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(54) **Titre : Pharmaceutical composition of non-enveloped virus.**

(57) **Abrégé :**

The present invention relates to the field of pharmaceuticals, gene therapy and medicine, specifically to pharmaceutical compositions of a vector based on recombinant non-enveloped virus, in particular the recombinant adeno-associated virus (rAAV), which compositions may be aqueous or lyophilized compositions and may be used for the treatment and prevention of various diseases.

PHARMACEUTICAL COMPOSITION OF NON-ENVELOPED VIRUS

Field of the invention

The present invention relates to the field of pharmaceuticals, gene therapy and medicine, specifically to pharmaceutical compositions of a vector based on a recombinant non-enveloped virus, in particular the recombinant adeno-associated virus (rAAV), which compositions may be used for the treatment and prevention of various diseases.

Background of the invention

Typical representatives of non-enveloped viruses are parvoviruses, noroviruses, as well as rotaviruses and adenoviruses.

Simple or non-enveloped viruses consist of a nucleic acid and a protein envelope called a capsid. The capsid consists of repeating morphological subunits called capsomers. The nucleic acid and the capsid interact with each other to form a nucleocapsid. In each virus, oligomerization of capsid proteins during capsid assembly typically leads to a certain type of symmetrical quaternary structure. Most viruses have capsids of spiral or icosahedral structure (Lidmar J, Mirny L, Nelson Dr. Virus shapes and buckling transitions in spherical shells. Phys Rev E Stat Nonlin Soft Matter Phys. 2003 Nov;68(5 Pt 1):051910. doi: 10.1103/PhysRevE.68.051910. Epub 2003 Nov 25. PMID: 14682823).

Unlike non-enveloped viruses, the capsid of enveloped viruses is covered by a lipid membrane known as a supercapsid. The envelope is acquired by the capsid from an intracellular membrane in the virus' host; examples include the inner nuclear membrane, the Golgi apparatus membrane, and the cell's outer membrane. The presence of a lipid envelope makes viruses less resistant to physical and chemical stresses (Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD (1994). Molecular Biology of the Cell (4th ed.). p. 280).

The capsid of non-enveloped viruses performs the function of protecting the genome during long-term storage, as well as from chemical and physical stresses, such as UV radiation, extreme pH or temperatures, proteolytic and nucleolytic agents. Non-enveloped viruses may retain their properties in a dried state for a long time on various surfaces, both porous and non-porous (Abad FX, Pinto RM, Bosch A. Survival of enteric viruses on environmental fomites. Appl Environ Microbiol. 1994 Oct;60(10):3704-10. doi: 10.1128/aem.60.10.3704-3710.1994. PMID: 7986043; PMCID: PMC201876).

Viruses stably introduce genetic material thereof into the genome of a host cell, so it was proposed to use them as vectors for delivering genetic information to cells. Vectors based on

retrovirus, adenovirus, adenoassociated virus (AAV), herpes simplex virus, etc. are employed. Among these viruses, adenovirus and AAV refer to non-enveloped viruses.

Vectors based on recombinant AAV and recombinant adenovirus are currently the most widely used and developed gene therapy products, and they are further used in vaccine development.

Currently, gene therapy is considered as a potentially universal approach to treatment of almost the entire spectrum of diseases: infectious diseases, genetic diseases, malignant neoplasms, etc.

Viruses or viral vectors (viral particles) must maintain structural integrity thereof in order to be infectious and biologically active. The structural integrity of a viral vector is typically destructed during preparation of vaccines or products containing the viral vector, which excludes usage thereof as a delivery vector.

Viral vector products are produced in a frozen form, requiring a storage temperature below -20 °C, often even below -80 °C. Lyophilisates solve this problem, having an optimal storage and transportation temperature regime of 2-8 °C. Also, lyophilization is widely used to improve the stability of various viral vaccines and products containing a recombinant virus vector.

The prior art provides lyophilized pharmaceutical compositions of AAV (WO2018/128689, WO2020/014479, WO2020/214929), lyophilized pharmaceutical compositions of adenovirus (WO00/29024, WO2017/013169, WO2018/103601).

However, lyophilisates are typically relatively expensive, since they require large time and material costs to be produced, and the biological activity of a viral vector may be lost to a certain extent during lyophilization and reconstitution of the lyophilisate. In addition, residual water (moisture) content in the end product significantly affects viral vector stability during storage.

Thus, there is a need for stable pharmaceutical compositions comprising a recombinant non-enveloped virus vector, including pharmaceutical compositions that allow obtaining a stable lyophilized dosage form without loss of biological activity of the viral vector, as well as with minimal lyophilization time.

Detailed description of the invention

The authors of the present invention surprisingly found that the addition of hydroxypropyl-beta-cyclodextrin increases the glass transition temperature of a pharmaceutical composition comprising a recombinant non-enveloped virus vector during freeze-drying, which fact allows to reduce the duration of the freeze-drying process, reduce residual water content in the lyophilisate, thus resulting in the production of a lyophilized pharmaceutical composition comprising a recombinant non-enveloped virus vector, which composition has stability during storage (for

example, during long-term storage). The authors of the present invention also surprisingly found that the above formulation and the lyophilisate production process make it possible to maintain the activity of the recombinant non-enveloped virus vector unchanged for a long time. Also, the authors of the present invention surprisingly found that the prelyophilization solution (an aqueous pharmaceutical composition) is stable and may act as a finished dosage form.

Definitions

Unless defined otherwise herein, all technical and scientific terms used in connection with the present invention will have the same meaning as is commonly understood by those skilled in the art.

Furthermore, unless otherwise required by context, singular terms shall include plural terms, and the plural terms shall include the singular terms.

As used in the present description and claims that follow, unless otherwise dictated by the context, the words "include" and "comprise," or variations thereof such as "includes", "including", "comprises," or "comprising," will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

The term "pharmaceutical composition" refers to a composition and/or formulation comprising the nonenveloped virus vector in a therapeutically effective amount and excipients or auxilliary substances (carriers, diluents, fillers, solvents, etc.), the choice and proportions of which depend on the type and route of administration and dosage.

The term "aqueous composition" as used herein refers to a water-based composition, the water in the composition may be: water, water for injections, physiologic saline (0.9%-1.0% aqueous solution of sodium chloride).

The term "freeze-dried" as used herein refers to a formulation that has been subjected to a process known in the art as freeze-drying, which includes freezing the formulation followed by removal of ice from the frozen contents.

The pharmaceutical composition is "stable" if the active agent retains physical stability and/or chemical stability and/or biological activity thereof during the specified shelf life at storage temperature, for example, of 2-8 °C. Further, the active agent may retain both physical and chemical stability, as well as biological activity. Storage period is adjusted based on the results of stability test in accelerated or natural aging conditions.

The term "vector" as used herein means a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked.

The term “long-term storage” or “long term stability” should be understood to mean that a pharmaceutical composition may be stored for three months or more, for six months or more, for one year or more, and the composition may have a minimum stable shelf life of at least two years

as well. Generally speaking, the terms "long term storage" and "long term stability" further include stable storage durations that are at least comparable to or better than the stable shelf life typically required for currently available commercial formulations, without losses in stability that would render the formulation unsuitable for its intended pharmaceutical application.

The term "buffering agent" refers to an acid or base component (typically a weak acid or weak base) of the buffer or buffer solution. A buffering agent helps to maintain the pH value of a given solution at or near to a pre-determined value, and the buffering agents are generally chosen to complement the pre-determined value. A buffering agent may be a single compound which gives rise to a desired buffering effect, especially when said buffering agent is mixed with (and suitably capable of proton exchange with) an appropriate amount (depending on the pre-determined value desired) of corresponding "acid/base conjugate" thereof.

The term “buffer” or “buffer solution” refers to an aqueous solution comprising a mixture of an acid (typically a weak acid, such as e.g. acetic acid, citric acid) and a conjugated base thereof (such as e.g. an acetate or citrate salt, e.g. sodium acetate, sodium citrate, as well as hydrates of said salts, e.g. sodium acetate trihydrate) or alternatively a mixture of a base (typically a weak base, e.g. histidine) and a conjugated acid thereof (e.g. histidine hydrochloride or histidine hydrochloride monohydrate or L-histidine hydrochloride (h/c) monohydrate (m/h) or L-histidine h/c m/h or histidine h/c m/h). The pH value of a “buffer solution” changes only slightly upon addition thereto of a small quantity of strong base or strong acid, as well as upon dilution or concentration due to the “buffering effect” imparted by a “buffering agent”.

Typically, amino acids are L-amino acids. For example, if histidine and histidine hydrochloride monohydrate are used, it is typically L-histidine and L-histidine hydrochloride monohydrate. For example, if arginine is used, it is typically L-arginine. Amino acid equivalents, for example, pharmaceutically acceptable proline salts (for example, proline hydrochloride) may also be used.

Abbreviations

PCR – polymerase chain reaction

EIA – enzyme immunoassay

FA – functional activity

Ct – number of cycles

TU – transduction units

T'g – glass transition temperature

Vg – viral genomes

GFP – green fluorescent protein gene

FAM – carboxyfluorescein dye

BHQ-1 – fluorescence extinguisher used in PCR

MOI – multiplicity of infection

The present invention discloses stable pharmaceutical compositions of the vector based on a recombinant non-enveloped virus, in particular the recombinant adeno-associated virus (rAAV), which compositions may be used for treatment and prevention of various diseases.

The developed formulations make it possible to produce a lyophilisate of the vector based on a recombinant non-enveloped virus, in particular rAAV, despite the high content of sodium chloride in same that is necessary for non-enveloped virus stabilization. A low water content is achieved in the lyophilisate, ensuring stability thereof during both long-term and accelerated storage conditions. In addition, the developed formulations make it possible to optimize the freeze drying process by reducing duration thereof.

In one aspect, the present invention relates to a pharmaceutical composition of a recombinant non-enveloped virus vector, said composition comprising:

(i) a recombinant non-enveloped virus vector,

(ii) a buffering agent,

(iii) hydroxypropyl beta cyclodextrin,

(iv) a surfactant,

(v) sodium chloride,

(vi) magnesium chloride,

(vii) water for injection.

Any pharmaceutically acceptable buffer agent may be used as a buffering agent. Buffering agents are well known in the art and include, without limitation, histidine, sodium citrate, tris(hydroxymethyl)aminomethane (TRIS), glycine, N-glycylglycine, sodium acetate, sodium carbonate, lysine, arginine, sodium phosphate, potassium phosphate, and mixtures thereof.

In some embodiments of the invention, the buffering agent is tris(hydroxymethyl)aminomethane and hydrochloric acid.

In some embodiments of the invention, tris(hydroxymethyl)aminomethane is present at a concentration of 1.9 – 8.7 mg/ml.

In some embodiments of the invention, hydrochloric acid is added to pH 6.0 – 8.0.

In some embodiments of the invention, the buffering agent is L-histidine and L-arginine.

In some embodiments of the invention, L-histidine is present at a concentration of 0.4 – 10.0 mg/ml.

