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(54) Title: ADENOVIRUS FORMULATIONS

(57) Abstract: The present invention provides pharmaceutical adenovirus formulations, in particular liquid pharmaceutical formulations comprising adenoviruses.

ADENOVIRUS FORMULATIONS

FIELD OF THE INVENTION

The present invention relates to adenovirus formulations and related pharmaceutical products for use in e.g. gene therapy and/or vaccine applications. In particular, liquid formulations for adenoviruses are disclosed herein, which improve the adenoviral stability by preserving quantity, potency (infectivity) and quality of the contained adenovirus when stored in about the 2-8°C range or higher while also being compatible with parenteral administration.

BACKGROUND OF THE INVENTION

Adenovirus vectors are considered the most efficient and widely used vehicles for gene delivery. An ongoing challenge in the field of gene therapy and vaccine research is to generate liquid adenovirus formulations which are able to stabilize these viruses for longer periods of time within a realistic storage temperature range for pharmaceutical products, such as from about 2°C to about 8°C.

Biological activity of an adenovirus depends upon the conformational integrity of at least a core sequence of nucleotides surrounded by an icosahedral capsid structure consisting of the capsid proteins. Unlike traditional organic and inorganic drugs, these are highly complex biological structures and minor chemical or physical stressors can contribute to the degradation of the adenoviral particle. A good formulation of adenovirus preparations is therefore of crucial importance to ensure a reasonable shelf-life, but stabilizing these vectors poses particular challenges. Adenoviruses may lose potency as a result of physical instabilities, including denaturation, aggregation (both soluble and insoluble aggregate formation), precipitation and adsorption, as well as chemical instabilities, including hydrolysis, deamidation, and oxidation. Any of these degradation routes can lead to lowered biologically activity, but can potentially also result in the formation of by-products or derivatives having increased toxicity, and/or altered immunogenicity.

It therefore needs a tailored approach to find a robust formulation for adenoviruses ensuring stability over a wide range of conditions. Buffer type, pH and specialized excipients will need to be meticulously optimized to keep an adenovirus chemically, physically and biologically stable. In view of all the factors that can be varied, finding optimal conditions for

formulating adenoviruses is burdened with challenges, and the composition of a good formulation is a priori unpredictable.

Lyophilized formulations exist and are stable. However they tend to be relatively expensive, require time consuming handling before administration, and potency might to a certain extent be lost in the lyophilization process. Liquid formulations that are stable under frozen conditions (-80°C) exist, but these require specialized shipment and expensive storage facilities, making a reliable cold chain almost impossible, especially at the periphery of the distribution network. A preferred formulation for adenoviruses is therefore a liquid formulation that offers adenoviral stability at a temperature range between 2-8°C or higher. Such a formulation can be stored in a regular fridge and can be administered quickly and easily.

Liquid formulations for adenoviruses have been described previously, for instance in Evans et al. 2004. The best formulations exemplified in said reference are Tris buffered formulations having a pH ranging between 7.5 and 8.5. Other formulations disclosed in Evans et al. contain a histidine buffer, sucrose and $MgCl_2$. We have found herein that said formulations are suboptimal for adenoviruses. Formulations for adenoviruses have also been disclosed in US6225289, in particular a Tris buffered formulation at pH 7.8 containing trehalose has been disclosed therein. We have found that said formulations are suboptimal for adenoviruses.

Accordingly, there is a need in the art to find formulations which improve the adenoviral stability by preserving quantity and potency of the contained adenovirus during storage over a prolonged period of time. The adenoviral stability should also be retained in the case of agitation stress during transport or shear forces during production or clinical use, and under wide-ranging climatic conditions, in particular at elevated temperature or after repeated freeze/thaw cycles. Furthermore, the formulation should be suitable for the intended route of administration, should be well tolerated and should preferably have a composition with as little components as possible. It is an object of the invention to provide such formulations for adenoviruses.

SUMMARY OF THE INVENTION

We have found and describe herein, formulations for adenoviruses which improve the adenoviral-stability by preserving quantity and potency (infectivity) and quality of the adenovirus as compared to previously disclosed formulations. Remarkably, the combination of a histidine buffered solution having a pH ranging between 6 and 7 together with trehalose resulted in an outstanding formulation for the preservation of quantity, potency (infectivity) and quality of adenoviruses, therewith improving overall adenoviral-stability as compared to other formulations known in the art.

As with all excipients used for formulation development, some of the components present in the formulation according to the present invention are separately cited in the prior art. However, it is the very specific combination of several components that gives the present formulation its outstanding properties and stabilizing potential. The exact formulation according to the present invention was not disclosed in the prior art. In addition, it could not have been foreseen, based on the prior art in this inherently unpredictable field, that said formulation would provide such improved stability to adenoviruses.

The present invention therefore relates to stabilized adenovirus formulations and related pharmaceutical products that can e.g. be used in gene therapy and/or vaccine applications.

The adenovirus formulations according to the present invention comprise histidine as buffer at a pH ranging between 6 and 7, and further comprise trehalose. The formulation additionally comprises a salt and a non-ionic detergent. The adenoviral formulations of the present invention are amenable to prolonged storage at 2°C to 8°C or ≤-65°C, for more than 6 months, 1 year, 1.5 year, 2 years, or more.

The adenovirus formulations of the present invention comprise a) a recombinant adenovirus in a b) histidine buffered solution, which further comprises c) trehalose; d) a salt; and e) a non-ionic detergent. In order to preserve the stability of the adenovirus it is essential that the pH of this formulation ranges between 6 and 7.

Preferably the formulation according to the present invention comprises adenovirus at a titer ranging between about $1x10^7$ vp/mL and about $1x10^{13}$ vp/mL.

In another preferred embodiment according to the present invention the histidine concentration in the formulation ranges between about 5 mM and about 30 mM.

Trehalose is the preferred cryoprotectant and is preferably present in a concentration ranging between about 1% (w/w) and about 10% (w/w).

Sodium chloride (NaCl) is the preferred salt, which is preferably present at a concentration ranging between about 20 mM and about 200 mM.

Polysorbate-80 is the preferred non-ionic detergent which preferably has a concentration ranging between about 0.005% (w/w) and about 0.5% (w/w).

The formulation according to the invention can optionally include one or more additional stabilizer. To this end, the virus formulation according to the present invention further contains at least one stabilizer selected from the group consisting of a 2-carbon alcohol, a 4-carbon alcohol, EDTA and an EDTA/ethanol combination.

In a preferred embodiment according to the present invention, the virus formulation further contains a 2 or 4-carbon alcohol. In an even more preferred embodiment, the virus formulation further contains ethanol.

In a preferred embodiment according to the present invention the virus formulation further comprises ethanol with a concentration ranging between about 0.1% (w/w) to about 1% (w/w).

In one embodiment according to the present invention the formulation has a pH ranging between about 6 and about 7, and comprises histidine as buffer at a concentration ranging between about 5 and about 30 mM; trehalose at a concentration ranging between about 1% (w/w) and about 10% (w/w); NaCl at a concentration ranging between about 20 mM and about 200 mM; Polysorbate-80 at a concentration ranging between about 0.005% (w/w) and about 0.5% (w/w).

In a preferred embodiment according to the present invention the formulation has a pH ranging between about 6.2 and about 6.8, and comprises histidine as buffer at a concentration ranging between about 15 and about 25 mM; trehalose at a concentration ranging between about 3% (w/w) and about 8% (w/w); NaCl at a concentration ranging between about 50 mM and about 100 mM; and Polysorbate-80 at a concentration ranging between about 0.01% (w/w) and about 0.05% (w/w).

In a even more preferred embodiment the formulation according to the invention, has a pH of about 6.6 and comprises about 20 mM histidine; trehalose in a concentration of about

5% (w/w); NaCl at about 75 mM, and Polysorbate-80 at a concentration of about 0.02% (w/w).

Therefore in a preferred embodiment, the formulation according to the invention further comprises a stabilizer selected from the group consisting of a 2-carbon alcohol, a 4-carbon alcohol, EDTA and a combination of ethanol/EDTA. In a preferred embodiment the formulation according to the invention further comprises a 2 or 4-carbon alcohol. In a preferred embodiment said 2 or 4-carbon alcohol is ethanol. The ethanol concentration is preferably ranging between about 0.1% (w/w) to about 1% (w/w).

In a preferred embodiment according to the present invention the formulation has a pH ranging between about 6 and about 7, and comprises histidine as buffer at a concentration ranging between about 5 and about 30 mM; trehalose at a concentration ranging between about 1% (w/w) and about 10% (w/w); NaCl at a concentration ranging between about 20 mM and about 200 mM; Polysorbate-80 at a concentration ranging between about 0.01% (w/w) and about 0.05% (w/w); and ethanol at a concentration ranging between about 0.2% (w/w) and about 0.6% (w/w).

