Preferably, the stabiliser composition according to the invention is sterile. Sterilisation of the stabiliser according to the invention can be achieved by heat, filtration, irradiation or any suitable technique known in the art. Preferably the stabiliser composition is heat sterilised.

By way of non-limiting examples, chemically defined compounds suitable for use in the stabiliser composition according to the invention are listed in Table 1

Table 1: Chemically defined compounds suitable for use in the stabiliser composition according to the invention

Product	Supplier	Catalogue nr.
Na_2HPO_4 di-hydrate	Merck	6576
D-Sorbitol	Sigma	S 7547
Na-L-Glutamate mono-hydrate	Merck	6445
Glycine	Sigma	G 8790
Spermine	Fluka	85590
Spermine tetra-hydrochloride	Fluka	85610
Spermine di-hydrate	Fluka	85588
Spermidine	Fluka	85561
Spermidine tri-hydrochloride	Fluka	85580
Putrescine	Sigma	P 7505
Ethylene diamine	Aldrich	19,580-4
Tris-HCl (Trishydroxymethyl-aminomethane)	Merck	8382
tri-Na Citrate di-hydrate	Merck	6447
Sarcosine	Sigma	S 7672
Betaine	Sigma	B 7045
Di-glycine	Sigma	G 3915
Choline	Sigma	C 2004

As is known in the art, derivatives of the herein described compounds of the stabiliser composition according to the invention, are available, or can be synthesised. Such derivatives are also suitable for use as compounds of the stabiliser composition according to the invention. Therefore, such derivatives are within the scope of the invention.

Table 2: Overview of the preferred and most preferred stabiliser compositions according to the invention.

Amounts given correspond to those of a 4x concentrate:

		Amount in g/l in a 4x concentrate	
		Preferred	Most preferred
Buffer		0.1 - 100	
	Na_2HPO4	idem	1
	or Tris/Citrate	id.	50 mM / 10 mM
Sugar		10 - 200	
	Sorbitol	id.	75
Amino-acid		0.1 - 400	
	Glutamate	id.	20
	and/or Glycine	id.	160
Polyamine		0.1 - 100	
	Spermine	id.	10
	or Spermidine	id.	2.5
	or Putrescine	id.	10
	or Ethylene-diamine	id.	10
	or combinations: spermine and putrescine or spermine and ethylene diamine		 10 5 10 5
Compatible solute		0.1 - 400	
	Sarcosine	id.	160
	or Betaine	id.	160
	or Di-glycine	id.	160
	or Choline	id.	160
pH		6 - 8	6.9

A large number of experiments were performed with stabiliser compositions according to the invention. These were designed to incorporate all conditions under which the stabiliser composition according to the invention provides effective stabilisation:

- cooling; for storage of (bulk)product in liquid form at e.g. 4 °C
- freezing; for storage of (bulk)product deep frozen, at e.g. -20 °C, or -45 °C
- freeze-drying; incorporating: drying, heating, cooling
- freezing of freeze-dried product; for storage deep frozen, at e.g. -20 °C, or -45 °C
- cooling of freeze-dried product; for storage at 4 °C
- heating of freeze-dried product, to temperatures above room temperature, and
- reconstitution of freeze-dried product in a diluent.

When these experiments were performed with stabilised compositions comprising micro-organisms, the remaining number of live infective micro-organisms in the reconstituted products were determined using well known quantification techniques based on titration or limiting dilution. When performed with biological molecules, the quality and quantity was determined using specific assays such as titration, Elisa, or bio-assays.

These experiments and their results are summarised in the examples, and show that the stabiliser composition according to the invention provides for efficient stabilisation of micro-organisms and or biological molecules, not only for the various uses in and around freeze-drying, but also to stabilisation of bulk antigen in cooled or in frozen storage, prior to or in stead of freeze-drying.

In an alternate embodiment, the stabiliser composition of the invention is characterised in that no compounds of high molecular weight or large size are comprised.

For the invention, "high molecular weight" and "large" compounds, are defined as any compound having a molecular weight or size of the molecule

as its molecular ion has a molecular weight of only 202 Da, when excluding any crystal water, or salt-forms, such as hydrates, or (hydro) chlorides.

As described above, it was surprising to find none of the bulky compounds commonly used in the art are required to achieve efficient stabilisation, in fact no compound larger than spermine is required.

The advantages of this have also been mentioned; the high-molecular weight, large compounds often are ill-defined and heterogenous, or at best are compounds of which only an average molecular size or an average length of the side chains is known. Consequently, such ill-defined compounds used in a stabiliser composition will result in a stabilised product with unpredictable characteristics.

In an alternate preferred embodiment, the invention relates to a vaccine composition comprising a stabiliser composition according to the invention, and at least one biological molecule or at least one micro-organism, or a combination thereof.

A vaccine composition is commonly known in the art to represent a composition comprising an immunogenic compound in a pharmaceutically acceptable carrier, which immunogenic compound is capable of inducing a passive or active activation of a targets' immune system. The induced immune response is meant to interfere which the establishment or the progression of a certain infection, or to ameliorate symptoms of disease.

A pharmaceutically acceptable carrier can be e.g. sterile water or a sterile physiological salt solution. In a more complex form the carrier can e.g. be a buffer.

A "biological molecule" for the invention is understood to be a molecule that can be found in nature; in particular this refers to a protein, carbohydrate, lipid, or nucleic acid. The origin of the molecule may be biologic or synthetic, derived either ex vivo or ex vitro.

The term "protein" is meant to incorporate a molecular chain of two or more amino acids; therefore peptides, oligopeptides and polypeptides are included within the definition of protein.

The vaccine composition according to the invention may be in any form, e.g.: freeze-dried, liquid, or frozen; or may be formulated or processed to a liquid, a gel, an ointment, a powder, a tablet, a capsule, or a freeze-dried body depending on the desired method of storage, transportation or application to the target.