In some embodiments of the invention, L-arginine is present at a concentration of 0.05 – 10.0 mg/ml.

In some embodiments of the invention, hydroxypropyl-beta-cyclodextrin is present at a concentration of 10.0 – 200.0 mg/ml.

Any pharmaceutically acceptable surfactant may be used as a surfactant. Surfactants are well known in the art and include, without limitation, polysorbate 20, polysorbate 80, various poloxamers and pluronics, as well as mixtures thereof.

In some embodiments of the invention, the surfactant is present at a concentration of 0.01 – 2.0 mg/ml.

In some embodiments of the invention, the surfactant is poloxamer 188.

In some embodiments of the invention, sodium chloride is present at a concentration of 1.0 – 15.0 mg/ml.

In some embodiments of the invention, magnesium chloride is present at a concentration of 0.05 – 1.0 mg/ml.

Magnesium chloride may be used both in the form of anhydrous magnesium chloride and in the form of hydrates thereof. In some embodiments of the invention, magnesium chloride is magnesium chloride hexahydrate.

In some embodiments of the invention, the pharmaceutical composition further comprises a disaccharide.

Any pharmaceutically acceptable disaccharide may be used as a disaccharide. Disaccharides are well known in the art and include, without limitation, trehalose, sucrose, as well as mixtures thereof.

In some embodiments of the invention, disaccharide is present at a concentration of 10.0 – 200.0 mg/ml.

In some embodiments of the invention, disaccharide is trehalose.

Trehalose may be used both in the form of anhydrous trehalose and in the form of hydrates thereof. In some embodiments of the invention, trehalose is trehalose dihydrate.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i) a recombinant non-enveloped virus vector,

(ii) a buffering agent being

tris(hydroxymethyl)aminomethane	1.9 – 8.7 mg/ml and
hydrochloric acid	to pH 6.0 – 8.0
Or	
L-histidine	0.4 – 10.0 mg/ml and
L-arginine	0.05 – 10.0 mg/ml,

(iii) hydroxypropyl beta cyclodextrin 10.0 – 200.0 mg/ml,

(iv)	a surfactant	0.01 – 2.0 mg/ml,
(v)	sodium chloride	1.0 – 15.0 mg/ml,
(vi)	magnesium chloride	0.05 – 1.0 mg/ml,
(vii)	water for injection	to 1.0 ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	1.9 – 8.7 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0,
(iii)	hydroxypropyl beta cyclodextrin	10.0 – 200.0 mg/ml,
(iv)	a surfactant	0.01 – 2.0 mg/ml,
(v)	sodium chloride	1.0 – 15.0 mg/ml,
(vi)	magnesium chloride	0.05 – 1.0 mg/ml,
(vii)	water for injection	to 1.0 ml.

In some embodiments of the invention, tris(hydroxymethyl)aminomethane is present at a concentration of 2.22 – 7.76 mg/ml, or 2.22 – 7.46, or 2.42 – 7.26 mg/ml, or 1.9 – 3.0 mg/ml, or 2.22 – 2.66 mg/ml, or 2.42 mg/ml, or 5.7 – 8.7 mg/ml, or 6.76 – 7.76 mg/ml, or 7.06 – 7.46 mg/ml, or 7.26 mg/ml.

In some embodiments of the invention, hydrochloric acid is added to pH 7.0 – 8.0, or to pH 7.3 – 7.8, or to pH 7.4 – 7.6, or to pH 7.5.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	L-histidine	0.4 – 10.0 mg/ml and
	L-arginine	0.05 – 10.0 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	10.0 – 200.0 mg/ml,
(iv)	a surfactant	0.01 – 2.0 mg/ml,
(v)	sodium chloride	1.0 – 15.0 mg/ml,
(vi)	magnesium chloride	0.05 – 1.0 mg/ml,
(vii)	water for injection	to 1.0 ml.

In some embodiments of the invention, L-histidine is present at a concentration of 2.0 – 9.24 mg/ml, or 2.58 – 8.74 mg/ml, or 2.79 – 8.37 mg/ml, or 2.0 – 3.58 mg/ml, or 2.29 – 3.08 mg/ml, or 2.58 – 3.0 mg/ml, or 2.79 mg/ml, or 6.0 – 10.0 mg/ml, or 7.5 – 9.24 mg/ml, or 8.0 – 8.74 mg/ml, or 8.37 mg/ml.

In some embodiments of the invention, L-arginine is present at a concentration of 0.248 – 1.344 mg/ml, or 0.3 – 1.2 mg/ml, or 0.348 – 1.044 mg/ml, or 0.248 – 0.448 mg/ml, or 0.3 – 0.396 mg/ml, or 0.348 mg/ml, or 0.744 – 1.344 mg/ml, or 0.888 – 1.2 mg/ml, or 1.044 mg/ml.

In some embodiments of the invention, hydroxypropyl-beta-cyclodextrin is present at a concentration of 30.0 – 110 mg/ml, or 40.0 – 105.0 mg/ml, or 50.0 – 100.0 mg/ml, 30.0 – 70.0 mg/ml, or 40.0 – 60.0 mg/ml, or 45.0-55.0 mg/ml, or 50.0 mg/ml, or 90.0 – 200.0 mg/ml, or 90.0 – 110.0 mg/ml, or 95.0 – 105.0 mg/ml, or 100.0 mg/ml.

In some embodiments of the invention, the surfactant is present at a concentration of 0.035 – 1.8 mg/ml, or 0.05 – 1.5 mg/ml, or 0.01 – 0.1 mg/ml, or 0.035 – 0.065 mg/ml, or 0.05 mg/ml, or 0.1 – 1.0 mg/ml, or 0.35 – 0.65 mg/ml, or 0.5 mg/ml, or 1.0 – 2.0 mg/ml, or 1.2 – 1.8 mg/ml, or 1.5 mg/ml.

In some embodiments of the invention, the surfactant is poloxamer 188.

In some embodiments of the invention, sodium chloride is present at a concentration of 2.5 – 14.14 mg/ml, or 3.88 – 13.64 mg/ml, or 3.0 – 13.14 mg/ml, or 4.38 – 6.5 mg/ml, or 4.5 – 6.0 mg/ml, or 1.0 – 7.0 mg/ml, or 1.0 – 6.0 mg/ml, or 2.5 – 3.5 mg/ml, or 3.0 mg/ml, or 3.88 – 4.88 mg/ml, or 4.38 mg/ml, or 4.5 mg/ml, or 5.5 – 6.5 mg/ml, or 6.0 mg/ml, or 9.0 – 15.0 mg/ml, or 12.14 – 14.14 mg/ml, or 12.64 – 13.64 mg/ml, or 13.14 mg/ml.

In some embodiments of the invention, magnesium chloride is present at a concentration of 0.15 – 0.7 mg/ml, or 0.173 – 0.659 mg/ml, or 0.203 – 0.609 mg/ml, or 0.15 – 0.50 mg/ml, or 0.173 – 0.233 mg/ml, or 0.203 mg/ml, or 0.5 – 0.7 mg/ml, or 0.559 – 0.659 mg/ml, or 0.609 mg/ml.

In some embodiments of the invention, magnesium chloride is magnesium chloride hexahydrate.

In some embodiments of the invention, the above pharmaceutical composition further comprises a disaccharide at a concentration of 10 – 200 mg/ml, or 20.0 – 150.0 mg/ml, or 25.0 – 100.0 mg/ml, or 25.0 – 50.0 mg/ml, or 20.0 – 30.0 mg/ml, or 23.0 – 27.0 mg/ml, or 25.0 mg/ml, or 30.0 – 70.0 mg/ml, or 30.0 – 36.6 mg/ml, or 32.3 – 34.3 mg/ml, or 33.3 mg/ml, or , or 45.0 – 55.0 mg/ml, or 50.0 mg/ml, or 90.0 – 200.0 mg/ml, or 100.0 mg/ml.

In some embodiments of the invention, disaccharide is trehalose.

In some embodiments of the invention, trehalose is trehalose dihydrate.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i) a recombinant non-enveloped virus vector,

(ii) a buffering agent being

tris(hydroxymethyl)aminomethane	2.42 mg/ml and
hydrochloric acid	to pH 6.0 – 8.0

Or

	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	6.0 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	6.0 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	6.0 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	

	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	Or	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	4.5 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)		
	further comprises a disaccharide being trehalose	25.0 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	4.5 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)		
	further comprises a disaccharide being trehalose	25.0 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,

(v)	sodium chloride	4.5 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)		
	further comprises a disaccharide being trehalose	25.0 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	Or	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	3.0 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)		
	further comprises a disaccharide being trehalose	50.0 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	3.0 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)		
	further comprises a disaccharide being trehalose	50.0 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	3.0 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)		
	further comprises a disaccharide being trehalose	50.0 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	Or	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	33.3 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	4.38 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)		
	further comprises a disaccharide being trehalose	33.3 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
(iii)	hydroxypropyl beta cyclodextrin	33.3 mg/ml,

(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	4.38 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)		
	further comprises a disaccharide being trehalose	33.3 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	33.3 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	4.38 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)		
	further comprises a disaccharide being trehalose	33.3 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	7.26 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	Or	
	L-histidine	8.37 mg/ml and
	L-arginine	1.044 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	1.5 mg/ml,
(v)	sodium chloride	13.14 mg/ml,
(vi)	magnesium chloride	0.609 mg/ml,
(vii)	water for injection	to 1.0 ml and

(viii)

further comprises a disaccharide being trehalose 100.0 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i) a recombinant non-enveloped virus vector,

(ii) a buffering agent being

tris(hydroxymethyl)aminomethane 7.26 mg/ml and

hydrochloric acid to pH 6.0 – 8.0

(iii) hydroxypropyl beta cyclodextrin 100.0 mg/ml,

(iv)

a surfactant being poloxamer 188 1.5 mg/ml,

(v) sodium chloride 13.14 mg/ml,

(vi) magnesium chloride 0.609 mg/ml,

(vii) water for injection to 1.0 ml and

(viii)

further comprises a disaccharide being trehalose 100.0 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i) a recombinant non-enveloped virus vector,

(ii) a buffering agent being

L-histidine 8.37 mg/ml and

L-arginine 1.044 mg/ml,

(iii) hydroxypropyl beta cyclodextrin 100.0 mg/ml,

(iv)

a surfactant being poloxamer 188 1.5 mg/ml,

(v) sodium chloride 13.14 mg/ml,

(vi) magnesium chloride 0.609 mg/ml,

(vii) water for injection to 1.0 ml and

(viii)

further comprises a disaccharide being trehalose 100.0 mg/ml.

In some embodiments of the invention, trehalose is trehalose dihydrate.