In another preferred embodiment according to the present invention the formulation has a pH ranging between about 6.2 and about 6.8, and comprises histidine as buffer at a concentration ranging between about 15 and about 25 mM; trehalose at a concentration ranging between about 3% (w/w) and about 8% (w/w); NaCl at a concentration ranging between about 50 mM and about 100 mM; Polysorbate-80 at a concentration ranging between about 0.01% (w/w) and about 0.03% (w/w); and ethanol at a concentration ranging between about 0.2% (w/w) and about 0.6% (w/w).

In an even more preferred embodiment the formulation according to the invention has a pH of about 6.6 and comprises histidine as buffer at a concentration of about 20 mM; trehalose at a concentration of about 5% (w/w); NaCl at a concentration of about 75 mM, Polysorbate-80 at a concentration of about 0.02% (w/w) and ethanol at a concentration of about 0.4% (w/w).

In another preferred embodiment of the present invention the formulations are (frozen) liquid formulations. In yet another embodiment, the formulations of the present invention is suitable for parenteral use.

In one embodiment, the formulations according to the present invention are contained in a vial. In another embodiment, the formulations are contained in a bag or a bottle. In yet another embodiment the formulations are contained in a syringe or cartridge.

The present invention also relates to a method of preserving an adenovirus which comprises preparing a formulation according to the present invention.

In yet another embodiment, the present invention relates to a method of preserving an adenovirus which comprises preparing a formulation as described herein and storing said formulation at a temperature ranging between 2°C and 8°C.

The enhanced long-term stability over a wide temperature range results in an extended shelf life of the virus formulations disclosed herein, allowing for storage and eventual host administration of these formulations over preferably about a 1-2 year period, or more with acceptable losses in virus potency (i.e. not more than 0.3log per two years at 2-8°C). In addition, formulations of the present invention show stability during exposure to elevated temperatures, extended freeze/thaw cycles and agitation.

DESCRIPTION OF THE FIGURES

Figure 1. Potency (in log IU/mL) loss (Δ) of Ad26 during accelerated temperature at 25°C in Formulation D (open squares) and control formulation (closed circles). Mean Δpotency (n=4) is shown, reflecting $potency_{stressed\ sample}$-$potency_{control\ sample}$.

Figure 2. Potency (in log IU/mL) loss (Δ) of Ad35 during accelerated temperature at 25°C in Formulation D (open squares) and control formulation (closed circles). Mean Δpotency (n=4) is shown, reflecting $potency_{stressed\ sample}$-$potency_{control\ sample}$.

Figure 3. Thermal melting point of Ad26 in Formulation D and control formulation. TMA analysis (n=3) for Ad26 was performed on t=0 samples.

Figure 4. Thermal melting point of Ad35 in Formulation D and control formulation. TMA analysis (n=3) for Ad35 was performed on t=0 samples.

Figure 5. Dynamic Light Scattering plot showing the particle size obtained during a stepwise temperature increase of Ad26 in Formulation D (open squares) and control formulation (closed circles). Inset: zoom in on onset of aggregation.

Figure 6. Dynamic Light Scattering plot showing the particle size obtained during a stepwise temperature increase of Ad35 in Formulation D (open squares) and control formulation (closed circles). Inset: zoom in on onset of aggregation.

Figure 7. Potency (in log IU/mL) loss (Δ) of Ad35 during accelerated temperature at 25°C in the optimal formulation (open squares) and control formulation (closed circles). Mean potency (n=4) is shown, reflecting $potency_{stressed\ sample}$-$potency_{control\ sample}$.

DETAILED DESCRIPTION OF THE INVENTION

The formulations of the invention comprise at least one recombinant adenovirus. The construction of adenoviral vectors is well understood in the art and involves the use of standard molecular biological techniques, such as those described in, for example, Sambrook et al., Molecular Cloning, a Laboratory Manual, 2d ed., Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989), Watson et al., Recombinant DNA, 2d ed., Scientific American Books (1992), and Ausubel et al., Current Protocols in Molecular Biology, Wiley Interscience Publishers, NY (1995), and other references mentioned herein. In short, the adenoviral vector can be deficient in at least one essential gene function of the E1 region, e.g., the E1a region and/or the E1b region, of the adenoviral genome that is required for viral replication. As known to the skilled person for producing adenovirus, in case of deletions of essential regions from the adenovirus genome, the functions encoded by these regions have to be provided in trans, preferably by the producer cell, for instance integrated in the genome, or in the form of so-called helper adenovirus or helper plasmids, when producing the recombinant adenovirus.

Propagation of a recombinant adenovirus has been described in detail in: US patent 6,492,169 or in WO 03/104467 U.S. Pat. Nos. 5,559,099, 5,837,511, 5,846,782, 5,851,806, 5,994,106, 5,994,128, 5,965,541, 5,981,225, 6,040,174, 6,020,191, and 6,113,913, and Thomas Shenk, "Adenoviridae and their Replication", M. S. Horwitz, "Adenoviruses", Chapters 67 and 68, respectively, in Virology, B. N. Fields et al., eds., 3d ed., Raven Press, Ltd., New York (1996), which is incorporated here by reference. The replication-deficient adenoviral vector can be generated by using any species, strain, subtype, or mixture of species, strains, or subtypes, of an adenovirus or a chimeric adenovirus as the source of vector DNA (see for instance WO 96/26281, WO 00/03029). In certain embodiments of the present invention, serotypes of human adenovirus include any one of serotypes 2, 4, 5, 7, 11, 26, 34, 35, 36, 48, 49 or 50 or any hybrid or mutated adenovirus serotypes. In a preferred

embodiment of the present invention the recombinant adenovirus is from human adenovirus serotype 5, 26 or 35.

In further embodiments, the adenovirus of the invention is a simian adenovirus, preferably a chimpanzee or gorilla adenovirus. These adenoviruses generally have a low seroprevalence and/or low pre-existing neutralizing antibody titers in the human population.

In further embodiments, the adenovirus of the invention further comprises heterologous nucleic acid. Suitable heterologous nucleic acid is well known to the skilled person, and for instance may include transgene open reading frames, for instance open reading frames coding for polypeptides against which an immune response is desired when the vector is used for vaccination purposes, e.g. transgenes suitable to generate an immune response against malaria (see e.g. WO 2004/055187), HIV, tuberculosis (see e.g. WO 2006/053871), certain viruses, etc, all well known to the skilled person. In fact, the nature of the transgene is not critical to the current invention, it may be any heterologous nucleic acid sequence, and hence needs no further elaboration here.

The term "stability" as used herein refers to the relative resistance to degradation of adenovirus particles in a formulation retaining its potency on the timescale of its expected usefulness. Preferably, the potency shows a decrease of not more than 0.3log per two years at 2-8°C.

The term "potency" as used herein refers to a measure of adenovirus activity expressed in terms of infectious units measured in a cell-based potency assay, which is described hereunder.

A composition according to the invention shows a decrease in potency of not more than 0.5log per 70 days and a decrease in titer of not more than 0.4log per 70 days in an accelerated stability study which study is performed by incubation of the formulations at 25°C ± 2°C during 1 to 3 months.

A composition according to the invention also shows a decrease in potency of not more than 0.2log per 10 cycles in a study wherein vials are subjected to repeated freeze/thawing cycles followed by 24 hours of agitation at room temperature in a horizontal orientation at 200 rpm.

By "pharmaceutically acceptable excipient" is meant any inert substance that is combined with an active molecule such as a virus for preparing an agreeable or convenient

dosage form. The “pharmaceutically acceptable excipient” is an excipient that is non-toxic to recipients at the dosages and concentrations employed, and is compatible with other ingredients of the formulation comprising the viral preparation. Examples of excipients are cryoprotectants, non-ionic detergents, buffers, salts and inhibitors of free radical oxidation.

The term "by-product" includes undesired products, which detract or diminish the proportion of therapeutic/prophylactic adenovirus in a given formulation. Typical by-products include aggregates of the adenovirus and fragments of the adenovirus, resulting from e.g. protein denaturation, deamidation, hydrolysis or combinations thereof. Typically, aggregates are complexes that have a molecular weight greater than the isolated virus particle.