The vaccine composition according to the invention may comprise single immunogenic micro-organisms and biological molecules or combinations thereof.

In preferred embodiments, the invention relates to a vaccine composition:

- wherein the micro-organism is a virus or a bacterium, or wherein both a virus and a bacterium are comprised; more preferably the virus is at least one viral species selected from the group consisting of herpes-, paramyxo-, orthomyxo-, adeno-, rhabdo-, birna-, corona-, pneumo-, and poxvirus; even more preferably the virus is selected from the group consisting of Bovine herpes virus, bovine parainfluenzavirus 3, pseudorabies virus, human respiratory syncytial virus, and human or animal influenza virus; more preferably, the bacterium is selected from the group consisting of the bacterial species Streptococcus, Staphylococcus, Escherichia, Mycoplasma, Edwardsiella, Campylobacter and Salmonella.
- wherein the biological molecule is at least one selected from the group consisting of a protein, a carbohydrate, a lipid, and a nucleic acid; more preferably the protein is at least one selected from the group consisting of an antibody, an antibody-fragment, a cytokine, a hormone, and an enzyme.

Antibody fragments are e.g. Fab fragments and single chain antibodies. Hormones for use with the stabiliser composition of the invention are for example insulin, gonadotrophic hormones such as follicle stimulating hormone, human chorion gonadotrophin and erythropoeitin.

The vaccines and stabiliser compositions of the invention are applicable with a wide variety of viruses, of any genomic or morphologic type, infective to any species of animal, or to humans. In particular, any results presented on stabilisation of a particular animal virus can be extrapolated directly to predict the results of stabilisation of the corresponding virus infecting an other host species, either animal or human, and to the other viral species within its viral family.

Viruses having a viral envelope are particularly sensitive to chemical and physical influences. Consequently, as such viruses can be effectively stabilised by the stabiliser composition of the invention, this proves its efficacy even more.

Table 3: Examples of viruses for use with the stabiliser composition of the invention

family	example	genome	enveloped
herpes	bovine herpes virus (BHV) *	ds DNA	yes
id.	pseudorabies virus (PRV)	id.	yes
pox	myxomavirus	id.	yes
paramyxo	bovine parainfluenzavirus 3 (PI3)	ss RNA, negative strand	yes
pneumo	bovine respiratory syncytial virus (BRSV)	id.	yes
orthomyxo	equine influenza virus (EIV)	id., segmented	yes
corona	bovine coronavirus (BCV)	ss RNA, positive stranded	yes

* Bovine herpes virus is also known as infectious bovine rhinotracheitis virus

The bacteria for use with the stabiliser according to the invention can be of any type, for instance as characterised by staining Gram positive or Gram negative.

Table 4: Examples of bacteria for use with the stabiliser composition of the invention:

characteristic	name
Gram positive	Streptococcus equi
id.	Staphylococcus carnosus
Gram negative	Escherichia coli
id	Edwardsiella ictaluri
id.	Salmonella gallinarum

Results obtained in stabilising these bacteria can be extrapolated to other bacterial species within the genuses mentioned.

The production system used for producing biological molecules and micro-organisms for use with the stabiliser of the invention can be any in vitro or an in vivo system. For instance viruses can be produced in a cell culture, on fertilized chicken eggs, or in a host animal; bacteria can be produced using similar techniques, and additionally in culture broth, and biological molecules can be produced by isolation from an animal or micro-organism, especially recombinant expression systems can be used advantageously. Many other embodiments are known in the art.

However, the optimal safety and predictability of the stabilised end-product is achieved by removing unknown, heterogenous and ill-defined constituents from both the production stage and the stabiliser composition.

Therefore, in a more preferred embodiment of the vaccine composition according to the invention, the biological molecule or the micro-organism, or both, were produced animal-component free.

ACF production systems for instance are viral cultures on cells in ACF media; bacterial cultures in an ACF culture broth; and recombinant expression cultures using ACF media, e.g. the baculovirus expression system using insect cells.

In a further preferred embodiment, the vaccine composition according to the invention additionally comprises an adjuvant.

Adjuvants are commonly used to boost the immune response to a vaccine component, by stimulating the immune system of a target human or animal, in a non-specific manner. Many different adjuvants are known in the art, for example: mineral or biological oils, saponins, peptides, alumina and derivatives, etc.

In a further preferred embodiment, the vaccine composition according to the invention is freeze-dried.

In freeze-dried form the stabiliser composition of the invention will be most benificiary; the freeze-dried vaccine compositions according to the invention are stable at for instance +4°C for months to several years.

Procedures for freeze-drying are known in the art; equipment for freeze-drying at different scales is available commercially. Typically vaccine compositions comprising a stabiliser according to the invention are filled in containers such as glass vials to a certain volume, e.g. between 0.5 and 50 ml, preferably 1, 2, or 5 ml. Subsequently these vials are frozen, and submitted to a freeze-drying process.

An outline of an exemplary freeze-drying process comprises the steps of:

- deep freezing for 10 - 60 minutes, to fix the liquid into a certain structure;
- first drying step at -20 to 20 °C, for 1 - 12 hours, at high vacuum, to sublimate the crystallised water;
- secondary drying at 0 to 40 °C, for ½ - 6 hours, at medium vacuum, to desorb the unfrozen water;
- packing the freeze dried bodies (e.g. closing glass vials by a rubber stopper) to maintain dry conditions, e.g under vacuum, or under nitrogen gas; and
- hold on + 4°C.

The skilled person is very well capable of routine variations and optimisations to such a process in order to achieve the best results for a specific type of sample.

The freeze-drying process can be optimised by monitoring the temperature, and the water content in the apparatus during drying. Also, differential scanning calorimetry is routinely used to assess the characteristics of the product before and after freeze-drying.