In some embodiments of the invention, magnesium chloride is magnesium chloride hexahydrate.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i) a recombinant non-enveloped virus vector,

(ii) a buffering agent being

	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	Or	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	6.0 mg/ml,
(vi)	magnesium chloride hexahydrate	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	6.0 mg/ml,
(vi)	magnesium chloride hexahydrate	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	6.0 mg/ml,
(vi)	magnesium chloride hexahydrate	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	Or	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	4.5 mg/ml,
(vi)	magnesium chloride hexahydrate	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)	further comprises a disaccharide being trehalose dihydrate	25.0 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	4.5 mg/ml,
(vi)	magnesium chloride hexahydrate	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)	further comprises a disaccharide being trehalose dihydrate	25.0 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,

(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	4.5 mg/ml,
(vi)	magnesium chloride hexahydrate	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)	further comprises a disaccharide being trehalose dihydrate	25.0 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	Or	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	3.0 mg/ml,
(vi)	magnesium chloride hexahydrate	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)	further comprises a disaccharide being trehalose dihydrate	50.0 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	3.0 mg/ml,
(vi)	magnesium chloride hexahydrate	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and

(viii) further comprises a disaccharide being trehalose dihydrate 50.0 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i) a recombinant non-enveloped virus vector,

(ii) a buffering agent being

L-histidine 2.79 mg/ml and

L-arginine 0.348 mg/ml,

(iii) hydroxypropyl beta cyclodextrin 100.0 mg/ml,

(iv)

a surfactant being poloxamer 188 0.5 mg/ml,

(v) sodium chloride 3.0 mg/ml,

(vi) magnesium chloride hexahydrate 0.203 mg/ml,

(vii) water for injection to 1.0 ml and

(viii) further comprises a disaccharide being trehalose dihydrate 50.0 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i) a recombinant non-enveloped virus vector,

(ii) a buffering agent being

tris(hydroxymethyl)aminomethane 2.42 mg/ml and

hydrochloric acid to pH 6.0 – 8.0

Or

L-histidine 2.79 mg/ml and

L-arginine 0.348 mg/ml,

(iii) hydroxypropyl beta cyclodextrin 33.3 mg/ml,

(iv)

a surfactant being poloxamer 188 0.5 mg/ml,

(v) sodium chloride 4.38 mg/ml,

(vi) magnesium chloride hexahydrate 0.203 mg/ml,

(vii) water for injection to 1.0 ml and

(viii) further comprises a disaccharide being trehalose dihydrate 33.3 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i) a recombinant non-enveloped virus vector,

(ii) a buffering agent being

	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
(iii)	hydroxypropyl beta cyclodextrin	33.3 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	4.38 mg/ml,
(vi)	magnesium chloride hexahydrate	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)	further comprises a disaccharide being trehalose dihydrate	33.3 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	33.3 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	4.38 mg/ml,
(vi)	magnesium chloride hexahydrate	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)	further comprises a disaccharide being trehalose dihydrate	33.3 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	7.26 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	Or	
	L-histidine	8.37 mg/ml and
	L-arginine	1.044 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	1.5 mg/ml,

(v)	sodium chloride	13.14 mg/ml,
(vi)	magnesium chloride hexahydrate	0.609 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)	further comprises a disaccharide being trehalose dihydrate	100.0 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	7.26 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	1.5 mg/ml,
(v)	sodium chloride	13.14 mg/ml,
(vi)	magnesium chloride hexahydrate	0.609 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)	further comprises a disaccharide being trehalose dihydrate	100.0 mg/ml.

In some embodiments of the invention, the pharmaceutical composition comprises:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	L-histidine	8.37 mg/ml and
	L-arginine	1.044 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	1.5 mg/ml,
(v)	sodium chloride	13.14 mg/ml,
(vi)	magnesium chloride hexahydrate	0.609 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)	further comprises a disaccharide being trehalose dihydrate	100.0 mg/ml.

In some embodiments of the invention, hydrochloric acid is added to pH 7.0 – 8.0, or to pH 7.3 – 7.8, or to pH 7.4 – 7.6, or to pH 7.5.

In some embodiments of the invention, the above pharmaceutical compositions are suitable for lyophilization, i.e. they can act as a prelyophilization solution.

In one aspect, the present invention relates to a pharmaceutical composition of a recombinant non-enveloped virus vector, which composition is provided in dry (i.e. powder or granular) form to be dissolved in a suitable solvent (e.g. water) prior to administration. Such pharmaceutical composition may be prepared by, for example, lyophilisation, i.e. a process which is known in the art as freeze drying, which involves freezing a product followed by removal of solvent from the frozen contents.

In one aspect, the present invention relates to a **lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector, said composition being produced by lyophilizing any pharmaceutical composition described above.** Accordingly, the pharmaceutical compositions according to the present invention may be either aqueous pharmaceutical compositions or lyophilized pharmaceutical compositions (lyophilisates).

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector is produced by lyophilizing a pharmaceutical composition comprising:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	1.9 – 8.7 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	Or	
	L-histidine	0.4 – 10.0 mg/ml and
	L-arginine	0.05 – 10.0 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	10.0 – 200.0 mg/ml,
(iv)	a surfactant	0.01 – 2.0 mg/ml,
(v)	sodium chloride	1.0 – 15.0 mg/ml,
(vi)	magnesium chloride	0.05 – 1.0 mg/ml,
(vii)	water for injection	to 1.0 ml.

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector is produced by lyophilizing a pharmaceutical composition comprising:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	Or	

	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	6.0 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml.

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector is produced by lyophilizing a pharmaceutical composition comprising:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	Or	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	4.5 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)		
	further comprises a disaccharide being trehalose	25.0 mg/ml.

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector is produced by lyophilizing a pharmaceutical composition comprising:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	Or	
	L-histidine	2.79 mg/ml and

	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	3.0 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)		
	further comprises a disaccharide being trehalose	50.0 mg/ml.

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector is produced by lyophilizing a pharmaceutical composition comprising:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	Or	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	33.3 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	4.38 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)		
	further comprises a disaccharide being trehalose	33.3 mg/ml.

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector is produced by lyophilizing a pharmaceutical composition comprising:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	7.26 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0

	Or	
	L-histidine	8.37 mg/ml and
	L-arginine	1.044 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	1.5 mg/ml,
(v)	sodium chloride	13.14 mg/ml,
(vi)	magnesium chloride	0.609 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)		
	further comprises a disaccharide being trehalose	100.0 mg/ml.

Lyophilisates are used to produce other dosage forms. For example, a lyophilisate for preparing an injectable solution, a lyophilisate for preparing an infusion solution, lyophilisate for preparing a concentrate for preparing an infusion solution. Lyophilisates are reconstituted by dissolving same in a suitable solvent, most typically in water for injection. Also, lyophilized compositions are first reconstituted in the required volume of solvent (most typically in water) and then further diluted in a suitable solvent (e.g. 5% glucose solution, 0.9% sodium chloride solution).

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector is intended for preparing an injectable solution.

In some embodiments of the invention, the lyophilized pharmaceutical composition of the recombinant non-enveloped virus vector is intended for preparing a solution for intramuscular, intravenous or subcutaneous administration.

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector is present in a vial.

In some embodiments of the invention, the vial is a glass or plastic vial.

In one aspect, the present invention relates to **a lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector, said composition following being reconstituted in an aqueous solvent comprises:**

(i) a recombinant non-enveloped virus vector,

(ii) a buffering agent,

(iii) hydroxypropyl beta cyclodextrin,

(iv) a surfactant,

(v) sodium chloride,

(vi) magnesium chloride,

(vii) water for injection.

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector, following being reconstituted in an aqueous solvent, comprises.

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	1.9 – 3.0 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	Or	
	L-histidine	0.4 – 10.0 mg/ml and
	L-arginine	0.05 – 10.0 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	20.0 – 100.0 mg/ml,
(iv)	a surfactant	0.01 – 1.0 mg/ml,
(v)	sodium chloride	1.0 – 6.0 mg/ml,
(vi)	magnesium chloride	0.05 – 1.0 mg/ml,
(vii)	water for injection	to 1.0 ml.

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector, following being reconstituted in an aqueous solvent, comprises.

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	1.9 – 3.0 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
(iii)	hydroxypropyl beta cyclodextrin	20.0 – 100.0 mg/ml,
(iv)	a surfactant	0.01 – 1.0 mg/ml,
(v)	sodium chloride	1.0 – 6.0 mg/ml,
(vi)	magnesium chloride	0.05 – 1.0 mg/ml,
(vii)	water for injection	to 1.0 ml.

In some embodiments of the invention, tris(hydroxymethyl)aminomethane is present at a concentration of 2.22 – 2.66 mg/ml, or 2.42 mg/ml.

In some embodiments of the invention, hydrochloric acid is added to pH 7.0 – 8.0, or to pH 7.3 – 7.8, or to pH 7.4 – 7.6, pH 7.5.

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector, following being reconstituted in an aqueous solvent, comprises.

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	L-histidine	0.4 – 10.0 mg/ml and
	L-arginine	0.05 – 10.0 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	20.0 – 100.0 mg/ml,
(iv)	a surfactant	0.01 – 1.0 mg/ml,
(v)	sodium chloride	1.0 – 6.0 mg/ml,
(vi)	magnesium chloride	0.05 – 1.0 mg/ml,
(vii)	water for injection	to 1.0 ml.

In some embodiments of the invention, L-histidine is present at a concentration of 2.0 – 3.58 mg/ml, or 2.29 – 3.08 mg/ml, or 2.58 – 3.0 mg/ml, or 2.79 mg/ml.

In some embodiments of the invention, L-arginine is present at a concentration of 0.248 – 0.448 mg/ml, or 0.3 – 0.396 mg/ml, or 0.348 mg/ml.

In some embodiments of the invention, hydroxypropyl-beta-cyclodextrin is present at a concentration of 30.0 – 70.0 mg/ml, or 30.0 – 36.6 mg/ml, or 33.3 mg/ml, or 40.0 – 60.0 mg/ml, or 45.0-55.0 mg/ml, or 50.0 mg/ml, or 90.0 – 100.0 mg/ml, 100.0 mg/ml.

In some embodiments of the invention, the surfactant is present at a concentration of 0.01 – 0.1 mg/ml, or 0.035 – 0.065 mg/ml, or 0.05 mg/ml, or 0.1 – 1.0 mg/ml, or 0.35 – 0.065 mg/ml, or 0.5 mg/ml.

In some embodiments of the invention, the surfactant is poloxamer 188.

In some embodiments of the invention, sodium chloride is present at a concentration of 1.0 – 7.0 mg/ml, or 1.0 – 6.0 mg/ml, or 2.5 – 3.5 mg/ml, or 3.0 mg/ml, or 3.88 – 4.88 mg/ml, or 4.38 mg/ml, or 4.5 mg/ml, or 5.5 – 6.5 mg/ml, or 6.0 mg/ml.

In some embodiments of the invention, magnesium chloride is present at a concentration of 0.15 – 0.50 mg/ml, or 0.173 – 0.233 mg/ml, or 0.203 mg/ml.

In some embodiments of the invention, magnesium chloride is magnesium chloride hexahydrate.