A formulation which improves the adenoviral stability, also named a “stable formulation” as used herein is a formulation in which the adenovirus therein essentially retains its physical and/or chemical integrity and/or biological activity upon storage. Stability can be assessed by determining different characteristics such as the quantity (of adenovirus in a formulation), the potency, and/or other quality aspects of the adenovirus in the formulation over a period of time and under certain storage conditions. These characteristics of an adenovirus formulation can be measured at elevated temperatures (predictive for real-time temperatures) or under other stress conditions, for instance formulations can be subjected to incubation at 25˚C or subjected to freeze/thaw cycles and agitation in order to study effects of different formulations maximizing shelf-life. Said characteristics which determine the stability may be determined by at least one of the methods selected from the group consisting of visual inspection, virus particle quantitative polymerase chain reaction (vp-QPCR), QPCR-based Potency Assay (QPA), Reverse Phase High Performance Liquid Chromotography (RP-HPLC) and Differential Centrifugal Sedimentation (DCS), Thermal Melting Assay (TMA), Turbidimetry, and Intrinsic Fluorescence.

Virus particle quantitative polymerase chain reaction (vp-QPCR)

The vp-QPCR was developed for the quantification of adenovirus particles using primers that target a 100 bp region of the CMV promoter of the transgene cassette present within the adenovirus vector. Briefly, this QPCR method relies on the exonuclease activity of Taq polymerase, which results in degradation of a specific fluorescent probe annealed in the middle of the 100 bp amplicon. The probe is covalently linked to a light emitter and a

quencher, and its degradation frees the emitter from the quencher with a consequent fluorescence emission proportional to the amount of template. Quantitative values are obtained from the threshold cycle (Ct), the cycle at which an increase in fluorescence signal exceeds a threshold value. The threshold for detection of DNA-based fluorescence is set slightly above background. The number of cycles at which the fluorescence exceeds the threshold is called the threshold cycle (Ct) or, according to the MIQE guidelines, quantification cycle (Cq) (Bustin et al, 2009). During the exponential amplification phase, the target DNA sequence doubles every cycle. For example, a DNA sample of which the Ct precedes that of another sample by 3 cycles contained $2^3 = 8$ times more template. Consequently, a higher Ct value represents a lower amount of target DNA and a lower Ct value represents a high availability of target DNA. Absolute quantification can be performed by comparing a standard curve generated by a serial dilution of a stock adenovirus of which the concentration has been determined by the optical density at 260 nm (OD_{260}). The Ct values of the test material are plotted against the Ct values of the standard curve, which generates an accurate and precise number of vector particles.

When used as readout after incubation on E1 competent cells (QPA, see below), more degraded samples will lead to higher delta (t=0 subtracted) Ct values and more stabilizing formulations will lead to lower Ct values.

QPCR-based Potency Assay (QPA)

To quantify adenovirus potency, the QPA combines QPCR with a tissue culture-based infectivity assay. The assay is based on the experimental observation that the appearance of newly synthesized viral DNA is very rapid after inoculation of a cell-monolayer, and is proportional to the virus input concentration over a large range of multiplicity of infection (MOI). Dilutions of samples (non-endpoint diluted) are inoculated onto HEK293 cell monolayers in a 96-well plate. The infection is allowed to proceed for 3 hours at 35°C. Wells are aspirated and replenished with medium that does not contain adenoviruses. Plates are incubated for an additional 42 hours prior to cell lysis by means of Triton X-100 solution and a single freeze/ thaw step in order to release adenovirus DNA. A QPCR is performed on diluted cell lysates according to the method described above. The infectivity titer is calculated by comparison to a standard curve generated by the Ct values of a sample of known

infectivity, which is determined by endpoint titration. Alternatively, the delta potency can be expressed directly as Ct values since the infectivity titer, or potency, is directly correlated to the Ct values. Especially in comparing relative differences in potency between formulations, this is a quick and reliable method.

Reverse Phase High Performance Liquid Chromatography (RP-HPLC)

In order to determine some quality aspects of an adenovirus, one can analyze adenoviral protein profiles by Reverse Phase High Performance Liquid Chromatography (RP-HPLC). HPLC separates components of a mixture by using a variety of chemical interactions between the sample, the mobile phase (a buffer or solvent) and the stationary phase (a chromatographic packing material in a column). A high-pressure pump moves the mobile phase through the column and a detector shows the retention times (t_R; time between sample injection and the appearance of the peak maximum) of the molecules using UV absorbance detection at 280 nm. The separation of RP-HPLC is based on differences in hydrophobicity. The non-polar stationary phase is made up of hydrophobic alkyl chains (chain lengths: C4, C8 and C18). The polar mobile phase is water with 0.1% trifluoroacetic (TFA). Compounds that bind to the columns are eluted using an increasing concentration of acetonitrile with 0.1% TFA. In general, an analyte with a larger hydrophobic surface area has a longer retention time, whereas the presence of polar groups reduce retention time. A typical adenoviral RP-HPLC profile consists of 10 or 14 proteins, including core protein (VII), penton base (III) and hexon (II).

Differential Centrifugal Sedimentation (DCS)

DCS is a method to measure particle size distributions (aggregation) by sedimentation. In a disc centrifuge, particles settle in a sucrose gradient (of known viscosity and density) under high gravitational forces according to Stokes' law. Sedimentation velocity increases with the square of the particle diameter, so particles that differ in size by only a few percent settle at significantly different rates. The time needed to reach the detector is used to calculate the size of the particles. The measurement range for this method is about 0.02 to 30 microns.

Thermal melting assay (TMA)

The thermal melting assay (TMA) can be used to determine the melting temperature (T_m) of adenovirus in experimental formulations, which is the temperature where the viral capsid denatures. This viral disintegration can be measured real-time using a dsDNA intercalating fluorescent dye. This fluorescent dye only gives a fluorescence signal when bound to DNA, which is released when the viral particle disintegrates. The exponential fluorescence increase upon capsid melting can be measured using a common QPCR machine during a stepwise increase in temperature. Samples are diluted to the same concentration (range is $4x10^9$ to $1x10^{12}$ vp/mL) in the specific formulations and mixed with SYBRGreen dye (1X final concentration) in a volume of 50 µL. The temperature was increased 0.5°C per 30 seconds starting from 30°C up to 79°C. From the fluorescent raw data first and second derivatives are calculated and melting temperature is read at the intercept of the second derivative with the x-axis. Higher melting temperatures (T_m) may be indicative of a more stabilizing formulation.

An adenovirus "retains its physical stability" in a pharmaceutical formulation, if it, amongst others, shows minimal loss (i.e. 0.3log/2 years) in terms of quantity and potency, and displays no major protein modifications. Additionally, no signs of aggregation, precipitation, change of colour and/or clarity upon visual examination should be observed.

"About" as used in the present application means ±10%, unless stated otherwise.

The present invention relates to formulations which stabilize an adenovirus and to related pharmaceutical products, preferably for use in gene therapy and/or vaccine applications. A preferred stabilized virus containing formulation disclosed herein is a liquid adenovirus formulation, which shows improved adenoviral-stability when stored in about the 2-8°C range while also being compatible with parenteral administration. These pH-stable formulations can however also be stored at lower temperatures, e.g. -20°C or lower, -40°C or lower, -65°C or lower, -80°C or lower. They may also be more stable at temperatures above 8°C, e.g. 25°C or even higher.

These formulations which are able to stabilize an adenovirus comprise histidine as buffer, trehalose as a cryoprotectant, a salt, a non-ionic detergent, as well as optional additional components which enhance stability to the added virus, including but not limited to

a two or four carbon alcohol, EDTA and/or EDTA. The pH of said buffer lies between 6 and 7.

The formulations of the present invention provide stability to adenoviruses at varying virus concentrations, mono- or multivalent, and may be administered to a variety of vertebrate organisms, preferably mammals and especially humans. The stabilized viral formulations of the present invention are adenoviral-based compositions, which can, for instance, be administered as a vaccine that may offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect.

A preferred aspect of the invention is a formulation for recombinant adenoviruses (i.e., an adenovirus containing a whole or a portion of a transgene which is expressed within the target host subsequent to host administration, such as in any mammalian/human gene therapy- or gene vaccination-based methodology available to the skilled artisan) which shows enhanced stability characteristics described herein with a virus concentration in the range from about $1x10^7$ vp/mL (virus particles/mL) to about $1x10^{13}$ vp/mL. A more preferred range is from about $1x10^9$ to about $lxl0^{l3}$ vp/mL, with an especially preferred virus concentration being from about $1x10^{10}$ to about $5xl0^{l2}$ vp/mL. Therapeutic or prophylactic compositions of the formulations of the present invention can be administered to an individual in amounts sufficient to treat or prevent the respective disorder. The effective amount for human administration may, of course, vary according to a variety of factors such as the individual's condition, weight, sex and age. Other factors include the mode of administration. In a preferred embodiment, the formulations of the present invention are suitable for parenteral use.

The formulations of the present invention are histidine buffered solutions having a pH ranging between 6 and 7, and further comprise trehalose, a salt and a non-ionic detergent (also named surfactant). Unexpectedly said combination has proven to be an outstanding formulation for the preservation of quantity, potency (infectivity) and quality of adenoviruses, as demonstrated herein.