Residual water content (rwc) in the end product is an important parameter determining the freeze-dried products' stability. Preferably the rwc of freeze-dried samples and vaccines according to the invention is below 5 %. To determine the rwc, Karl Fischer titration known in the art can advantageously be employed.

In a further preferred embodiment, a freeze-dried vaccine according to the invention is prepared in the form of a lyosphere as described in European patent EP 799.613.

The freeze-dried body produced of a vaccine composition according to the invention can be in any form. The stabilising composition according to the invention provides beneficiary characteristics to the vaccine composition to achieve an elegant and attractive appearance of the freeze-dried body. Preferably the freeze-dried cake in a glass vial is of shiny, homogenous appearance, and preferably stays attached to the wall of the vial upon flicking it, and is not so brittle that it becomes pulverised under normal transportation conditions.

Consequently, the desired appearance of the freeze-dried vaccine body not only relates to subjective or aesthetic characteristics, but also comprises genuine advantageous technical effects; in particular the stabilisation efficacy, the resistance to the rigours of transportation, and the redissolution speed are involved.

Of special interest is to avoid an appearance known in the art as "collapsed", as freeze-dried bodies having such an amorphous and clumped appearance redissolve slowly and incompletely, and do not provide adequate stabilisation of the biological molecules and/or micro-organisms that are to be conserved.

In a further preferred embodiment, a ready for use vaccine solution is obtainable by reconstituting a freeze-dried vaccine composition according to the invention in a pharmaceutically acceptable carrier.

As the freeze-dried vaccine compositions according to the invention dissolve rapidly, preferably the reconstitution is performed immediately before the vaccine solution is to be applied to a target. This according the

The vaccine composition according to the invention can be administered to a human or animal target according to methods known in the art. For instance by parenteral applications such as through all routes of injection into or through the skin: e.g. intramuscular, intravenous, intraperitoneal, intradermal, sub mucosal, or subcutaneous. Alternative routes of application that are feasible are by topical application as a drop, spray, gel or ointment to the mucosal epithelium of the eye, nose, mouth, anus, or vagina, or onto the epidermis of the outer skin at any part of the body; by spray as aerosol, or powder. Alternatively, application can be via the alimentary route, by combining with the food, feed or drinking water e.g. as a powder, a liquid, or tablet, or by administration directly into the mouth as a liquid, a gel, a tablet, or a capsule, or to the anus as a suppository. Also immersion vaccination can be applied advantegeously.

The preferred application routes are intramuscular or subcutaneous injection, or intranasal spray.

It goes without saying that the optimal route of application will depend on the specific particularities of the infection or symptoms that are to be prevented or ameliorated, the characteristics of the vaccine formulation that is used, and the particular characteristics of the target species.

The scheme of the application of the vaccine composition according to the invention to the target human or animal can be in single or multiple doses, which may be given at the same time or sequentially, in a manner compatible with the dosage and formulation, and in such an amount as will be immunologically effective, for instance as a single yearly dose.

In an alternate embodiment, the invention relates to a method for preparing a pharmaceutical composition, comprising admixing a stabiliser composition according to the invention with a composition comprising at least one biological molecule or at least one micro-organism, or a combination thereof.

The pharmaceutical composition prepared according to the method of the invention can be in any form: liquid or dry, frozen, freeze-dried, etc.

The admixing can be performed using any suitable technique available in the art.

Preferably the method according to the invention comprises admixing 1 part of the stabiliser composition according to the invention as a sterile aqueous buffered solution at 4x concentration, with 3 parts of a composition comprising at least one biological molecule and/or at least one micro-organism, to produce a pharmaceutical composition according to the invention.

The composition comprising at least one biological molecule or at least one micro-organism, or a combination thereof, that is to be admixed with the stabiliser composition according to the invention, is preferably in liquid form. This composition can be obtained from a viral or bacterial culture, or an expression system culture. This composition can for instance be obtained after downstream-processing, such as centrifugation, e.g. the culture supernatant, or the (resuspended) centrifuged pellet (comprising virus-infected cells or bacteria); or the unprocessed whole culture may be used.

Preferably the admixing of stabiliser composition and composition comprising a biological molecule and/or a micro-organism according to the method of the invention, is performed shortly after production and down-stream-processing have finished, in order to achieve the best stabilisation results.

Preferably the stabilised pharmaceutical composition thus obtained is than stored cooled or frozen, awaiting further processing and the results of quality control on the production harvest.

In preferred embodiments of the method according to the invention:

- the micro-organism is a virus or a bacterium, or both a virus and a bacterium are comprised.
- the virus is at least one viral species selected from the group consisting of herpes-, paramyxo-, orthomyxo-, adeno-, rhabdo-, birna-, corona-, pneumo-, and poxvirus.
- the biological molecule is at least one selected from the group consisting of a protein, a carbohydrate, a lipid, and a nucleic acid

- the protein is at least one selected from the group consisting of an antibody, an antibody-fragment, a cytokine, a hormone, and an enzyme.
- the biological molecule or the micro-organism, or both, were produced animal-component free.
- the resulting pharmaceutical composition is subjected to freeze-drying.

In a more preferred embodiment, the invention relates to a pharmaceutical composition obtainable by the method according to the invention, wherein the composition is a vaccine.

In an even more preferred embodiment, the invention relates to a method of preparing a vaccine solution by reconstituting a pharmaceutical composition according to the invention, in a pharmaceutically acceptable carrier.

In a further alternate embodiment, the invention relates to the use of a stabiliser composition according to the invention for stabilising the quality and/or the quantity of at least one biological molecule or at least one micro-organism, or a combination thereof, in a composition.