In some embodiments of the invention, the above pharmaceutical composition further comprises a disaccharide at a concentration of 20.0 – 100.0 mg/ml, 20.0 – 30.0 mg/ml, or 23.0 – 27.0 mg/ml, or 25.0 mg/ml, or 30.0 – 70.0 mg/ml, or 30.0 – 36.6 mg/ml, or 32.3 – 34.3 mg/ml, or 33.3 mg/ml, or , or 45.0 – 55.0 mg/ml, or 50.0 mg/ml.

In some embodiments of the invention, disaccharide is trehalose.

In some embodiments of the invention, trehalose is trehalose dihydrate.

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector, following being reconstituted in an aqueous solvent, comprises.

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	Or	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	6.0 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml.

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector, following being reconstituted in an aqueous solvent, comprises.

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	6.0 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml.

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector, following being reconstituted in an aqueous solvent, comprises.

(i) a recombinant non-enveloped virus vector,

(ii) a buffering agent being

	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	6.0 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml.

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector, following being reconstituted in an aqueous solvent, comprises.

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	Or	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	4.5 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)		
	further comprises a disaccharide being trehalose	25.0 mg/ml.

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector, following being reconstituted in an aqueous solvent, comprises.

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,

(iv)

	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	4.5 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)		
	further comprises a disaccharide being trehalose	25.0 mg/ml.

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector, following being reconstituted in an aqueous solvent, comprises.

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	4.5 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)		
	further comprises a disaccharide being trehalose	25.0 mg/ml.

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector, following being reconstituted in an aqueous solvent, comprises.

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	Or	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)	a surfactant being poloxamer 188	

		0.5 mg/ml,
(v)	sodium chloride	3.0 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)		
	further comprises a disaccharide being trehalose	50.0 mg/ml.

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector, following being reconstituted in an aqueous solvent, comprises.

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	3.0 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)		
	further comprises a disaccharide being trehalose	50.0 mg/ml.

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector, following being reconstituted in an aqueous solvent, comprises.

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	3.0 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and

(viii)

further comprises a disaccharide being trehalose 50.0 mg/ml.

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector, following being reconstituted in an aqueous solvent, comprises.

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	Or	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	33.3 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	4.38 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)		
	further comprises a disaccharide being trehalose	33.3 mg/ml.

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector, following being reconstituted in an aqueous solvent, comprises.

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
(iii)	hydroxypropyl beta cyclodextrin	33.3 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	4.38 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)	further comprises a disaccharide being trehalose	

33.3 mg/ml.

In some embodiments of the invention, the lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector, following being reconstituted in an aqueous solvent, comprises.

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	33.3 mg/ml,
(iv)		
	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	4.38 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)		
	further comprises a disaccharide being trehalose	33.3 mg/ml.

The described pharmaceutical compositions are suitable for delivering therapeutic agents to subjects for the treatment or prevention of various diseases or disorders. They may be used in gene therapy for the treatment of diseases such as hemophilia A, hemophilia B, malignant neoplasms, spinal muscular atrophy, etc., as well as in the development of vaccines for the prevention of, for example, infectious diseases.

The pharmaceutical compositions according to the invention may comprise the recombinant non-enveloped virus vector at various concentrations. The concentration of the viral vector may depend, for example, on the disease to be prevented or treated using the above pharmaceutical compositions, as well as on the age, weight and state of health of the patient, and thus may vary from patient to patient. A suitable dose can be adjusted by the decision of the attending physician so that it can be administered to the patient once or through several injections.

In some embodiments of the invention, the recombinant non-enveloped virus vector is present at a concentration of $1.0*10^5 - 1.0*10^{14}$ viral genomes/ml, or $1.0*10^9 - 1.0*10^{14}$ viral genomes/ml, or $1.0*10^9 - 5.0*10^{13}$ viral genomes/ml, or $1.0*10^9 - 1.0*10^{13}$ viral genomes/ml, or $(1.0 \pm 0.5)*10^5$ viral genomes/ml, or $(1.0 \pm 0.5)*10^6$ viral genomes/ml, or $(1.0 \pm 0.5)*10^7$ viral genomes/ml, or $(1.0 \pm 0.5)*10^8$ viral genomes/ml, or $(1.0 \pm 0.5)*10^9$ viral genomes/ml, or $(1.0 \pm 0.5)*10^{10}$ viral genomes/ml, or $(1.0 \pm 0.5)*10^{11}$ viral genomes/ml, or $(1.0 \pm 0.5)*10^{12}$ viral genomes/ml, or $(1.0 \pm 0.5)*10^{13}$ viral genomes/ml, or $(1.0 \pm 0.5)*10^{14}$ viral genomes/ml.

The recombinant non-enveloped viruses in the above pharmaceutical compositions may be rAAV, recombinant adenoviruses and other types of recombinant non-enveloped viruses.

The recombinant adenovirus vector may be produced using any species, strain, serotype or any combination of species, strains or serotypes of adenovirus or chimeric adenovirus. Human adenovirus serotypes include any of the serotypes 2, 4, 5, 7, 11, 26, 34, 35, 36, 48, 49 or 50 or combinations thereof, derivatives, modifications or pseudotypes.

In some embodiments of the invention, the recombinant non-enveloped virus vector is a rAAV vector. It may be produced using any strain, AAV serotype (for example, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15 and AAV16) or any combination of strains, serotypes (for example, a rAAV vector that includes two or more serotypes); may contain an AAV capsid protein (capsid) of any serotype (AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15 and AAV16) or combinations thereof, derivatives, modifications or pseudotypes.

In some embodiments of the invention, the rAAV vector comprises a AAV5 or AAV9 capsid.

In some embodiments of the invention, the AAV5 or AAV9 capsid may be a modified capsid.

The rAAV vector may be modified genetically and/or chemically.

The rAAV vector may be genetically modified to produce rAAV vectors with altered receptor usage, antigenicity, transduction efficiency and/or tissue tropism, and to insert peptide ligands, antibodies, antibody fragments, MHC (major histocompatibility complex) and/or receptors into the viral capsid. For example, the rAAV vector may be genetically modified by introducing one or more amino acid mutations, such as point mutations.

The phrase "more point mutations" refers to two, three, four, five, six, seven, eight, nine, or ten point substitutions.

Particularly preferred embodiments include substitutions (mutations) that are conservative in nature, i.e. substitutions that take place within a family of amino acids that are joined in their side chains. In particular, amino acids are typically divided into four families: (1) acidic amino acids are aspartate and glutamate; (2) basic amino acids are lysine, arginine, histidine; (3) non-polar amino acids are alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, and (4) uncharged polar amino acids are glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. For example, it is reasonably predictable that an isolated substitution of leucine for isoleucine or valine, an aspartate for a glutamate, a threonine for a serine, or a similar

conservative substitution of an amino acid for a structurally related amino acid, will not have a major effect on the biological activity. For example, the polypeptide of interest may include up to about 5-10 conservative or non-conservative amino acid substitutions, so long as the desired function of the molecule remains intact.

A variant of point mutations in the sequences of AAV proteins VP1, VP2, or VP3 using amino acid substitutions is a substitution of at least one amino acid residue in the AAV protein VP1, VP2, or VP3 with another amino acid residue.

Conservative substitutions are shown in Table A under "preferred substitutions".

Table A

Initial residue	Exemplary substitutions	Preferred substitutions
Ala (A)	Val; Leu; Ile	Val
Arg(R)	Lys; Gln; Asn	Lys
Asn(N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln(Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly(G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe(F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser(S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp(W)	Tyr; Phe	Tyr
Tyr(Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

For example, the international application WO2012/145601 describes adeno-associated virus (AAV) virions with variant capsid protein, where the AAV virions exhibit greater infectivity

of retinal cells, when administered via intravitreal injection, compared to wild-type AAV. The international application WO2013/158879 describes an adenoassociated virus (AAV) vector for delivering to a subject a heterologous nucleic acid sequence comprising the VP1 capsid protein comprising one or more lysine substitutions, wherein one lysine substitution is K137R, wherein said lysine substitution is effective for inhibiting ubiquitination of said capsid protein, thereby increasing transduction efficiency of said AVV vector in target cells.

The rAAV vector may be chemically modified to alter tissue tropism. Also, chemically modified rAAV-based vectors may exhibit altered receptor usage, antigenicity, transduction efficiency, and/or tissue tropism. Chemically modified rAAV vectors are created by using, for example, chemoselective reactions which may target specific side chains of amino acids, may be used to change the charge, polarity, hydrophobicity and potential of hydrogen bonds inside the receptor binding domains on AAV capsids.

In some embodiments of the invention, said pharmaceutical composition of a recombinant nonenveloped virus vector according to the present invention is intended for parenteral administration.

In some embodiments of the invention, said pharmaceutical composition of a recombinant nonenveloped virus vector according to the present invention is intended for intramuscular, intravenous or subcutaneous administration.

In some embodiments of the invention, said pharmaceutical composition of a recombinant nonenveloped virus vector according to the present invention may be administered intravenously as an infusion.

The pharmaceutical composition of a recombinant nonenveloped virus vector according to the present invention may be used following dilution. To this end, the required volume of the composition is transferred from a vial to an infusion container comprising a sterile 0.9% sodium chloride solution or a sterile 5% dextrose solution. The resulting solution is stirred by gently turning the infusion container over.

In some embodiments of the invention, the subject of treatment, or patient, is a mammal, preferably a human subject. Said subject may be either male or female, of any age.

Pharmaceutical compositions according to the present invention may be stored in any suitable container. For example, a glass or plastic container, vial, ampoule, syringe, cartridge, or bottle of the desired volume. The containers may be provided with additional means for administration, such as droppers, auto-injectors.

A pharmaceutical composition according to the invention may be manufactured, packaged, or widely sold in the form of a single unit dose or a plurality of single unit doses in the form of a ready formulation. The term "single unit dose" as used herein refers to discrete quantity of a

pharmaceutical composition containing a predetermined quantity of an active ingredient. The quantity of the active ingredient typically equals the dose of the active ingredient to be administered in a subject, or a convenient portion of such dose, for example, half or a third of such dose.

The pharmaceutical compositions may be administered as a single therapeutic agent or in combination with additional therapeutic agents as needed. Thus, in one embodiment, the present methods for treatment and/or prophylaxis are used in combination with administration of a therapeutically effective amount of another active agent. The other active agent may be administered before, during or following the administration of the pharmaceutical compositions according to the present invention. The other active agent may be administered as part of the present composition or, alternatively, as a separate formulation.

Examples

The following examples are provided for better understanding of the invention. These examples are for purposes of illustration only and are not to be construed as limiting the scope of the invention in any manner.

All publications, patents, and patent applications cited in this specification are incorporated herein by reference. Although the foregoing invention has been described in detail by way of illustration and example for purposes of clarity of understanding, it will be apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended embodiments.

Methods

1. Determination of thermal transitions by differential scanning calorimetry.

Thermal transitions were determined by differential scanning calorimetry on the DSC4000 differential scanning calorimeter, Perkin Elmer, USA, in capped steel crucibles with rubber rings. The test used 50 µl of the sample. An excipient solution or a process solution was used as the test solution. Temperature transitions were studied in the range from (25)°C to (-85)°C in increments in the range of 0.5 – 10 °C/min. The phase transition temperatures were determined using the Pyris Series DSC 4000 software.