In a preferred embodiment, the concentration of histidine is ranging between about 5 mM and about 30 mM, e.g. between about 10 mM and about 25 mM, e.g. between about 15 mM and about 25 mM, e.g. about 20 mM.

Another essential component in these formulations which contributes to virus stabilization over large temperature ranges and for prolonged storage periods is trehalose, which is used as a cryoprotectant. In a preferred embodiment, the concentration of trehalose is ranging between about 1% (w/w) to about 10% (w/w), e.g. between about 3% (w/w) to about 8% (w/w), e.g. between about 4% (w/w) to about 6% (w/w), e.g. about 5% (w/w).

An additional component of the formulations of the present invention is salt. Salt enhances viral stability. A purpose of inclusion of a salt in the formulation is to attain the desired ionic strength or osmolality and additionally optimize electrostatic interactions. Salt is present at an ionic strength which is physiologically acceptable to the host. Contributions to ionic strength may come from ions produced by the buffering compound as well as from the ions of non-buffering salts. Salts that are appropriate for the formulations of the present invention include but are not limited to sodium chloride (NaCl), Calcium chloride ($CaCl_2$) or manganese chloride ($MnCl_2$). Magnesium chloride ($MgCl_2$) was shown to be detrimental to adenoviral-stability. Therefore, in a preferred embodiment the formulation according to the present invention is free from magnesium chloride.

In a preferred embodiment, the virus formulation according to the present invention comprises sodium chloride (NaCl). In a preferred embodiment, the concentration of sodium chloride is ranging between about 10 mM and about 250 mM, e.g. between about 20 mM and about 200 mM, e.g. between about 30 mM and about 150 mM, e.g. between about 50 mM and about 100 mM, e.g. about 75 mM.

The formulations of the present invention comprise at least one non-ionic detergent (also named non-ionic surfactant) added to reduce adsorption to container surfaces as well as possibly providing increased virus stabilization. Non-ionic detergents for use in the formulations of the present invention include but are not limited to polyoxyethylene sorbitan fatty acid esters, including but not limited to Polysorbate-80 (Tween 80®), Polysorbate-60 (Tween 60®), Polysorbate-40 (Tween 40®) and Polysorbate-20 (Tween 20®), and the Pluronic series of non-ionic surfactants (e.g. Pluronic 121).

In a preferred embodiment, the concentration of non-ionic detergent is ranging between about 0.001% (w/w) to about 1% (w/w), e.g. between about 0.005% (w/w) to about 0.5% (w/w), e.g. between about 0.01% (w/w) to about 0.1% (w/w), e.g. between about 0.01%

(w/w) to about 0.05% (w/w), e.g. between about 0.015% (w/w) to about 0.03% (w/w), e.g. about 0.02% (w/w).

In a preferred embodiment, the virus formulation according to the present invention comprises Polysorbate-80. The concentration of Polysorbate-80 is preferably ranging between about 0.001% (w/w) to about 1% (w/w), e.g. between about 0.005% (w/w) to about 0.5% (w/w), e.g. between about 0.01% (w/w) to about 0.1% (w/w), e.g. between about 0.01% (w/w) to about 0.05% (w/w), e.g. between about 0.015% (w/w) to about 0.03% (w/w), e.g. about 0.02% (w/w).

In a preferred embodiment, the virus formulation according to the present invention further comprises EDTA. In a more preferred embodiment, the concentration of EDTA is ranging between about 0.05 mM to about 0.2 mM, e.g. between about 0.05 mM to about 0.15 mM, e.g. between about 0.08 mM to about 0.12 mM, e.g. about 0.1 mM.

In another preferred embodiment, instead of EDTA, the virus formulation according to the present invention further comprises ethanol. In a more preferred embodiment, the concentration of ethanol is ranging between about 0.1% (w/w) to about 1% (w/w), e.g. between about 0.2% (w/w) to about 0.8% (w/w), e.g. between about 0.2% (w/w) to about 0.6% (w/w), e.g. about 0.4% (w/w).

In a preferred embodiment, when the virus formulation according to the present invention comprises ethanol it must not comprise EDTA at the same time.

In view of the discussion above, the present invention relates to a formulation containing an adenovirus, such as a recombinant Ad5, Ad26 or Ad35, that can e.g. be used in gene therapy and/or vaccination applications, which show improved stability properties as compared to the best performing formulation known in the art (disclosed in Evans et al. 2004) and which at least contains histidine as buffer, trehalose as a cryoprotectant, a salt and a surfactant.

A particular embodiment of the present invention relates to such a recombinant adenovirus formulation which is buffered with histidine to a pH ranging between 6 and 7, and further comprises trehalose, a salt, a non-ionic detergent, and optionally a 2 or 4-carbon alcohol.

In a preferred embodiment according to the present invention, the formulation comprises histidine as buffer with a pH ranging from about pH 6.2 to about pH 6.8, comprises

trehalose as the cryoprotectant, NaCl as the salt, Polysorbate-80 as the surfactant and optionally a 2- or 4-carbon alcohol as an additional cryoprotectant.

In another preferred embodiment according to the present invention, the formulation comprises histidine as buffer with a pH ranging from about pH 6.2 to about pH 6.8, comprises trehalose as the cryoprotectant, NaCl as the salt, Polysorbate-80 as the surfactant and EDTA.

In another preferred embodiment according to the present invention, the formulation comprises histidine as buffer with a pH ranging from about pH 6.2 to about pH 6.8, comprises trehalose as the cryoprotectant, NaCl as the salt, Polysorbate-80 as the surfactant and ethanol.

In another preferred embodiment according to the present invention, the formulation comprises histidine as buffer with a pH ranging from about pH 6.2 to about pH 6.8, comprises trehalose as the cryoprotectant, NaCl as the salt, Polysorbate-80 as the surfactant. This formulation further comprises ethanol and is free of EDTA.

In another preferred embodiment according to the present invention the formulation has a pH ranging between about 6.2 and about 6.8, and comprises histidine at a concentration ranging between about 15 and about 25 mM; trehalose at a concentration ranging between about 3% (w/w) and about 8% (w/w); NaCl at a concentration ranging between about 50 mM and about 100 mM; Polysorbate-80 at a concentration ranging between about 0.01% (w/w) and about 0.03% (w/w); and EDTA at a concentration ranging between about 0.05 mM to about 0.15 mM.

In an even more preferred embodiment of the present invention the formulation is buffered with about 20 mM histidine to a pH of about 6.6; trehalose is present at a concentration of about 5% (w/w); NaCl is present at a concentration of about 75 mM; the surfactant is Polysorbate-80 at a concentration of about 0.02% (w/w); and EDTA is present at a concentration of about 0.1 mM.

In another preferred embodiment according to the present invention, the formulation comprises histidine as buffer with a pH ranging from about pH 6.2 to about pH 6.8, comprises trehalose as the cryoprotectant, NaCl as the salt, Polysorbate-80 as the surfactant and a combination of ethanol/EDTA.

In another preferred embodiment according to the present invention the formulation has a pH ranging between about 6.2 and about 6.8, and comprises histidine at a concentration

ranging between about 15 and about 25 mM; trehalose at a concentration ranging between about 3% (w/w) and about 8% (w/w); NaCl at a concentration ranging between about 50 mM and about 100 mM; Polysorbate-80 at a concentration ranging between about 0.01% (w/w) and about 0.03% (w/w); EDTA at a concentration ranging between about 0.05 mM and about 0.15 mM; and ethanol at a concentration ranging between about 0.2% (w/w) and about 0.6% (w/w).

In another preferred embodiment of the present invention the formulation is buffered with about 20 mM histidine to a pH of about 6.6; trehalose is present at a concentration of about 5% (w/w); NaCl is present at a concentration of about 75 mM; the surfactant is Polysorbate-80 at a concentration of about 0.02% (w/w); EDTA at a concentration of about 0.1 mM; and ethanol is present at a concentration of about 0.4% (w/w).

In an even more preferred embodiment of the present invention the formulation is buffered with about 20 mM histidine to a pH of about 6; trehalose is present at a concentration of about 5% (w/w); NaCl is present at a concentration of about 75 mM; the surfactant is Polysorbate-80 at a concentration of about 0.02% (w/w); and ethanol is present at a concentration of about 0.4% (w/w). Additionally, combinations of the above mentioned factors can be used.

In one embodiment the formulations according to the present invention are contained in a vial such as e.g. DIN 2R type I borosilicate glass vial. In another embodiment, the formulations are contained in a bag. Bags that contain the formulations of the present invention may comprise layers made of e.g. Ethylene Vinyl Acetate Copolymer (EVA) or Ethyl Vinyl Alcohol (EVOH). In yet another embodiment of the present invention, the formulations are contained in a syringe.