In preferred embodiments of the use according to the invention:

- the micro-organism is a virus or a bacterium, or both a virus and a bacterium are comprised.
- the virus is at least one viral species selected from the group consisting of herpes-, paramyxo-, orthomyxo-, adeno-, rhabdo-, birna-, corona-, pneumo-, and poxvirus.
- the biological molecule is at least one selected from the group consisting of a protein, a carbohydrate, a lipid, and a nucleic acid.
- the protein is at least one selected from the group consisting of an antibody, an antibody-fragment, a cytokine, a hormone, and an enzyme.
- the biological molecule or the micro-organism, or both, were produced animal-component free.

In a more preferred embodiment, the invention relates to the use according to the invention, of a vaccine solution according to the invention, or a vaccine solution obtainable by the method of the invention, for immunisation of a target human or animal.

The invention will now be further described with reference to the following, non-limiting, examples.

Examples

As mentioned above, freeze-drying experiments were performed incorporating all conditions under which an effective stabiliser must be able to stabilise: cooling, freezing, drying, heating.

The general outline of the freeze-drying experiments performed in stabilising micro-organisms was as follows:

- compositions comprising micro-organisms were obtained from a culture, processed if required and stored at 4°C or below zero °C.
- a stabiliser composition according to the invention was prepared by weighing the required compounds in the desired quantities, adding these to a required volume of sterile distilled water, and mixing until dissolved. The stabiliser composition was then heat sterilised at 121 °C for 20 minutes.
- at start of the experiment the sample was thawed, mixed with a volume of a 4x concentrated stabiliser that was one third of the sample volume. Either the stabiliser according to the invention, or reference stabiliser compositions were used. Also samples without any stabiliser were comprised in the experiments.
- stabilised samples were put into standard 10 ml glass vials at a certain filling volume: BHV, and PI3 virus at 2 ml, and PRV, myxoma virus, BRSV, EIV and BCV at 1 ml. Bacterial samples were fillled at 2.5 ml per vial.
- samples were freeze-dried using a standard two-stage program, to a residual water content below 5%, and then sealed under vacuum with a rubber stopper.
- subsequently samples were stored at 4°C or at higher temperatures for a certain period of time; results in the columns "direct 4°C" are results of titrations on samples stored at 4°C for less than 1 month after freeze-drying; results in columns "3 days 28 °C" are titration results of samples stored at 28 °C for 3 days subsequently to possible storage at 4°C after freeze-drying, and then quantified immediately after these three days; results in columns "1 year 4°C" are titration results of samples stored at 4 °C for at least one year after freeze-drying.

Storage at 28 °C for three days is incorporated as an accelerated stability assay; most times these results are indicative for the consequences of storage over longer time at ambient or cooled conditions.

In order to determine the amount and the quality of the micro-organism that remained after these treatment conditions, virus samples were infectivity-titrated, and bacterial samples were plate-counted.

BRSV titration assay:
Stabilised samples of BRS virus was diluted in standard cell culture medium supplemented with FCS 10 % (v/v), to dilutions between 10^{-1} and 10^{-7}, in 0.2 ml. The dilutions are inoculated on BEL cells in the wells of a micro titre plate: 1.5 x 10^5 BEL cells/ml, at 0.2 ml/well. 10 wells were used per viral dilution. The micro titre plates are incubated during 8 days (3 - 5 % CO_2, 37°C). The plates are fixed using Acetone/PBS 70/30 % v/v, during 10-15 minutes at room temperature. The fixed monolayer is incubated initially with a monoclonal antibody specific for BRSV and secondary with an anti-antibody FITC-conjugate + Evans blue (as counter stain). Those dishes in which positive fluorescent cells have developed are scored as positive. Virus titres are calculated according to Reed and Muench, and expressed as viral titre in $TCID_{50}$.

In all experiments a homologues standard virus sample and negative control was included for every run of virus titrations. All virus titrations were carried out in duplo on one day. The averaged titres are presented.

Myxoma virus titration assay:
RK13 cells are seeded in 96 well plates, at $4x10^5$ cells per ml, at 100 µl per well, in standard cell-culture medium with 5% foetal calf serum (FCS) and appropriate antibiotics. The plates are incubated overnight at 37 °C, in 5% CO_2 atmosphere.

Next day a stabilised sample containing myxomavirus is diluted from 10^{-1} through 10^{-8}. 20 µl of each viral dilution is inoculated onto a well of the RK13 cell plate, in triplicate. After an incubation of an hour at 37°C/5% CO_2, 100 µl of fresh culture medium with FCS is added to the wells, and the plates are

Plates are then emptied, stained with a dilution of Naphtalene black, incubated for 1 hour at roomtemperature. The plates are emptied again, washed with tap water, dried, and read using lightmicroscopy.

The number of live infective myxoma viruses in the original sample is calculated by Spearman-Kärber algorithm, and expressed as a $TCID_{50}$ value.

BHV, PI3, and PRV virus titration assay:

Stabilised samples of BHV, PI3, and PRV virus were titrated in a manner similar to that for myxoma virus, except that for BHV and PI3 virusses JCK cells, and for PRV Vero cells were used. CPE was also determined by counting plaques using light microscopy.

BCV titration assay:

A cell suspension of MDBK cells is plated in 96 well plates, at 200 µl/well of $1x10^5$ cells/ml in DMEM medium with 10 % FCS and appropriate antibiotics. Plates are incubated for 3 days at 37 °C in 5 % CO_2 atmosphere. After three days a confluent monolayer has formed. Medium is removed, fresh 200 µl medium without FCS is added, and plates are incubated for 2 hours. Dilutions of BCV from stabilised samples are prepared in steps of 10 fold dilutions. The microtiter plates are emptied again, and dilutions between 10^{-3} and 10^{-8} are inoculated on the MDBK cell-monoloayer. Plates are incubated for 5-6 days. Viral plaques are visualised for immunofluoresecnce counting, using a polyclonal rabbit anti BCV serum, and a Goat-anti-rabbit-FITC labeled conjugated antibody. After counterstaining with Evans blue, plaques are counted using UV-light microscopy. Calculation of live viral $TCID_{50}$ titre in the stabilised sample is by Reed-Muench algorithm.