2. Determination of water content.

Water content was measured by the K.Fischer method according to RF SP GM.1.2.3.0002.15.

3. Determination of concentration of viral genomes (GFP gene) in the AAV product by PCR.

The concentration of the GFP gene in the AAV product was determined by quantitative polymerase chain reaction using a forward, reverse primer and a probe conjugated with FAM dye and BHQ1 quencher. The primers are matched to a region of the gene encoding the GFP protein. During sample preparation, the sample was first treated with DNAase in order to get rid of residual plasmid DNA, then with proteinase in order to destroy the capsid. A linearized plasmid (pAAV_GFP CellBioLab) was used to plot a calibration line. To plot a calibration line of log concentration as a function of number of cycles (Ct), standards with an increment of 10 were used. The concentration of viral genomes/ml (vg/ml) or copies/ml in the product was determined by the calibration line of log concentration as a function of Ct.

4. Determination of concentration of viral particles by enzyme immunoassay.

Concentration of viral vector particles (adeno-associated virus (AAV) capsids) was measured by means of enzyme immunoassay using commercial kits: PROGEN AAV5 titration ELISA and PROGEN AAV9 titration ELISA.

High binding 96-well plates are coated with anti-AAV capsid binding monoclonal mouse antibodies recognizing the conformational epitope of a particular serotype. AAV capsid proteins bind to antibodies under temperature incubation, unbound components of the sample are removed by washing the plate multiple times.

Biotinylated antibodies that bind to AAV capsid proteins under temperature incubation of the plate were used as detecting antibodies. The unbound components are removed by washing the plate multiple times.

Streptavidin-horseradish peroxidase conjugate was applied to detect the color. Streptavidin bound to biotin, unbound components were removed by washing the plate multiple times.

Tetramethylbenzidine acts as a chromogen. Interaction of horseradish peroxidase, hydrogen peroxide and tetramethylbenzidine chromogen develops a color reaction. EIA reaction was stopped by adding a stop solution (1 normal H_2SO4), and the optical density of the solutions in the wells was measured at a wavelength of 450 nm. The concentration of viral vector particles in the product was determined by a calibration graph plotted using optical density data of standard solutions with known concentration of AAV particles.

5. Determination of functional activity of the product based on recombinant adeno-associated viral vector comprising the GFP gene.

Transduction activity of the product based on the recombinant adeno-associated viral vector comprising the GFP gene was measured by GFP expression in product-induced CHO-K1 cells.

For transduction, on the day of analysis, the CHO-K1 cells were plated into 24-well plates in a growth medium with an antibiotic (MQD - medium for quantitative determination) at the rate of $1x10^4$ cells per cm^2 (19,000 cells per well) and incubated at a temperature of +37 °C in an atmosphere with 5% CO_2 for 4-8 h for attachment of the cells to plastic surface. The test samples at 3 doses, 3 replicates for each, were then introduced into the wells with cells. Product dosages (MOI) for each AAV serotype were pre-selected so as to obtain, following transduction, about 5, 10 and 20% of GFP-positive cells, respectively. Product volume required for transduction of a well was calculated using the following formula:

$$V = \frac{MOI \times S \times K}{C},$$

where V is the required product volume per well (ml), S is the area of one well (cm^2), MOI is the required dose of the product, *multiplicity of infection* (vg/cl), K is cell seeding density in the wells (cells/cm^2), C is the concentration of genome-containing capsids in the product (vg/ml).

The plates with transduced cells were incubated in a CO_2 incubator (5% CO_2, +37 °C) for 2-3 days. Following the incubation period, culture fluid was collected from the wells, the cells were washed with Hanks solution, and the cells were removed from the plastic using TrypLE solution (at 150 µl/well). After detaching the cells from the plastic, Hanks solution (1:1 with TrypLE) was introduced into the wells, the cells were collected into 1.5 ml microtubes and centrifuged for 5 min at 300g. The cell precipitate was resuspended in 150 µl of Hanks solution. 50 ml of a working dye solution for determination of viable cells in Hanks solution was added to each sample, except for 1 isotype control well, the cells were incubated for 20 minutes in the dark at room temperature. The stained cells were centrifuged for 5 minutes at 300g, the supernatant was carefully selected and 100 µl of BD Cytofix fixing buffer was added to each sample. The cells were resuspended, and the entire volume of the resulting cell suspension was transferred into a 96-well V-bottom plate for flow cytometer, the plate with cells was incubated for 20 minutes in the dark at room temperature. Following the incubation period, the plate was centrifuged for 5 minutes at 1200 rpm, a fixing buffer was collected, and the cells were washed with Stain Buffer (PBS with 0.1% Sodium azide and 0.5% BSA) at 100 µl/well. The plate was centrifuged again and the buffer was collected, following which Stain Buffer was introduced at 150 µl/well and the cells were carefully resuspended.

The cells were analyzed on a flow cytometer. Cell viability and GFP expression were evaluated. The value of FA (functional activity) or TU (transduction unit)/ml was calculated for each product dose according to the following formula:

$$TU = \frac{P \times N}{V} \times D,$$

where P is the number of GFP-positive viable cells as a percentage, N is the number of initially plated cells (N=S×K), V is the introduced volume of the product per well (ml), D is the product predilution factor.

The mean TU value for 3 product doses was considered the final result.

6. Preparation of formulations.

Samples with the target content of viral genomes were prepared in Stirred Cell (Millipore) concentration cells under pressure. To this end, the initial solution formulation containing viral particles was placed in a diafiltration container, then at least 10-fold volume of an aqueous solution with the target formulation including buffering, osmotic agents and, if necessary, additional water-soluble stabilizers was introduced into the cell. Following diafiltration process, the solution was concentrated to an optical density exceeding the target density, unloaded from the instrument, the exact optical density was determined by UV spectrophotometry and the concentration of viral genomes was determined by PCR. An appropriate solution of excipients was then added to the sample to prepare a solution with target viral genome content.

Also, samples of solutions containing viral particles were obtained in Pellicon (Millipore) cassettes in the tangential flow mode. To this end, the initial solution formulation was placed in a diafiltration tank, then at least 10-fold volume of the solution with the target formulation comprising buffering, osmotic agents, and, if necessary, additional water soluble stabilizers was supplied to the system. The concentrate of osmotic agents and water-soluble stabilizers may alternatively be added following diafiltration. Following the diafiltration process, the solution was concentrated to an optical density exceeding the target density, unloaded from the system and the exact values of the optical density and concentration of viral genomes were determined. An appropriate solution of excipients was then added to the sample to prepare a solution with target viral genome content.

When preparing formulations comprising solubilizers, the surfactant concentrates were added to the solution following diafiltering and concentrating with the final dilution to the target viral genome content with a solution of excipients.

Before aseptic filling in the final container (for example, a glass/plastic vessel, vial or syringe), the solution was filtered using a 0.22 μm membrane.

Example 1. Lyophilization of formulations.

We performed freeze-drying of the formulations according to the following scheme: Freezing - duration up to 300 minutes, temperature up to -70°C. Initial drying - duration up to 4000 min, temperature from -40°C to 40°C. Vacuum from 0 mbar to 0.25 mbar. Secondary drying -

duration up to 4000 min, temperature from 0°C to 40°C. Vacuum from 0 to 0.25 mbar. Each of the steps included one or more stages.

Example 2. Selection of formulations to optimize the freeze-drying mode.

Optimization was aimed at reducing the duration of the freeze-drying process, as well as at reducing residual water content in the lyophilisate. For this purpose, it is suggested to approbate a number of formulations, followed by measurement of the glass transition temperature.

It is known that the addition of sodium chloride to a formulation helps to reduce the glass transition temperature of the formulation (Electrolyte-induced changes in glass transition temperature of freeze-concentrated solutes, Pharmaceutical Research, vol.12, no.5, 1995). However, this component is an effective stabilizer for viral particles, prevents aggregation thereof and cannot be excluded from the formulation (Pharmaceutical Development of AAV-Based Gene Therapy Products for the Eye. Gerard A. Rodrigues & Evgenyi Shalaev & Thomas K. Karami & James Cunningham & Nigel K. H. Slater & Hongwen M. Rivers, Pharm Res (2019) 36: 29.

The freeze-drying process is most effective when setting and maintaining the temperature of a freeze-dryer shelf at upper limits of system and product stability. Thus, the temperature should be set at a level that is a few degrees below the collapse temperature. Collapse means a process in which structures created during freeze drying are destroyed when passing the sublimation phase separation boundary. This may lead not only to altered functional properties of the lyophilisate, but also to decreased efficiency of freeze drying because of blocked evaporation pathways, resulting in unevenly retained and distributed moisture.

The resulting substance in the final excipient solution is an amorphous mixture. Glass transition temperature is the temperature at which a non-crystallizable substance or a substance that does not have time to crystallize becomes solid, passing into a glassy state. The collapse temperature of solutions is typically 1-3 °C higher than the glass transition temperature. Thus, the higher the glass transition temperature, the higher the temperature can be set for the shelves, resulting in accelerated freeze drying process.

The following excipients were selected for screening: tris(hydroxymethyl)aminomethane (buffering agent, maintenance of the required pH level), sodium chloride (stabilizer, osmolytic), magnesium chloride hexahydrate (stabilizer), trehalose dihydrate (stabilizer, osmolytic, lyoprotector), poloxamer p188 (solubilizer), polyvinylpyrrolidone K-30 (stabilizer), polyvinylpyrrolidone K-90 (stabilizer), sulfobutyl-ether-beta-cyclodextrin sodium salt (stabilizer, lyoprotector), hydroxypropyl-beta-cyclodextrin (stabilizer, lyoprotector), L-histidine (bufferring agent, maintenance of the required pH level, stabilizer), L-arginine (bufferring agent, maintenance of the required pH level, stabilizer).

The results of determination of glass transition temperatures for test excipient solutions are shown in Table 2.

Table 2 – Glass transition temperatures for different formulations.