The recombinant virus formulations described herein can be administered to the vertebrate host (preferably a mammalian host and especially a human recipient) by any means known in the art. These routes of delivery include but are not limited to intramuscular injection, intraperitoneal injection, intravenous injection, inhalation or intranasal delivery, oral delivery, sublingual administration, subcutaneous administration, transdermal administration, intradermal administration, intraductal salivary gland administration, transcutaneous administration or percutaneous administration. In a preferred embodiment, the formulation of the present invention is compatible with parenteral administration.

In accordance with the formulations disclosed herein, the present invention also relates to methods of preserving an adenovirus which comprise preparing virus containing formulations as disclosed herein, such formulations which result in improved viral stability when stored below -65˚C and in about the 2-8 °C range and possibly higher while also being compatible with parenteral administration, especially parenteral administration to humans.

Another aspect of the present invention therefore relates to methods of preserving an adenovirus which comprise preparing a formulation as disclosed herein and storing said formulation at a temperature ranging between 2°C and 8°C.

The following examples are provided to illustrate the present invention without, however, limiting the same hereto.

Example 1

Experimental design and methodology

After having tested several different formulations, one formulation outperformed most of the others. This new formulation was named "formulation D" and comprises 5% (w/w) Trehalose, 20 mM Histidine, 0.02% (w/w) PS-80 and 75 mM NaCl at a pH of 6.6.

Two Adenoviral (Ad35.TB-S and Ad26.Mos1.Gag-Pol) preparations (one comprising a serotype 35 adenovirus (Ad35) and one comprising a serotype 26 adenovirus (Ad26)) have been buffer-exchanged using PD-10 columns (GE Healthcare) into formulation D.

Both adenoviral preparations have also been formulated in a "control formulation" which was described in Evans et al. 2004 and which was the best formulation available thus far. Said "control formulation" comprises 10 mM Tris, 10 mM Histidine, 1 mM $MgCl_2$, 75 mM NaCl, 5% (w/w) sucrose, 0.02 % (w/w) PS-80, 0.1 mM EDTA, 0.4 % (w/w) EtOH, at a pH of 7.4.

Per formulation, 12 columns were used; eluates were pooled, sterile filtrated and stored at 2-8 °C in a glass bottle. Samples were taken for viral titer determination by vp-QPCR and all titers were adjusted with the appropriate buffer to $1.7x10^{11}$ vp/mL. Subsequently, the formulations were filled into glass vials (0.7 mL per vial), stoppered and capped.

The t=0 samples (control, 6 vials per group) were stored directly at ≤-65°C. Subsequently, six vials per group (n=6) were incubated at 25°C and frozen at ≤-65°C at t=10,

20, 30, 40, 50, 65, 70, 75, 80 and 90 days until sample analysis by QPA was performed in triplicate per sample.

In addition, t=0 samples for vectors in both formulations were analyzed by TMA (Thermal Melting Assay) and DLS to determine the capsid melting temperature and onset of aggregation, respectively. A higher melting temperature correlates to higher thermal stability of the capsid. A later onset of aggregation by DLS resembles a more stabilizing formulation in terms of preventing aggregation. End point samples (t=90 days) were also analyzed by Reverse Phase High Performance Liquid Chromatography (RP-HPLC) and Differential Centrifugal Sedimentation (DCS) and compared to t=0 controls.

Results and conclusion

After the completion of the study, all samples were analyzed by QPA and the loss in potency was expressed as deltas by subtracting the t=0 values. The formulation according to the present invention (open squares) significantly (p=1.22E-07) outperformed the control formulation (closed circles) leading to less degradation over time and a longer predicted shelf life (Table 1) for both adenoviruses (Figures 1 and 2). With *Statistical Analysis System* (SAS software), linear (Ad26) and quadratic (Ad35) models were fitted on the potency data using time and the square of the time as fixed effects. While the fixed 'time' effect represents the linear decrease in potency, the fixed 'time*time' effect represents the curvature of this decrease. Both effects were used in the model to evaluate their significance. The Akaike Information Criterion (AIC) is a measure for the relative quality of the statistical model, taking into account the complexity of the model and its accuracy of fit. The smaller the AIC, the better the model fits. In the linear model the slope represents the potency degradation rate. By comparing slopes, the best buffer (lowest slope) can be identified.

Shelf lives correspond to the time points for which the lower limit of this interval pass under a given specification (i.e. decrease in potency). For the quadratic model, to compute shelf life, the two-sided 95% confidence intervals around the mean were taken. Keeping only the lower bound for the shelf life assessment leads to a 97.5% confidence level for the univariate lower confidence interval. The intercepts (reflecting potency at t=0) were averaged and removed from the raw data and the model. The shelf lives were computed for both formulations and expressed as percentage difference compared to control.

Table 1: Shelf lives at 25°C derived from statistical analysis of degradation curves (Figures 1 and 2).

	Formulation D compared to control
Ad26-shelf life	73.6% longer
Ad35-shelf life	60.9% longer

The RP-HPLC did not show any signs of adenovirus protein modifications nor oxidation (data not shown). The DCS did not reveal any signs of aggregation (data not shown). In line with the previous data, the formulation according to the present invention led to a significantly increased melting temperature for both Ad26 (Figure 3) and Ad35 (Figure 4) compared to the control formulation, indicating increased thermal stability of the adenoviruses in formulation D.

With regard to aggregation as a degradation pathway, the onset of aggregation was determined by DLS during a stepwise temperature increase in Formulation D and control formulation. It was clearly demonstrated that the onset of aggregation was later in Formulation D compared to the control formulation for both Ad26 (Figure 5) and Ad35 (Figure 6). This clearly indicates that formulation D is better in stabilizing Ad26 and Ad35 in terms of delaying product aggregation.

Importantly, the VP/IU ratio, reflecting the infectious proportion of the viral preparation (indicative of the quality of the virus particles), revealed considerably higher values (less infectious particles per total amount of particles) in the control formulation compared to Formulation D after exposure to accelerated temperature (25°C) for both Ad26 and Ad35, see Table 2. This shows that formulation D is able to preserve the infectivity of adenoviruses in a much greater way as compared to the control formulation. Therefore formulation D is the ideal liquid formulation for adenoviruses.

Table 2: VP/IU ratios for Formulation D and control formulation at t=0 and t=90 at 25°C. Higher values represent more degradation and less infectious virus particles relative to the total amount of virus particles.

Ad26	*Unstressed*	*Stressed*
Control Formulation	36.3	208.9
Formulation D	34.7	75.9
Ad35		
Control Formulation	8.9	562.3
Formulation D	9.3	36.3

Example 2

Experimental design and methodology

A new formulation, resulting from the addition of 0.4 % (w/w) Ethanol to Formulation D, was also tested. This formulation named "optimal formulation" comprises 5% (w/w) Trehalose, 20 mM Histidine, 0.02% (w/w) PS-80, 75 mM NaCl, and 0.4% (w/w) Ethanol at a pH of 6.6.

A serotype 35 adenovirus preparation (Ad35.TB-S) has been buffer-exchanged using PD-10 columns (GE Healthcare) into the optimal formulation and into a "control formulation". The control formulation was described in Evans et al. 2004 and was the best formulation available thus far.

The eluates of the buffer-exchanged preparations were pooled, sterile filtrated and stored at 2-8 °C in a glass bottle. Samples were taken for viral titer determination by vp-QPCR. Titers were adjusted with the appropriate buffer to $2.4x10^{11}$ vp/mL. Subsequently, the formulations were filled into glass vials (0.7 mL per vial), stoppered and capped. The t=0 samples (control, 6 vials per group) were stored directly at ≤-65°C. Subsequently, six vials per group (n=6) were incubated at 25°C and frozen at ≤-65°C at t=30, 60, 75, and 90 days until sample analysis. Analysis by QPA was performed in four replicates per sample.

Results and conclusion

After the completion of the study, all samples were analyzed by QPA and the loss in potency was expressed as deltas by subtracting the t=0 values. The optimal formulation (open squares), derived from the addition of 0.4% (w/w) ethanol to Formulation D, clearly outperformed the control formulation (closed circles) leading to less degradation over time for Ad35 (Figure 7).This new optimal formulation unexpectedly outperformed the formulations as described in Evans et al, providing increased heat stability to an adenovirus and therewith allowing for a longer shelf life of the adenovirus composition at a refrigerated storage temperature.

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CLAIMS

1. A formulation for adenoviruses comprising:

a) a recombinant adenovirus;

b) a histidine buffered solution;

c) trehalose;

d) a salt;

e) a non-ionic detergent;

wherein said formulation has a pH ranging between 6 and 7.