Equine influenza virus

EIV is titrated on chicken eggs; dilutions of a stabilised EIV sample are inoculated into the alllantoic cavity of a fertilized live 10 day old chicken egg, and incubated at 40-42 °C for 3-5 days. Eggs are opened, and a sample of allantoic fluid is tested in heamagglutination assay using ckicken red blood cells. The amount of live infectious EIV in the original sample is calculated from the highest dilution giving a HA reaction.

In some experiments the quality of the freeze-dried cake was judged. This comprised visual inspection and shaking of the vial.
An arbitrary indication was given:
+ elegant appearance, good firm structure
0 adequate appearance and structure
- less than desired appearance and structure

Bacteria

Bacterial cultures were obtained by growing stock material in shaker flasks in appropriate standard growth media. Next, these cultures were quantified, by plating out in duplo a series of limiting dilution samples on blood-agar plates, for counting the number of colonies appearing after overnight incubation. For some experiments the number of colonies recovered after freeze drying were expressed as % of the number of colonies in the original culture before freeze drying.

The corresponding cultures were then mixed 1:4 with a 4x concentrated stabiliser composition according to the invention, and submitted to a standard two-stage freeze drying program.

For the experiments involving E. coli, a K12 strain was used, and different culture media were used: standard LB medium, LB with ampicillin, and blood-agar medium. The efficacy of the stabiliser comosition according to the invention was compared to that of a standard stabiliser containing albumin.

Samples were tested either before or directly after freeze-drying, or after 3 days of incubation at 28 or 30°C, again by way of accelerated stability assay.

Table 5: Effect of concentration of Glycine

Micro-organism used: BHV

Stabiliser composition used:

Compound		g/l (4x conc)
Buffer	Na_2HPO_4 di-hydrate	1
Sugar	D-Sorbitol	75
Amino-acid	Na-L-Glutamate mono-hydrate	20
	and Glycine	variable
Polyamine	Spermine tetra-hydrochloride	10

Exp. code 03AL	Virus titre (log_{10} $TCID_{50}$/vial)				Cake quality
Glycine g/l	Wet titre before freeze-drying	Titre after freeze drying			
		Direct	3 days 28°C	1 year 4°C	
240	9.0	8.2	7.9	7.9	0
200		8.4	8.1	7.9	0
160		8.2	8.1	8.2	+
120		8.4	8.1	8.2	0
80		8.4	8.3	8.3	-
40		8.5	7.9	7.8	-
0		8.0	7.7	7.9	-

Table 6: Effect of concentration of Glutamate

Micro-organism used: BHV

Stabiliser composition used:

Compound		g/l (4x conc)
Buffer	Na_2HPO_4 di-hydrate	1
Sugar	D-Sorbitol	75
Amino-acid	Na-L-Glutamate mono-hydrate	variable
	and Glycine	160
Polyamine	Spermine tetra-hydrochloride	10

Exp. code 03AL	Virus titre (log_{10} $TCID_{50}$/vial)				Cake quality
Glutamate g/l	Wet titre before freeze-drying	Titre after freeze drying			
		Direct	3 days 28°C	1 year 4°C	
0	9.0	8.2	7.9	8.4	0
20		8.4	8.1	7.7	+
100		8.2	8.1	7.9	-

Table 7: Type of polyamine and effect of concentration of polyamine

Micro-organism used: BHV

Stabiliser composition used:

Compound		g/l (4x conc)
Buffer	Na_2HPO_4 di-hydrate	1
Sugar	D-Sorbitol	75
Amino-acid	Na-L-Glutamate mono-hydrate	20
	and Glycine	160
Polyamine	Spermine tetra-hydrochloride	variable
	or Spermidine tri-hydrochloride	variable

NB: in these experiments either Spermine or Spermidine was comprised in the stabiliser composition.

For reference purposes, a prior art conventional stabiliser (Makoschey et al., supra) was included as comparative example, this is indicated as "Conv. stab."

Table 7: Type of polyamine and effect of concentration of polyamine (continued)

Exp. code 03AL	Virus titre (log_{10} $TCID_{50}$/vial)				Cake quality
Spermine g/l	Wet titre before freeze-drying	Titre after freeze drying			
		Direct	3 days 28°C	1 year 4°C	
0	9.0	8.0	7.8	n.a.	+
0.5		8.3	7.8	8.0	0
2.5		8.2	8.0	8.2	+
10		8.2	8.1	8.2	+
30		8.3	8.0	8.5	+
50		8.1	8.2	7.9	0
Spermidine g/l	before freeze-dr.	Direct	3 days 28°C	1 year 4°C	Cake quality
0.5	9.0	8.7	8.1	7.5	0
2.5		8.5	8.1	8.1	0
10		8.4	7.8	8.4	0
30		8.2	8.0	7.9	-
50		n.a.	n.a.	8.1	n.a.
	before freeze-dr.	Direct	3 days 28°C	1 year 4°C	Cake quality
Conv. stab	9.0	8.2	8.0	7.9	+

n.a. = not available

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Table 8: Type of polyamine-salts and hydrates and effect of concentration

Micro-organism used: BHV

Stabiliser composition used:

Compound		g/l (4x conc)
Buffer	Na_2HPO_4 di-hydrate	1
Sugar	D-Sorbitol	75
Amino-acid	Na-L-Glutamate mono-hydrate	20
	and Glycine	160
Polyamine	Spermine tetra-hydrochloride	variable
	or Spermine di-hydrate	variable
	or Putrescine*	variable
	or Ethylene diamine*	variable

* in these experiments either one polyamine was comprised or a combination; this is indicated in the table