No.	Pharmaceutical composition	T'g, °C
1	Tris(hydroxymethyl)aminomethane - 7.26 mg/ml Sodium chloride - 13.14 mg/ml Magnesium chloride hexahydrate - 1.22 mg/ml Trehalose dihydrate - 150 mg/ml Poloxamer p188 - 1.5 mg/ml Hydrochloric acid - to pH 7.5	-35.55
2	Tris(hydroxymethyl)aminomethane - 7.26 mg/ml Sodium chloride - 13.14 mg/ml Magnesium chloride hexahydrate - 1.22 mg/ml Trehalose dihydrate - 50 mg/ml Sulfobutyl-ether-beta-cyclodextrin sodium salt - 100 mg/ml Poloxamer p188 - 1.5 mg/ml Hydrochloric acid - to pH 7.5	-37.83
3	Tris(hydroxymethyl)aminomethane - 7.26 mg/ml Sodium chloride - 13.14 mg/ml Magnesium chloride hexahydrate - 1.22 mg/ml Sulfobutyl-ether-beta-cyclodextrin sodium salt - 150 mg/ml Poloxamer p188 - 1.5 mg/ml Hydrochloric acid - to pH 7.5	-41.37
4	Tris(hydroxymethyl)aminomethane - 7.26 mg/ml Sodium chloride - 13.14 mg/ml Magnesium chloride hexahydrate - 1.22 mg/ml Polyvinylpyrrolidone K-30 - 150 mg/ml Poloxamer p188 - 1.5 mg/ml Hydrochloric acid - to pH 7.5	-28.65
5	Tris(hydroxymethyl)aminomethane - 7.26 mg/ml Sodium chloride - 13.14 mg/ml Magnesium chloride hexahydrate - 1.22 mg/ml Polyvinylpyrrolidone K-90 - 60 mg/ml Poloxamer p188 - 1.5 mg/ml Hydrochloric acid - to pH 7.5	-38.69
6	Tris(hydroxymethyl)aminomethane - 7.26 mg/ml Sodium chloride - 13.14 mg/ml Magnesium chloride hexahydrate - 1.22 mg/ml Trehalose dihydrate - 50 mg/ml Hydroxypropyl-beta-cyclodextrin - 100 mg/ml Poloxamer p188 - 1.5 mg/ml Hydrochloric acid - to pH 7.5	-26.37
7	Tris(hydroxymethyl)aminomethane - 7.26 mg/ml Sodium chloride - 13.14 mg/ml Magnesium chloride hexahydrate - 1.22 mg/ml Polyvinylpyrrolidone K-90 - 60 mg/ml Trehalose dihydrate - 90 mg/ml	-31.10

No.	Pharmaceutical composition	T'g, °C
	Poloxamer p188 - 1.5 mg/ml Hydrochloric acid - to pH 7.5	
8	Tris(hydroxymethyl)aminomethane - 7.26 mg/ml Sodium chloride - 13.14 mg/ml Magnesium chloride hexahydrate - 1.22 mg/ml Hydroxypropyl-beta-cyclodextrin - 150 mg/ml Poloxamer p188 - 1.5 mg/ml Hydrochloric acid - to pH 7.5	-20.52
9	Tris(hydroxymethyl)aminomethane - 7.26 mg/ml Sodium chloride - 13.14 mg/ml Magnesium chloride hexahydrate - 1.22 mg/ml Trehalose dihydrate - 50 mg/ml Poloxamer p188 - 1.5 mg/ml Hydrochloric acid - to pH 7.5	-37.15

According to the results, it was found that hydroxypropyl-beta-cyclodextrin significantly increases the glass transition temperature of a mixture comprising sodium chloride.

Further development was aimed at determining the optimal ratio of the contents of trehalose dihydrate, hydroxypropyl beta-cyclodextrin and sodium chloride, as well as evaluating the applicability of L-histidine and L-arginine buffer solutions to produce a lyophilic form. The study was performed for samples containing approximately $1\times^{10-11}$ vg/ml of recombinant AAV5 comprising the GFP gene in a complete 3-factor mixture design experiment with two levels and the central point repeated 3 times. The concentration of trehalose dihydrate (from 0.0 to 100.0 mg/ml), sodium chloride (from 3.0 to 12.0 mg/ml) and the concentration of hydroxypropyl-beta-cyclodextrin (from 0.0 to 100.0 mg/ml) were studied as quantitative factors. Poloxamer 188 (in the range of 0.05 – 2.0 mg/ml) and magnesium chloride hexahydrate (in the range of 0.15 – 0.70 mg/ml) do not have a statistically significant effect on the glass transition temperature.

The results of the experiment are shown in Table 3.

Table 3 – Results of experiment for AAV5-based formulations at a concentration of 1×10^{11} vg/ml

No.	Excipient concentration in formulation				T'g, °C
	Trehalose dihydrate, mg/ml	Sodium chloride, mg/ml	Hydroxypropyl-beta-cyclodextrin, mg/ml	Buffer solution, ml[1]	
10	0	3.0	0	to 1.0	-34.58
11	100.0	3.0	0	to 1.0	-35.07

12	0	12.0	0	to 1.0	-40.78
13	100.0	12.0	0	to 1.0	-43.29
14	0	3.0	100.0	to 1.0	-16.77
15	100.0	3.0	100.0	to 1.0	-25.48
16	0	12.0	100.0	to 1.0	-27.98
17	100.0	12.0	100.0	to 1.0	-29.92
18	50.0	7.5	50.0	to 1.0	-31.72
19	50.0	7.5	50.0	to 1.0	-30.88
20	50.0	7.5	50.0	to 1.0	-31.04
[1] Buffer solution formulation: L-histidine - 2.79 mg/ml; L-arginine - 0.348 mg/ml; magnesium chloride hexahydrate - 0.203 mg/ml; poloxamer p188 – 0.5 mg/ml.					

The resulting data was processed in the MODDE software.

According to the results, formulation No. 14 (L-histidine – 2.79 mg/ml; L-arginine – 0.348 mg/ml; magnesium chloride hexahydrate – 0.203 mg/ml; poloxamer p188 – 0.5 mg/ml; sodium chloride – 3.0 mg/ml; hydroxypropyl-beta-cyclodextrin - 100.0 mg/ml) showed the maximum glass transition temperature.

Further, the effect of the freeze drying process on recombinant AAV5 and recombinant AAV9 viral particles comprising the GFP gene was evaluated, the working range for the concentration of viral particles was established, as well as a comparative assessment of applicability of L-arginine, L-histidine and tris(hydroxymethyl)aminomethane buffer solutions to produce a lyophilic form was performed. The data are shown for the compositions listed in Table 4.

Table 4 – List of formulations for freeze drying

Component	Formulation 21	Formulation 22	Formulation 23	Formulation 24	Formulation 25	Formulation 26
AAV5 or AAV9	1×10^{11} vg/ml	1×10^{11} vg/ml	1×10^{11} vg/ml	1×10^{11} vg/ml	1×10^{11} vg/ml	1×10^{11} vg/ml
L-histidine	2.79 mg/ml	2.79 mg/ml	2.79 mg/ml	-	-	-
L-arginine	0.348 mg/ml	0.348 mg/ml	0.348 mg/ml	-	-	-
Sodium chloride	6.0 mg/ml	4.5 mg/ml	3.0 mg/ml	6.0 mg/ml	4.5 mg/ml	3.0 mg/ml
Magnesium chloride hexahydrate	0.203 mg/ml	0.203 mg/ml	0.203 mg/ml	0.203 mg/ml	0.203 mg/ml	0.203 mg/ml
Trehalose dihydrate	-	25 mg/ml	50 mg/ml	-	25 mg/ml	50 mg/ml

Hydroxypropyl beta cyclodextrin	100 mg/ml	100 mg/ml	100 mg/ml	100 mg/ml	100 mg/ml	100 mg/ml
Poloxamer p188	0.5 mg/ml	0.5 mg/ml	0.5 mg/ml	0.5 mg/ml	0.5 mg/ml	0.5 mg/ml
Tris(hydroxymethyl) aminomethane	-	-	-	2.42 mg/ml	2.42 mg/ml	2.42 mg/ml
Hydrochloric acid	-	-	-	to pH 7.5	to pH 7.5	to pH 7.5

The results of quality control of AAV5 and AAV9 lyophilisates are shown in Tables 5, 6.

Table 5 –Results of quality control of AAV5 lyophilisates

No.	T'g, °C	Hydrodynamic radius, nm	Concentration (PCR), vg/ml	Concentration (EIA), particles/ml	Activity, FA/ml	Water, %	Reconstitution time, sec
21	-21.51	10.1	2.1×10^{11}	2.6×10^{11}	5.6×10^{6}	1.03	Less than 10
22	-20.88	9.7	2.5×10^{11}	3.1×10^{11}	6.2×10^{6}	0.98	Less than 10
23	-21.13	10.5	2.4×10^{11}	2.8×10^{11}	5.3×10^{6}	1.01	Less than 10
24	-21.32	11.3	1.8×10^{11}	2.0×10^{11}	3.6×10^{6}	0.84	Less than 10
25	-20.03	9.8	3.0×10^{11}	3.6×10^{11}	7.0×10^{6}	0.92	Less than 10
26	-20.84	10.0	3.1×10^{11}	3.4×10^{11}	6.8×10^{6}	1.10	Less than 10

Table 6 –Results of quality control of AAV9 lyophilisates

No.	T'g, °C	Hydrodynamic radius, nm	Concentration (PCR), vg/ml	Concentration (EIA), particles/ml	Activity, FA/ml	Water, %	Reconstitution time, sec
21	-21.92	17.3	2.6×10^{11}	3.0×10^{11}	3.5×10^{6}	1.39	Less than 10
22	-20.76	16.9	3.4×10^{11}	3.9×10^{11}	3.0×10^{6}	1.21	Less than 10
23	-21.64	16.7	2.9×10^{11}	3.2×10^{11}	5.1×10^{6}	1.13	Less than 10
24	-21.12	15.6	2.5×10^{11}	2.9×10^{11}	3.9×10^{6}	1.09	Less than 10
25	-20.25	17.1	2.9×10^{11}	3.3×10^{11}	5.0×10^{6}	1.12	Less than 10
26	-20.66	17.3	2.7×10^{11}	3.2×10^{11}	4.3×10^{6}	1.09	Less than 10

It has been shown that all test formulations are suitable for producing a lyophilised dosage form. Water content in all test samples does not exceed 1.4%, lyophilisate reconstitution time does not exceed 10 seconds, and the freeze drying time is only 48 hours. Following reconstitution, no reduction in titer and functional activity of the samples was observed.

The formulations were then produced having different viral genome contents. The results of quality control of AAV5 and AAV9 lyophilisates with different viral genome contents are shown in Table 7. The data is shown for formulation No. 25.

Table 7 – Results of quality control of AAV5 and AAV9 lyophilisates with different viral genome contents

No.	T'g, °C	Concentration (PCR), vg/ml	Concentration (EIA), particles/ml	Activity, FA/ml	Water, %	Reconstitution time, sec
AAV5						
25-1	-24.03	1.9×10^{9}	2.2×10^{9}	3.6×10^{4}	1.53	Less than 10
25-2	-20.11	2.6×10^{12}	3.0×10^{12}	5.2×10^{7}	1.00	Less than 10
25-3	-19.50	9.0×10^{13}	9.4×10^{13}	7.3×10^{8}	0.89	Less than 10
AAV9						
25-4	-24.25	3.2×10^{9}	3.6×10^{9}	2.9×10^{4}	1.33	Less than 10
25-5	-20.59	2.7×10^{12}	3.1×10^{12}	5.5×10^{7}	0.98	Less than 10
25-6	-18.37	8.7×10^{13}	9.2×10^{13}	7.0×10^{9}	0.87	Less than 10

To further assess the possibility of lyophilization of 3-fold concentrated solutions, prelyophilization compositions for AAV5 and AAV9 were prepared, as shown in Tables 8 and 9. The results of control of the resulting samples following reconstitution of lyophilisates are shown in Table 10.