2. A formulation according to claim 1, wherein the histidine concentration is ranging between about 5 mM and about 30 mM.

3. A formulation according to claim 1 or 2, wherein the concentration of trehalose is ranging between about 1% (w/w) to about 10% (w/w).

4. A formulation according to any one of claims 1-3, wherein the salt is sodium chloride.

5. A formulation according to claim 4, wherein the sodium chloride concentration is ranging between about 20 mM to about 200 mM.

6. A formulation according to any one of claims 1-5, wherein the non-ionic detergent is Polysorbate-80.

7. A formulation according to claim 6, wherein the Polysorbate-80 concentration is ranging between about 0.005% (w/w) to about 0.5% (w/w).

8. A formulation according to any one of claims 1-7, wherein said formulation has a pH ranging between about 6.2 and about 6.8, and comprises histidine at a concentration ranging between about 15 and about 25 mM; trehalose at a concentration ranging between about 3% (w/w) and about 8% (w/w); NaCl at a concentration ranging between about 50 mM and about 100 mM; Polysorbate-80 at a concentration ranging between about 0.01% (w/w) and about 0.05% (w/w).

9. A formulation according to any one of claims 1-8, at pH 6.6, which comprises histidine at a concentration of about 20 mM; trehalose at a concentration of about 5% (w/w); NaCl at a concentration of about 75 mM and Polysorbate-80 at a concentration of about 0.02% (w/w).

10. A formulation according to any one of claims 1-9, wherein the formulation further comprises a stabilizer selected from the group consisting of a 2-carbon alcohol, a 4-carbon alcohol, EDTA and a combination of ethanol/EDTA.

11. A formulation according to claim 10, wherein the stabilizer is a 2 or 4-carbon alcohol.

12. A formulation according to claim 11, wherein the 2 or 4-carbon alcohol is ethanol.

13. A formulation according to claim 12, wherein the ethanol concentration is ranging between about 0.1% (w/w) to about 1% (w/w).

14. A formulation according to any one of claims 10-13, wherein said formulation has a pH ranging between about 6.2 and about 6.8 , and comprises histidine at a concentration ranging between about 5 and about 30 mM; trehalose at a concentration ranging between about 1% (w/w) and about 10% (w/w); NaCl at a concentration ranging between about 20 mM and about 200 mM; Polysorbate-80 at a concentration ranging between about 0.01% (w/w) and about 0.05% (w/w); and ethanol at a concentration ranging between about 0.2% (w/w) and about 0.6% (w/w).

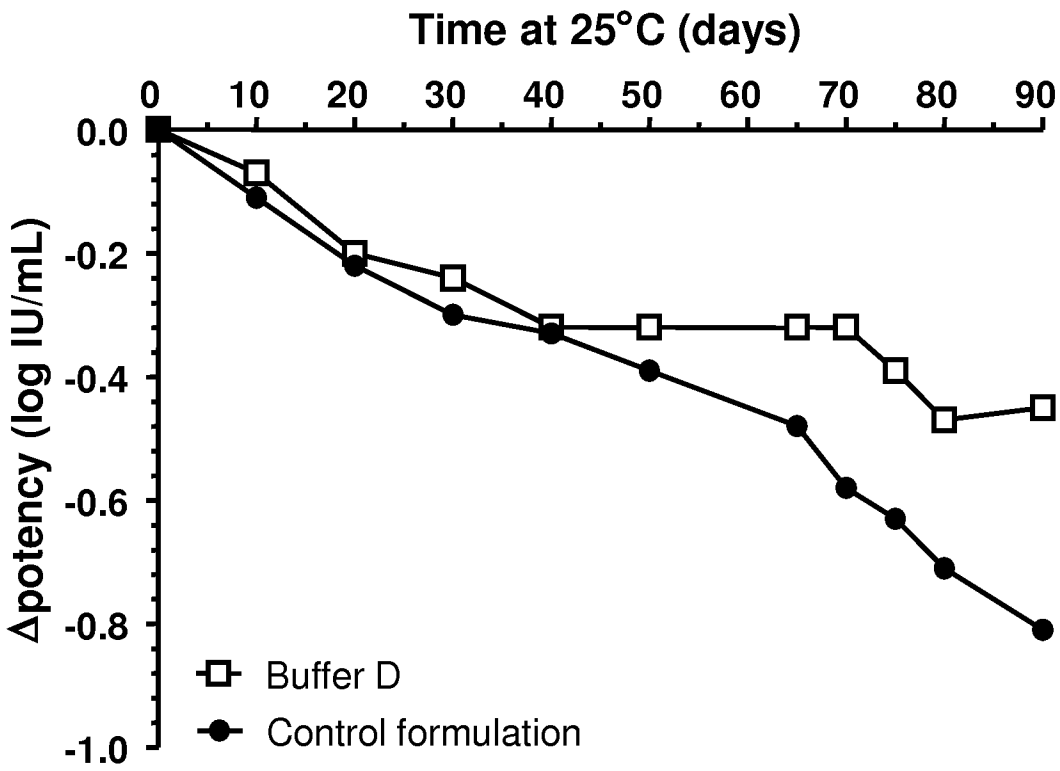
15. A formulation according to claim 14, wherein said formulation has a pH of about 6.6, and comprises histidine at a concentration of about 20 mM; trehalose at a concentration of about 5% (w/w); NaCl at a concentration of about 75 mM, Polysorbate-80 at a concentration of about 0.02% (w/w) and ethanol at a concentration of about 0.4% (w/w).

16. A formulation according to any one of claims 1-15, wherein said formulation is a liquid formulation.

17. A formulation according to any one of claims 1-16, wherein said formulation is suitable for parenteral use.

18. A method of preserving an adenovirus which comprises preparing a formulation according to any one of claims 1-17.

19. A method of preserving an adenovirus which comprises preparing a formulation according to any one of claims 1-17 and storing said formulation at a temperature ranging between 2°C and 8°C.


Time at 25°C (days)
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10
20
30
40
50
60
70
80
90
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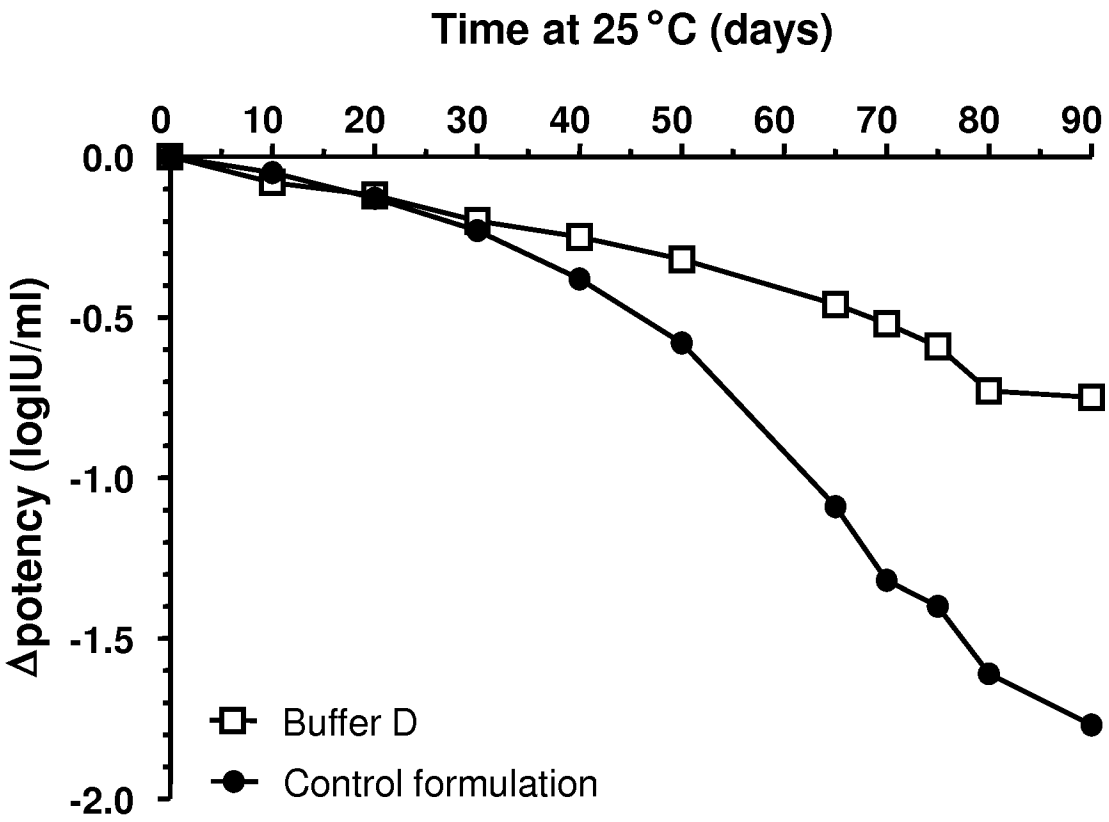
-0.4
-0.6
-0.8
-1.0
Δpotency (log IU/mL)
Buffer D
Control formulation