Table 8: Type of polyamine-salts and hydrates and effect of concentration (continued)

Exp. code 03AA	Virus titre (log_{10} $TCID_{50}$/vial)			
Spermine tetra-hydrchl. g/l	Wet titre before freeze-drying	Titre after freeze drying		
		Direct	3 days 28°C	1 year 4°C
10	n.a.	8.2	8.1	7.9
30		7.8	7.5	7.5

n.a. = not available

Exp. code 03AB	Virus titre (log_{10} $TCID_{50}$/vial)			
Spermine dihydrate g/l	Wet titre before freeze-drying	Titre after freeze drying		
		Direct	3 days 28°C	1 year +4°C
5	9.1	7.8	6.9	7.3
10		7.4	6.7	7.4
30		7.5	6.6	7.1
Spermine tetra-hydrchl. g/l	before freeze-dr.	Direct	3 days 28°C	1 year +4°C
5	9.1	8.1	7.5	7.6
10		8.0	7.7	7.8
30		7.7	7.0	7.4

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Table 8: Type of polyamine-salts and hydrates and effect of concentration (continued)

Exp. code 03AJ	Virus titre (log_{10} $TCID_{50}$/vial)			
Spermine tetra-hydrchl. g/l	Wet titre before freeze-drying	Titre after freeze drying		
		Direct	3 days 28°C	1 year +4°C
10	8.5	8.5	7.9	7.8
Putrescine g/l	before freeze-dr.	Direct	3 days 28°C	1 year +4°C
5	8.5	8.0	7.5	7.3
10		8.1	7.6	7.9
30		8.0	7.5	7.7
Ethylene-diamine g/l	before freeze-dr.	Direct	3 days 28°C	1 year +4°C
5	8.5	8.0	8.0	7.7
10		8.2	7.8	7.9
30		8.2	7.4	8.2
no polyam.	before freeze-dr.	Direct	3 days 28°C	1 year +4°C
0	8.5	8.1	7.3	7.4
Combined polyamines*	before freeze-dr.	Direct	3 days 28°C	1 year +4°C
A	8.5	8.4	7.8	8.2
B		8.5	8.2	8.2

* Polyamine combinations:

A = Spermine tetra-hydrochloride 10 g/l, and Putrescine 5 g/l.
B = Spermine tetra-hydrochloride 10 g/l, and Ethylene diamine 5 g/l.

Table 9: Stabilisation of different viruses, and effect of polyamine

Stabiliser compositions used:

"invention 1"

Compound		g/l (4x conc)
Buffer	Na_2HPO_4 di-hydrate	1
Sugar	D-Sorbitol	75
Amino-acid	Na-L-Glutamate mono-hydrate	20
	and Glycine	160
Polyamine	Spermine tetra-hydrochloride	10

"invention 2"

Compound		g/l (4x conc)
Buffer	Na_2HPO_4 di-hydrate	1
Sugar	D-Sorbitol	75
Amino-acid	Na-L-Glutamate mono-hydrate	20
	and Glycine	160
Polyamine	Spermidine tri-hydrochloride	2.5

"invention 3"

Compound		g/l (4x conc)
Buffer	Na_2HPO_4 di-hydrate	1
Sugar	D-Sorbitol	75
Amino-acid	Na-L-Glutamate mono-hydrate	20
	and Glycine	160
Polyamine	none	0

Comparative examples were performed with the prior art conventional stabiliser (Makoschey et al., supra), this is indicated as "Conv. stab".

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Table 9: Stabilisation of different viruses, and effect of polyamine (continued)

		Virus titre (log_{10} $TCID_{50}$/vial)			
		Wet titre before freeze-drying	Titre after freeze drying		
virus used and exp. code	Stabiliser		Direct	3 days 28°C	1 year +4°C
BRSV 04.20.001BD	no stab.	5.8	4.6	3.5	2.8
	Conv. stab		5.6	5.0	5.0
	invention 1		5.5	4.0	5.1
	invention 3		5.0	4.3	n.a.
BRSV 04.20.001BF	no stab.	6.2	3.6	3.6	2.6
	Conv. stab		5.5.	5.1	5.1
	invention 1		3.6	3.5	3.2
	invention 2		3.5	3.5	3.0
	invention 3		3.5	3.5	n.a.
BRSV 04.20.001BL	no stab.	5.8	4.6	4.2	3.4
	Conv. stab		5.4	5.2	5.0
	invention 1		n.a.	5.2	5.0
	invention 2		5.4	5.4	5.0
	invention 3		5.3	5.0	n.a.
EIV 03.20.003AA	no stab.	9.3	7.2	6.5	6.6
	Conv. stab		7.4	8.1	8.3
	invention 1		7.9	7.4	7.9
	invention 3		8.0	8.2	n.a.
EIV 03.20.203S	no stab.	9.1	6.9	5.6	4.3
	Conv. stab		8.9	8.3	7.7
	invention 1		8.8	8.3	7.1
	invention 3		8.9	8.4	n.a.

n.a. = not available

Table 9: Stabilisation of different viruses, and effect of polyamine (continued)