Table 8 – Concentrated AAV5 (No. 27) and AAV9 (No. 28) formulations based on tris(hydroxymethyl)aminomethane

Component	Prelyophilization solution	3-fold concentrated prelyophilization solution	Reconstituted solution
AAV5 or AAV9	1×10^{11} vg/ml	3×10^{11} vg/ml	1×10^{11} vg/ml
Sodium chloride	4.38 mg/ml	13.14 mg/ml	4.38 mg/ml
Magnesium chloride hexahydrate	0.203 mg/ml	0.609 mg/ml	0.203 mg/ml
Trehalose dihydrate	33.3 mg/ml	100.0 mg/ml	33.3 mg/ml
Hydroxypropyl beta cyclodextrin	33.3 mg/ml	100.0 mg/ml	33.3 mg/ml
Poloxamer p188	0.5 mg/ml	1.5 mg/ml	0.5 mg/ml
Tris(hydroxymethyl) aminomethane	2.42 mg/ml	7.26 mg/ml	2.42 mg/ml
Hydrochloric acid	to pH 7.5	to pH 7.5	to pH 7.5

Table 9 – Concentrated AAV5 (No. 29) and AAV9 (No. 30) formulations based on L-histidine and L-arginine

Component	Prelyophilization solution	3-fold concentrated prelyophilization solution	Reconstituted solution
AAV5 or AAV9	1×10^{11} vg/ml	3×10^{11} vg/ml	1×10^{11} vg/ml
Sodium chloride	4.38 mg/ml	13.14 mg/ml	4.38 mg/ml
Magnesium chloride hexahydrate	0.203 mg/ml	0.609 mg/ml	0.203 mg/ml

Trehalose dihydrate	33.3 mg/ml	100.0 mg/ml	33.3 mg/ml
Hydroxypropyl beta cyclodextrin	33.3 mg/ml	100.0 mg/ml	33.3 mg/ml
Poloxamer p188	0.5 mg/ml	1.5 mg/ml	0.5 mg/ml
L-histidine	2.79 mg/ml	8.37 mg/ml	2.79 mg/ml
L-arginine	0.348 mg/ml	1.044 mg/ml	0.348 mg/ml

Table 10 – Results of control of reconstituted lyophilisates obtained from concentrated prelyophilization AAV5 and AAV9 solutions (reconstituted in 3-fold solvent volume)

No.	Concentration (PCR), vg/ml	Concentration (EIA), particles/ml	Activity, FA/ml	Water, %	Reconstitution time, sec
AAV5					
27	1.8×10^{11}	2.0×10^{11}	3.5×10^{6}	1.02	Less than 10
29	2.2×10^{11}	2.5×10^{11}	2.9×10^{6}	1.24	Less than 10
AAV9					
28	2.3×10^{11}	2.7×10^{11}	3.2×10^{6}	1.10	Less than 10
30	1.6×10^{11}	1.8×10^{11}	3.1×10^{6}	1.16	Less than 10

Further, the formulations were produced for viral genomes (copies) concentration of about 1×10^{11} per ml (expressed in terms of prelyophilization formulation) and transferred to stability research.

The results of stability study for formulation No. 25 are shown in Tables 11, 12.

Table 11 – Results of AAV5 lyophilisate stability study (formulation No. 25)

Ageing point	Hydrodynamic radius, nm	Concentration (PCR), vg/ml	Concentration (EIA), particles/ml	Activity, FA/ml	Water, %	Reconstitution time, sec
Long–term stability study (ageing at a temperature of 2-8 °C)						
1 mon	11.3	2.6×10^{11}	3.0×10^{11}	6.1×10^{6}	0.96	Less than 10
2 mon	11.0	2.3×10^{11}	2.8×10^{11}	5.5×10^{6}	1.27	Less than 10
3 mon	12.2	2.7×10^{11}	3.2×10^{11}	4.9×10^{6}	1.11	Less than 10
6 mon	11.8	2.2×10^{11}	2.6×10^{11}	6.3×10^{6}	1.08	Less than 10
Accelerated stability study (ageing at temperature (25 ± 2) °C and relative humidity (60 ± 5)%						
1 mon	11.1	2.2×10^{11}	2.7×10^{11}	5.8×10^{6}	1.00	Less than 10
2 mon	11.8	2.7×10^{11}	3.2×10^{11}	4.9×10^{6}	1.16	Less than 10
3 mon	11.3	1.8×10^{11}	2.1×10^{11}	4.5×10^{6}	1.33	Less than 10
6 mon	12.0	2.3×10^{11}	2.7×10^{11}	5.3×10^{6}	1.10	Less than 10

Table 12 – Results of AAV9 lyophilisate stability study (formulation No. 25)

Ageing point	Hydrodynamic radius, nm	Concentration (PCR), vg/ml	Concentration (EIA), particles/ml	Activity, FA/ml	Water, %	Reconstitution time, sec
Long–term stability study (ageing at a temperature of 2-8 °C)						

1 mon	17.0	3.6×10^{11}	3.9×10^{11}	4.5×10^{6}	1.12	Less than 10
2 mon	17.2	2.7×10^{11}	3.2×10^{11}	4.0×10^{6}	1.45	Less than 10
3 mon	16.6	2.4×10^{11}	3.0×10^{11}	4.7×10^{6}	1.50	Less than 10
6 mon	17.9	3.1×10^{11}	3.6×10^{11}	3.8×10^{6}	1.13	Less than 10
Accelerated stability study (ageing at temperature (25 ± 2) °C and relative humidity (60 ± 5)%						
1 mon	17.3	2.8×10^{11}	3.1×10^{11}	4.3×10^{6}	1.30	Less than 10
2 mon	16.7	2.1×10^{11}	2.6×10^{11}	5.0×10^{6}	1.15	Less than 10
3 mon	16.9	1.7×10^{11}	2.0×10^{11}	4.8×10^{6}	1.33	Less than 10
6 mon	16.2	1.8×10^{11}	2.2×10^{11}	4.0×10^{6}	1.25	Less than 10

Thus, we developed stable lyophilisate formulations exhibiting shorter time for freeze drying process and a low content of residual water. The developed compositions may be applied to adeno-associated viruses and other types of non-enveloped viruses.

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Claims

1. A pharmaceutical composition of a recombinant non-enveloped virus vector comprising:

(i) a recombinant non-enveloped virus vector,

(ii) a buffering agent,

(iii) hydroxypropyl beta cyclodextrin,

(iv) a surfactant,

(v) sodium chloride,

(vi) magnesium chloride,

(vii) water for injection.

2. The pharmaceutical composition according to claim 1, wherein the buffering agent is tris(hydroxymethyl)aminomethane and hydrochloric acid.

3. The pharmaceutical composition according to claim 2, wherein tris(hydroxymethyl)aminomethane is present at a concentration of 1.9 – 8.7 mg/ml.

4. The pharmaceutical composition according to claim 2, wherein hydrochloric acid is added to pH 6.0 – 8.0.

5. The pharmaceutical composition according to claim 1, wherein the buffering agent is L-histidine and L-arginine.

6. The pharmaceutical composition according to claim 5, wherein L-histidine is present at a concentration of 0.4 – 10.0 mg/ml.

7. The pharmaceutical composition according to claim 5, wherein L-arginine is present at a concentration of 0.05 – 10.0 mg/ml.

8. The pharmaceutical composition according to claim 1, wherein hydroxypropyl-beta-cyclodextrin is present at a concentration of 10.0 – 200.0 mg/ml.

9. The pharmaceutical composition according to claim 1, wherein the surfactant is present at a concentration of 0.01 – 2.0 mg/ml.

10. The pharmaceutical composition according to claim 1, wherein the surfactant is poloxamer 188.

11. The pharmaceutical composition according to claim 1, wherein sodium chloride is present at a concentration of 1.0 – 15.0 mg/ml.

12. The pharmaceutical composition according to claim 1, wherein magnesium chloride is present at a concentration of 0.05 – 1.0 mg/ml.

13. The pharmaceutical composition according to claim 1, wherein magnesium chloride is magnesium chloride hexahydrate.

14. The pharmaceutical composition according to claim 1, the composition further comprising disaccharide.

15. The pharmaceutical composition according to claim 14, wherein disaccharide is present at a concentration of 10.0 – 200.0 mg/ml.

16. The pharmaceutical composition according to claim 14, wherein disaccharide is trehalose.

17. The pharmaceutical composition according to claim 16, wherein trehalose is trehalose dihydrate.

18. The pharmaceutical composition according to claim 1, comprising:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	1.9 – 8.7 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	or	
	L-histidine	0.4 – 10.0 mg/ml
		and
	L-arginine	0.05 – 10.0 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	10.0 – 200.0 mg/ml,
(iv)	a surfactant	0.01 – 2.0 mg/ml,
(v)	sodium chloride	1.0 – 15.0 mg/ml,
(vi)	magnesium chloride	0.05 – 1.0 mg/ml,
(vii)	water for injection	to 1.0 ml.

19. The pharmaceutical composition according to claim 2, wherein tris(hydroxymethyl)aminomethane is present at a concentration of 1.9 – 3.0 mg/ml or at a concentration of 5.7 – 8.7 mg/ml.

20. The pharmaceutical composition according to claim 2, wherein tris(hydroxymethyl)aminomethane is present at a concentration of 2.42 mg/ml or at a concentration of 7.26 mg/ml.

21. The pharmaceutical composition according to claim 2, wherein hydrochloric acid is added to pH 7.0 – 8.0.

22. The pharmaceutical composition according to claim 2, wherein hydrochloric acid is added to pH 7.5.

23. The pharmaceutical composition according to claim 5, wherein L-histidine is present at a concentration of 2.0 – 3.58 mg/ml or at a concentration of 6.0 – 10.0 mg/ml.

24. The pharmaceutical composition according to claim 5, wherein L-histidine is present at a concentration of 2.79 mg/ml or at a concentration of 8.37 mg/ml.

25. The pharmaceutical composition according to claim 5, wherein L-arginine is present at a concentration of 0.248 – 0.448 mg/ml or at a concentration of 0.744 – 1.344 mg/ml.

26. The pharmaceutical composition according to claim 5, wherein L-arginine is present at a concentration of 0.348 mg/ml or at a concentration of 1.044 mg/ml.

27. The pharmaceutical composition according to claim 1, wherein hydroxypropyl-beta-cyclodextrin is present at a concentration of 30.0 – 70.0 mg/ml or at a concentration of 90.0 – 200.0 mg/ml.

28. The pharmaceutical composition according to claim 1, wherein hydroxypropyl-beta-cyclodextrin is present at a concentration of 50.0 mg/ml or

at a concentration of 100.0 mg/ml.

29. The pharmaceutical composition according to claim 1, wherein the surfactant is present at a concentration of 0.01 – 0.1 mg/ml or at a concentration of 0.1 – 1.0 mg/ml, or at a concentration of 1.0 – 2.0 mg/ml.