Fig. 1


Time at 25 °C (days)
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30
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50
60
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Δpotency (logIU/ml)
Buffer D
Control formulation

Fig. 2

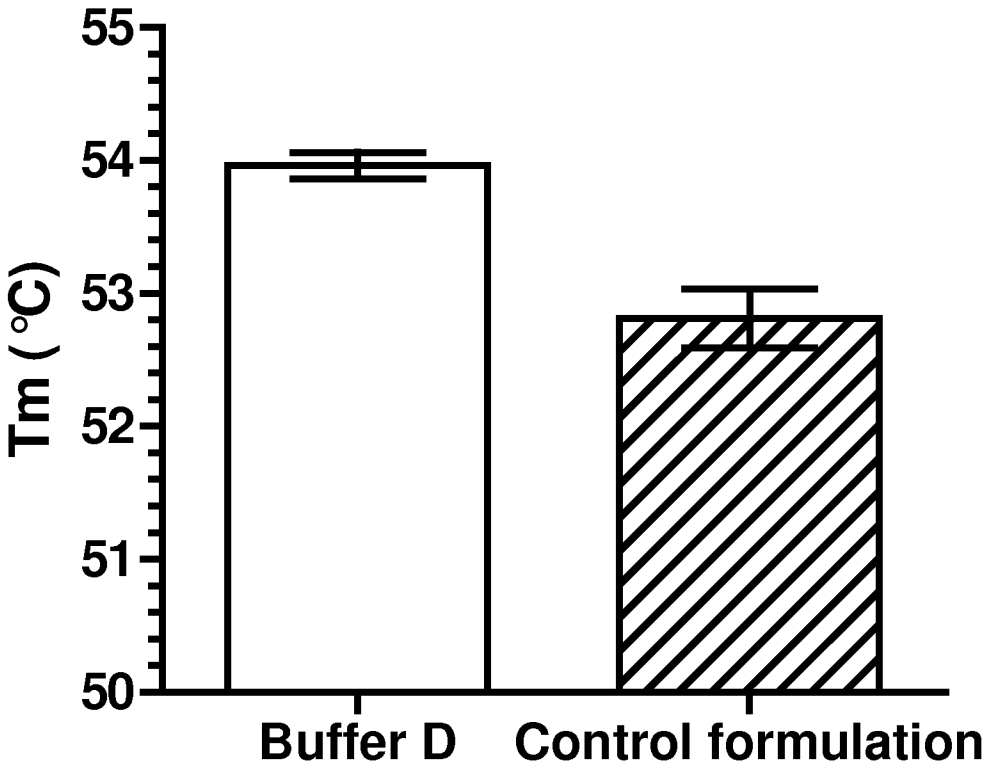


Fig. 3

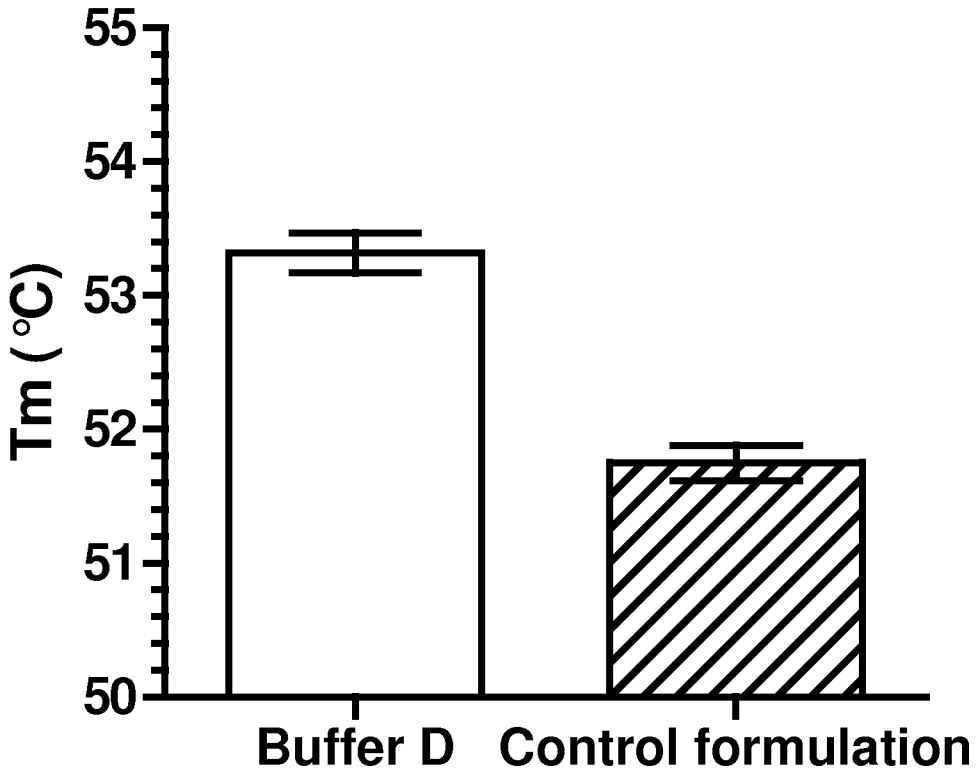
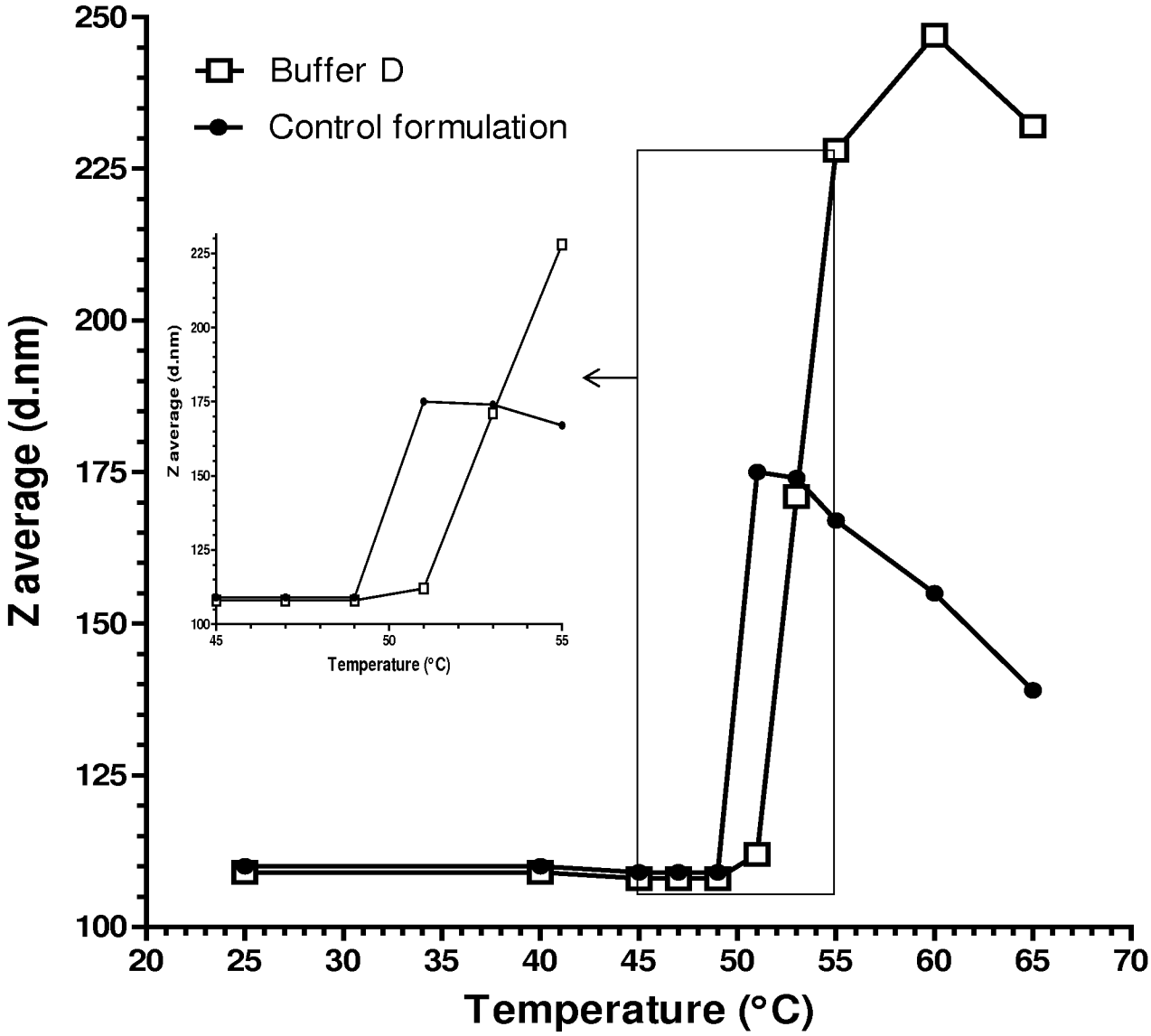