		Virus titre (log_{10} $TCID_{50}$/vial)			
		Wet titre before freeze-drying	Titre after freeze drying		
virus used and exp. code	Stabiliser		Direct	3 days 28°C	1 year +4°C
BCV 04.20.800AH	no stab.	7.0	4.7	< 3.5	3.2
	Conv. stab		6.1	5.8	5.5
	invention 1		5.7	4.0	4.2
	invention 3		5.6	< 3.5	n.a.
BCV 04.20.800AN	no stab.	6.6	5.9	4.4	3.9
	Conv. stab		5.7	5.3	5.1
	invention 1		5.9	5.4	5.2
	invention 2		6.0	5.3	4.9
	invention 3		5.9	5.4	n.a.
Myxoma 04.20.001R	invention 1	7.9	7.6	n.a.	n.a.
	invention 3		7.7		
PRV 04.20.004AB	no stab.	9.1	7.3	n.a.	n.a.
	Conv. stab		8.3		
	invention 1		8.3		
	invention 3		8.0		
PRV 03.20.003 G	Conv. stab	9.0	8.0	7.9	7.9
	invention 1		8.1	7.9	7.9
	invention 3		7.8	7.4	8.0

n.a. = not available

Table 9: Stabilisation of different viruses, and effect of polyamine (continued)

virus used and exp. code	Stabiliser	Virus titre (log_{10} $TCID_{50}$/vial)			
		Wet titre before freeze-drying	Titre after freeze drying		
			Direct	3 days 28°C	1 year +4°C
BHV 03AB	Conv. stab	9.1	8.4	8.1	8.0
	invention 1		8.0	7.7	7.8
	invention 3		7.7	6.7	n.a.
BHV 03AE	Conv. stab	8.7	7.7	n.a.	7.5
	invention 1		8.5	8.2	8.4
	invention 3		8.3	8.1	8.2
BHV 03 AN	Conv. stab	8.6	8.4	8.1	8.1
	invention 1		8.2	8.1	8.1
	invention 2		8.4	8.5	8.3
BHV 03 AO	no stab.	8.8	7.6	7.9	n.a.
	Conv. stab		8.3	8.1	8.0
	invention 1		8.0	8.2	8.0
	invention 2		7.9	8.2	7.9
	invention 3		7.5	7.9	n.a.
BHV 03 AR	no stab.	8.9	7.7	n.a.	n.a.
	Conv. stab		8.1	8.2	
	invention 1		8.3	8.1	
	Invention 2		8.3	8.1	
	invention 3		8.1	7.5	

n.a. = not available

Table 10: Stabilisation of different viruses, which were produced ACF

Stabiliser compositions used:
"invention 1", "invention 3", and conventional stabiliser "Conv. stab" (see Table 9).

		Virus titre (log_{10} $TCID_{50}$/vial)			
		Wet titre before freeze-drying	Titre after freeze drying		
ACF virus used and exp. code	Stabiliser		Direct	3 days 28°C	1 year +4°C
PI3 03H	Conv. stab	9.5	8.3	8.0	8.1
	invention 1		8.9	8.7	8.6
	invention 3		8.8	8.6	8.5
BHV 03AD	Conv. stab	8.8	8.3	7.9	n.a.
	invention 1		8.4	8.0	
	invention 3		8.0	7.8	
PRV 03N	Conv. stab	9.3	8.3	8.0	7.7
	invention 1		8.3	8.1	8.1
	invention 3		8.4	7.8	7.2
PRV 03P	invention 1	n.a.	8.5	8.2	n.a
	invention 3		8.3	7.8	
Myxoma 04.20.01L	Conv. stab	7.4	7.2	7.4	n.a
	invention 1		7.2	7.2	

n.a. = not available

Table 11: Replacement of glycine by compatible solute

Stabiliser compositions used:

"invention 3" and "Conv. stab" (see Table 9)

Modifications of invention 3:

Compound		g/l (4x conc)
Buffer	Na_2HPO_4 di-hydrate	1
Sugar	D-Sorbitol	75
Amino-acid	Na-L-Glutamate mono-hydrate	20
	no Glycine	none
Compatible solute	Sarcosine	80 or 160
	or Betaine	80 or 160
	or Di-glycine	80 or 160
	or Choline	80 or 160
Polyamine	none	0

Table 11: Replacement of glycine by compatible solute

(continued)

virus used and exp. code	Stabiliser	Virus titre (log_{10} $TCID_{50}$/vial) Wet titre before freeze-drying	Titre after freeze drying: Direct	Titre after freeze drying: 3 days 28°C
BHV 03AC	Conv. stab	9.0	8.1	8.1
	invention 3		7.8	7.7
	Invention 3 -/- glycine:			
	+ Sarcosine 80 g/l		7.5	7.5
	+ Sarcosine 160		8.1	7.6
	+ Betaine 80		8.4	7.1
	+ Betaine 160		8.1	7.1
	+ Di-glycine 80		7.9	7.3
	+ Di-glycine 160		8.0	7.7
	+ Choline 80		7.9	6.9
	+ Choline 160		7.5	5.7
PRV 03M	Conv. stab	9.0	8.1	7.6
	invention 3		7.6	7.0
	Invention 3 -/- glycine:			
	+ Sarcosine 80 g/l		7.8	7.4
	+ Sarcosine 160		8.2	7.2
	+ Betaine 80		7.2	6.8
	+ Betaine 160		7.4	7.1
	+ Di-glycine 80		7.6	7.4
	+ Di-glycine 160		8.2	8.3
	+ Choline 80		7.3	6.7
	+ Choline 160		7.4	5.7

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Table 12: Effect of buffer pH

Micro-organism used: BHV

Stabiliser composition used:

"invention 3" and "Conv. stab" (see Table 9)

Modifications of "invention 3" stabiliser

Compound		g/l (4x conc)
Buffer	Na_2HPO_4 di-hydrate	1
	or Tris HCl - Citraat	50 mM - 10 mM
Sugar	D-Sorbitol	75
Amino-acid	Na-L-Glutamate mono-hydrate	20
	and Glycine	160
Polyamine	none	0

Variations of pH are indicated in the table:

		Virus titre (log_{10} $TCID_{50}$/vial)		
		Wet titre before freeze-drying	Titre after freeze drying	
virus used and exp. code	Stabiliser		Direct	3 days 28°C
BHV 03U	Conv. stab, pH 7.1	8.8	8.0	7.8
	Conv. stab, pH 7.4		7.6	7.9
	invention 3, pH 6.9		7.9	7.3
	invention 3, pH 7.4		7.9	6.5
	invention 3 -/- phosphate + Tris/Citraat, pH 6.9		8.0	6.9
	invention 3 -/- phosphate + Tris/Citraat, pH 7.4		8.2	6.7

Table 13: Stabilisation of E coli bacteria

Micro-organism used: E. coli K12

Stabiliser composition used: "invention 1" (see Table 9). For comparison a standard bacterial stabiliser containing Albumin was used. This is indicated as "bac stab."