30. The pharmaceutical composition according to claim 18, wherein the surfactant is present at a concentration of 0.05 mg/ml or

at a concentration of 0.5 mg/ml; or

at a concentration of 1.5 mg/ml.

31. The pharmaceutical composition according to claim 1, wherein the surfactant is poloxamer 188.

32. The pharmaceutical composition according to claim 1, wherein sodium chloride is present at a concentration of 1.0 – 6.0 mg/ml or at a concentration of 9.0 – 15.0 mg/ml.

33. The pharmaceutical composition according to claim 1, wherein sodium chloride is present at a concentration of 3.0 mg/ml; or

34. at a concentration of 4.38 mg/ml; or

35. at a concentration of 4.5 mg/ml; or

36. at a concentration of 6.0 mg/ml; or

at a concentration of 13.14 mg/ml.

37. The pharmaceutical composition according to claim 1, wherein magnesium chloride is present at a concentration of 0.15 – 0.50 mg/ml or at a concentration of 0.5 – 0.7 mg/ml.

38. The pharmaceutical composition according to claim 1, wherein magnesium chloride is present at a concentration of 0.203 mg/ml or

at a concentration of 0.609 mg/ml.

39. The pharmaceutical composition according to claim 1, wherein magnesium chloride is magnesium chloride hexahydrate.

40. The pharmaceutical composition according to claim 18, the composition further comprising disaccharide at a concentration of 10 – 200 mg/ml.

41. The pharmaceutical composition according to claim 14, wherein disaccharide is present at a concentration of 30.0 – 70.0 mg/ml or

at a concentration of 90.0 – 200.0 mg/ml.

42. The pharmaceutical composition according to claim 14, wherein disaccharide is present at a concentration of 25.0 mg/ml; or

43. at a concentration of 33.3 mg/ml; or

at a concentration of 50.0 mg/ml; or

at a concentration of 100.0 mg/ml.

44. The pharmaceutical composition according to claim 14, wherein disaccharide is trehalose.

45. The pharmaceutical composition according to claim 44, wherein trehalose is trehalose dihydrate.

46. The pharmaceutical composition according to claim 1, comprising:

(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	or	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	6.0 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml.

47. The pharmaceutical composition according to claim 1, comprising:

(a)		
(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and

	hydrochloric acid	to pH 6.0 – 8.0
	or	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	4.5 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)	further comprises a disaccharide being trehalose	25.0 mg/ml; or
(b)		
(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	or	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)	a surfactant being poloxamer 188	0.5 mg/ml,
(v)	sodium chloride	3.0 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)	further comprises a disaccharide being trehalose	50.0 mg/ml; or
(c)		
(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	2.42 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	or	
	L-histidine	2.79 mg/ml and
	L-arginine	0.348 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	33.3 mg/ml,
(iv)	a surfactant being poloxamer 188	0.5 mg/ml,

(v)	sodium chloride	4.38 mg/ml,
(vi)	magnesium chloride	0.203 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)	further comprises a disaccharide being trehalose	33.3 mg/ml; or
(d)		
(i)	a recombinant non-enveloped virus vector,	
(ii)	a buffering agent being	
	tris(hydroxymethyl)aminomethane	7.26 mg/ml and
	hydrochloric acid	to pH 6.0 – 8.0
	or	
	L-histidine	8.37 mg/ml and
	L-arginine	1.044 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	100.0 mg/ml,
(iv)	a surfactant being poloxamer 188	1.5 mg/ml,
(v)	sodium chloride	13.14 mg/ml,
(vi)	magnesium chloride	0.609 mg/ml,
(vii)	water for injection	to 1.0 ml and
(viii)	further comprises a disaccharide being trehalose	100.0 mg/ml.

48. The pharmaceutical composition according to claim 47, wherein trehalose is trehalose dihydrate.

49. The pharmaceutical composition according to claim 46, wherein magnesium chloride is magnesium chloride hexahydrate.

50. The pharmaceutical composition according to claim 46, wherein hydrochloric acid is added to pH 7.5.

51. The pharmaceutical composition according to claim 1, wherein the recombinant non-enveloped virus vector is present at a concentration of $1.0*10^9 – 1.0*10^{14}$ viral genomes/ml.

52. The pharmaceutical composition according to claim 1, wherein the recombinant non-enveloped virus vector is present at a concentration of $1.0*10^9 – 1.0*10^{13}$ viral genomes/ml.

53. The pharmaceutical composition according to claim 1, wherein the recombinant non-enveloped virus vector is present at a concentration of $(1.0 \pm 0.5)*10^9$ viral genomes/ml, $(1.0 \pm 0.5)*10^{10}$ viral genomes/ml, $(1.0 \pm 0.5)*10^{11}$ viral genomes/ml, $(1.0 \pm 0.5)*10^{12}$ viral genomes/ml, $(1.0 \pm 0.5)*10^{13}$ viral genomes/ml или $(1.0 \pm 0.5)*10^{14}$ viral genomes/ml.

54. The pharmaceutical composition according to claim 1, wherein the recombinant non-enveloped virus vector is a rAAV vector.

55. The pharmaceutical composition according to claim 54, wherein the rAAV vector comprises a capside of AAV5 serotype or AAV9 serotype.

56. The pharmaceutical composition according to claim 55, wherein the capsid of AAV5 serotype or AAV9 serotype may be a modified capsid.

57. The pharmaceutical composition according to any one of claims 1-56, the composition being suitable for lyophilization.

58. A lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector produced by lyophilization of the pharmaceutical composition according to any one of claims 1-57.

59. The lyophilized pharmaceutical composition according to claim 58, the composition being intended for preparing an injectable solution.

60. The lyophilized pharmaceutical composition according to claim 58, the composition being intended for preparing a solution for intramuscular, intravenous or subcutaneous administration.

61. The lyophilized pharmaceutical composition according to claim 58, wherein the lyophilized pharmaceutical composition is present in a vial.

62. The lyophilized pharmaceutical composition according to claim 58, wherein the vial is a glass or polymer vial.

63. A lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector, said composition following being reconstituted in an aqueous solvent comprises:

(i) a recombinant non-enveloped virus vector,

(ii) a buffering agent,

(iii) hydroxypropyl beta cyclodextrin,

(iv) a surfactant,

(v) sodium chloride,

(vi) magnesium chloride,

(vii) water for injection.

64. The lyophilized pharmaceutical composition of a recombinant non-enveloped virus vector according to claim 63, said composition following being reconstituted in an aqueous solvent comprises:

(i) a recombinant non-enveloped virus vector,

(ii) a buffering agent being

tris(hydroxymethyl)aminomethane	1.9 – 3.0 mg/ml and
hydrochloric acid	to pH 6.0 – 8.0

	or	
	L-histidine	0.4 – 10.0 mg/ml and
	L-arginine	0.05 – 10.0 mg/ml,
(iii)	hydroxypropyl beta cyclodextrin	20.0 – 100.0 mg/ml,
(iv)	a surfactant	0.01 – 1.0 mg/ml,
(v)	sodium chloride	1.0 – 6.0 mg/ml,
(vi)	magnesium chloride	0.05 – 1.0 mg/ml,
(vii)	water for injection	to 1.0 ml.

65. The lyophilized pharmaceutical composition according to claim 63, wherein tris(hydroxylmethyl)aminomethane is present at a concentration of 2.42 mg/ml.

66. The lyophilized pharmaceutical composition according to claim 63, wherein hydrochloric acid is added to pH 7.5.

67. The lyophilized pharmaceutical composition according to claim 63, wherein L-histidine is present at a concentration of 2.79 mg/ml; or

68. at a concentration of 0.348 mg/ml.

69. The lyophilized pharmaceutical composition according to claim 63, wherein hydroxypropyl-beta-cyclodextrin is present at a concentration of 33.3 mg/ml; or

at a concentration of 100.0 mg/ml.

70. The lyophilized pharmaceutical composition according to claim 63, wherein the surfactant is present at a concentration of 0.05 mg/ml; or

at a concentration of 0.5 mg/ml.

71. The lyophilized pharmaceutical composition according to claim 63, wherein the surfactant is poloxamer 188.

72. The lyophilized pharmaceutical composition according to claim 63, wherein sodium chloride is present at a concentration of 3.0 mg/ml; or

at a concentration of 4.38 mg/ml; or

at a concentration of 4.5 mg/ml; or

at a concentration of 6.0 mg/ml.

73. The lyophilized pharmaceutical composition according to claim 63, wherein magnesium chloride is present at a concentration of 0.203 mg/ml.

74. The lyophilized pharmaceutical composition according to claim 63, wherein magnesium chloride is magnesium chloride hexahydrate.

75. The lyophilized pharmaceutical composition according to claim 63, the composition further comprising disaccharide.

76. The lyophilized pharmaceutical composition according to claim 75, wherein disaccharide is present at a concentration of 20 – 100 mg/ml.

77. The lyophilized pharmaceutical composition according to claim 75, wherein disaccharide is present at a concentration of 25.0 mg/ml; or

at a concentration of 33.3 mg/ml; or

at a concentration of 50.0 mg/ml.

78. The lyophilized pharmaceutical composition according to claim 75, wherein disaccharide is trehalose.

79. The lyophilized pharmaceutical composition according to claim 78, wherein trehalose is trehalose dihydrate.

80. The lyophilized pharmaceutical composition according to claim 63, wherein the recombinant non-enveloped virus vector is present at a concentration of $1.0*10^9 – 1.0*10^{14}$ viral genomes/ml.

81. The lyophilized pharmaceutical composition according to claim 63, wherein the recombinant non-enveloped virus vector is present at a concentration of $1.0*10^9 – 1.0*10^{13}$ viral genomes/ml.

82. The lyophilized pharmaceutical composition according to claim 63, wherein the recombinant non-enveloped virus vector is present at a concentration of $(1.0 \pm 0.5)*10^{11}$ viral genomes/ml, $(1.0 \pm 0.5)*10^{10}$ viral genomes/ml, $(1.0 \pm 0.5)*10^{11}$ viral genomes/ml, $(1.0 \pm 0.5)*10^{12}$ viral genomes/ml, $(1.0 \pm 0.5)*10^{13}$ viral genomes/ml или $(1.0 \pm 0.5)*10^{14}$ viral genomes/ml.

83. The lyophilized pharmaceutical composition according to claim 63, wherein the recombinant non-enveloped virus vector is a rAAV vector.

84. The lyophilized pharmaceutical composition according to claim 83, wherein the rAAV vector comprises a capsid of AAV5 serotype or AAV9 serotype.

85. The lyophilized pharmaceutical composition according to claim 84, wherein the capsid of AAV5 serotype or AAV9 serotype may be a modified capsid.

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Abstract

The present invention relates to the field of pharmaceuticals, gene therapy and medicine, specifically to pharmaceutical compositions of a vector based on recombinant non-enveloped virus, in particular the recombinant adeno-associated virus (rAAV), which compositions may be aqueous or lyophilized compositions and may be used for the treatment and prevention of various diseases.