Fig. 4


Buffer D
Control formulation
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200
175
150
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35
40
45
50
55
60
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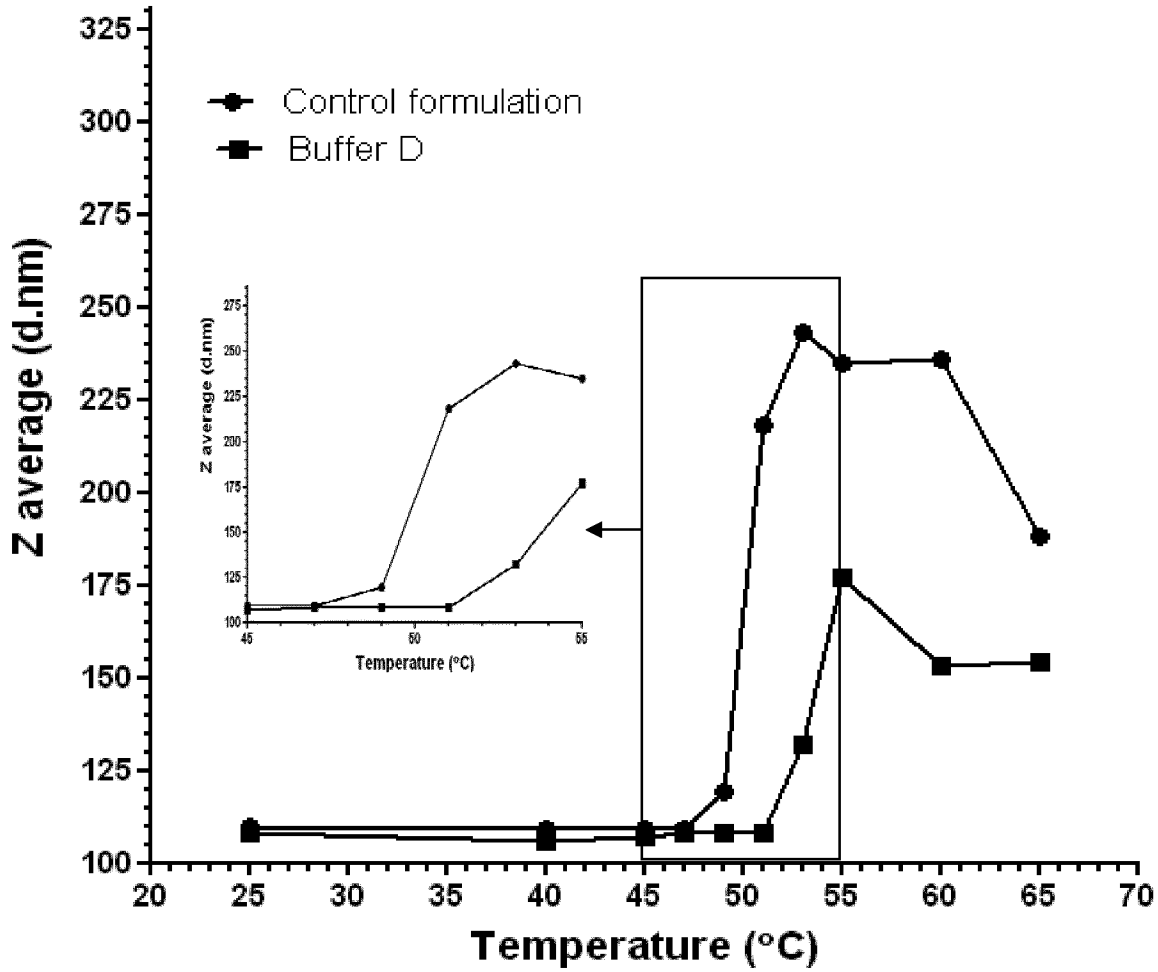
70
Z average (d.nm)
Temperature (°C)

Fig. 5


Control formulation
Buffer D
Z average (d.nm)
Temperature (°C)
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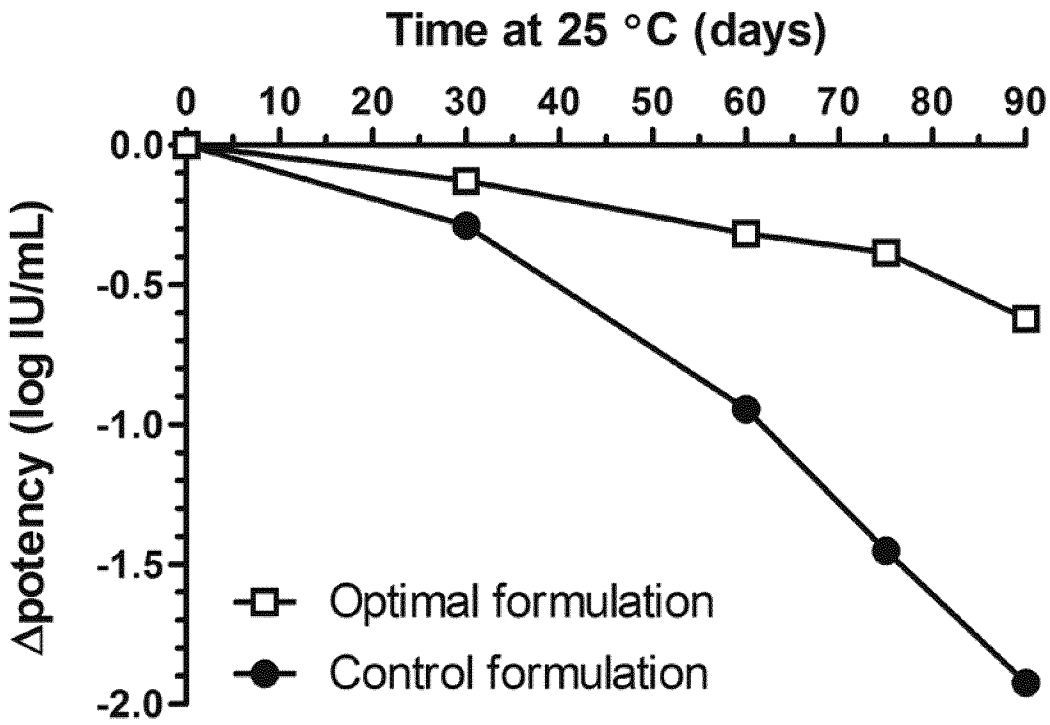
50
55
60
65
70
Z average (d.nm)
Temperature (°C)

Fig. 6


Time at 25 °C (days)
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10
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50
60
70
80
90
0.0
-0.5
-1.0
-1.5
-2.0
Δpotency (log IU/mL)
Optimal formulation
Control formulation

Fig. 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/070199

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N7/00 C12N15/861 A61K9/00 A61K47/02 A61K47/10
A61K47/18 A61K47/26 A61K9/08
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2005/186225 A1 (EVANS ROBERT K [US] ET AL) 25 August 2005 (2005-08-25)	1,2,4-7, 9-13, 16-19
Y	paragraph [0056]; claim 10; example 15	3,8,14, 15

Y	US 6 225 289 B1 (KOVESDI IMRE [US] ET AL) 1 May 2001 (2001-05-01) cited in the application claims 15-18	3,8,14, 15

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 December 2014	23/12/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Lonnoy, Olivier

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/070199

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CROYLE M A ET AL: "FACTORS THAT INFLUENCE STABILITY OF RECOMBINANT ADENOVIRAL PREPARATION FOR HUMAN GENE THERAPY", PHARMACEUTICAL DEVELOPMENT AND TECHNOLOGY, NEW YORK, NY, US, vol. 3, no. 3, August 1998 (1998-08), pages 373-383, XP000881617, ISSN: 1083-7450 abstract -----	1-19

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2014/070199

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2005186225 A1	25-08-2005	NONE	
US 6225289 B1	01-05-2001	AT 387492 T	15-03-2008
		AU 771112 B2	11-03-2004
		AU 2172400 A	26-06-2000
		CA 2351890 A1	15-06-2000
		DE 69938255 T2	12-02-2009
		DK 1137758 T3	09-06-2008
		EP 1137758 A2	04-10-2001
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