		Colony forming units	
Culture medium	Stabiliser	before freeze drying	direct after freeze drying
LB	bac stab	$3x10^{10}$	$6.5x10^{8}$
	invention 1		$2.4x10^{9}$
LB amp	bac stab	$3x10^{10}$	$3.5x10^{8}$
	invention 1		$2.4x10^{9}$
Blood agar	bac stab	$2.7x10^{11}$	$3.4x10^{9}$
	invention 1		$2.1x10^{10}$

Table 14: Stabilisation of different bacteria

Micro-organism used: Different bacteria.

Stabiliser composition used: "invention 1" and "invention 3" (see Table 9). For comparison a standard bacterial stabiliser containing Albumin was used. This is indicated as "bac stab."

		recovery %		Cake quality
Bacterium, starting CFU/ml	Stabiliser	direct after freeze drying	after 3 days 28°C	
E. coli K12, $2.4x10^9$	none	13	9	-
	invention 1	33	1	0
	invention 3	48	9	0
Salmonella gallinarum, $4.0x10^9$	none	24	16	-
	invention 1	18	14	+
	invention 3	34	17	+
	bac stab	100	57	+
Streptococcus equi, $1.2x10^9$	none	56	27	0
	invention 1	60	6	0
	invention 3	39	15	+
	bac stab	58	29	+
Staphylococcus carnosus, $2.4x10^9$	none	57	65	0
	invention 1	64	67	0
	invention 3	61	57	0

Claims:

1. Stabiliser composition comprising at least one amino acid, at least one sugar, and at least one polyamine wherein all compounds are chemically defined, characterised in that the polyamine is at least one selected from the group consisting of ethylene-diamine, cadaverine, putrescine, spermidine and spermine.

2. Stabiliser composition according to claim 1, wherein the sugar is at least one selected from the group consisting of glucose, lactose, sucrose, maltose, trehalose, sorbitol and mannitol.

3. Stabiliser composition according to any one of claims 1 or 2, wherein the amino acid is glutamate or glycine, or wherein both these amino acids are comprised.

4. Stabiliser composition according to any one of claims 1 to 3, wherein the composition is a buffered aqueous solution.

5. Stabiliser composition according to claim 4, wherein the buffer is phosphate based.

6. Vaccine composition comprising a stabiliser composition according to any one of claims 1 to 5, and at least one biological molecule or at least one micro-organism, or a combination thereof.

7. Vaccine composition according to claim 6, wherein the micro-organism is a virus or a bacterium, or wherein both a virus and a bacterium are comprised.

8. Vaccine composition according to claim 7, wherein the virus is at least one viral species selected from the group consisting of herpes-, paramyxo-,

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9. Vaccine composition according to claim 8, wherein the biological molecule is at least one selected from the group consisting of a protein, a carbohydrate, a lipid, and a nucleic acid.

10. Vaccine composition according to claim 9, wherein the protein is at least one selected from the group consisting of an antibody, an antibody-fragment, a cytokine, a hormone, and an enzyme.

11. Vaccine composition according to any one of claims 6 to 10, wherein the biological molecule or the micro-organism, or both, were produced animal-component free.

12. Vaccine composition according to any one of claims 6 to 11, additionally comprising an adjuvant.

13. Vaccine composition according to any one of claims 6 to 12, wherein the vaccine composition is in freeze-dried form.

14. Vaccine solution obtainable by reconstituting a freeze dried vaccine composition according to claim 13 in a pharmaceutically acceptable carrier.

15. Method for preparing a pharmaceutical composition, comprising admixing a stabiliser composition according to any one of claims 1 to 5 with a composition comprising at least one biological molecule or at least one micro-organism, or a combination thereof.

16. Method according to claim 15, wherein the micro-organism is a virus or a bacterium, or wherein both a virus and a bacterium are comprised.

17. Method according to claim 16, wherein the virus is at least one viral species selected from the group consisting of herpes-, paramyxo-, orthomyxo-, adeno-, rhabdo-, birna-, corona-, pneumo-, and poxvirus.

18. Method according to claim 15, wherein the biological molecule is at least one selected from the group consisting of a protein, a carbohydrate, a lipid, and a nucleic acid.

19. Method according to claim 18, wherein the protein is at least one selected from the group consisting of an antibody, an antibody-fragment, a cytokine, a hormone, and an enzyme.

20. Method according to any one of claims 15 to 19, wherein the biological molecule or the micro-organism, or both, were produced animal-component free.

21. Method according to any one of claims 15 to 20, wherein the resulting pharmaceutical composition is subjected to freeze-drying.

22. Pharmaceutical composition obtainable by the method of any one of claims 15 to 21, wherein the composition is a vaccine composition.

23. Method of preparing a vaccine solution by reconstituting a pharmaceutical composition according to claim 22 in a pharmaceutically acceptable carrier.

24. Use of a stabiliser composition according to any one of claims 1 to 5 for stabilising the quality and/or the quantity of at least one biological molecule or at least one micro-organism, or a combination thereof, in a composition.

25. Use according to claim 24, wherein the micro-organism is a virus or a bacterium, or wherein both a virus and a bacterium are comprised.

26. Use according to claim 25, wherein the virus is at least one viral species selected from the group consisting of herpes-, paramyxo-, orthomyxo-, adeno-, rhabdo-, birna-, corona-, pneumo-, and poxvirus.