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(12) **United States Patent**
Wright et al.

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(54) **COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION**

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CPC ***C12N 7/00*** (2013.01); *C12N 2750/14151* (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,138,287	A *	2/1979	Andersson et al.	435/239
6,146,874	A	11/2000	Zolotukhin et al.	435/235.1
6,194,191	B1	2/2001	Zhang et al.	435/235.1
6,566,118	B1	5/2003	Atkinson et al.	435/239
6,593,123	B1	7/2003	Wright et al.	435/239
2004/0166122	A1 *	8/2004	Evans et al.	424/204.1

FOREIGN PATENT DOCUMENTS

WO	WO99/61643	12/1999

OTHER PUBLICATIONS

Vihinen-Ranta M. et al., Intracellular Route of Canine Parvovirus Entry, Journal of Virology, Jan. 1998, vol. 72, No. 1, pp. 802-806.*
Adadeyoh et al., "Short-Term Field Use and Shipping Stability Study of a Wild Type AD5 Adenoviral Reference Material," BioProcessing, 1(3):62-9 (2002).
Braun et al., "Protein Aggregates Seem to Play a Key Role Among the Parameters Influencing The Antigenicity of Interferon Alpha (IFN-Alpha) in Normal and Transgenic Mice," Pharm. Res. 14(10):1472-1478 (1997).
Chen et al., "Strategies to Suppress Aggregation of Recombinant Keratinocyte Growth Factor During Liquid Formulation Development," J. Pharm. Sci. 83(12):1657-1661 (1994).
Chenuaud et al., "Autoimmune Anemia in Macaques Following Erythropoietin Gene Therapy," Blood 103(9):3303-3304 (2004).
Croyle et al., "Development of Formulations That Enhance Physical Stability of Viral Vectors for Gene Therapy," Gene Therapy 8(17):1281-1290 (2001).
Drittanti et al., "Optimised Helper Virus-Free Production of High Quality Adeno-Associated Virus Vectors", The Journal of Gene Medicine, 3:59-71 (2001).
Flotte, T. R., "Immune Responses to Recombinant Adeno-Associated Virus Vectors: Putting Preclinical Findings Into Perspective," Human Gene Ther. 15(7):716-717 (2004).
Gao et al., "Erythropoietin Gene Therapy Leads to Autoimmune Anemia in Macaques," Blood 103(9):3300-3302 (2004).
High et al., "Human Immune Responses to AAV-2 Capsid May Limit Duration of Expression in Liver-Directed Gene Transfer in Human With Hemophilis β," Blood, 104(11):121a, Abstract No. 413 (2004).
Huang et al., "ADAAV Support High-Titer Production of RAAV But Not Stable," Mol. Therapy 1:S286 (2000).
Meijer et al., "Targeting of Drugs to the Liver," Sem. Liver Dis. 15(3):202-256 (1995).
Qu et al., "Evidence That Ionic Interactions Are Involved in Concentration-Induced Aggregation of Recombinant Adeno-Associated Virus," Molecular Therapy, 7(5):S348, abstract No. 901 (2003).
Qu et al., "Scaling Up Production of Recombinant AAV Vectors for Clinical Applications," Curr Opin Drug Disc Dev. 3(6):750-755 (2000).
Shire et al., "Challenges in The Development of High Protein Concentration Formulations," J. Pharm. Sci. 93(6):1390-1402 (2004).
Sommer et al., "Quantification of Adeno-Associated Virus Particles and Empty Capsids by Optical Density Measurement," Mol Ther 7(1):122-128 (2003).
Steinbach et al., "Assembly of Adeno-Associated Virus Type 2 Capsids in Vitro," J Gen. Virol. 78(6):1453-1462 (1997).
Wang, W., "Instability, Stabilization, and Formulation of Liquid Protein Pharmaceuticals," Int. J. Pharm. 185(2):129-188 (1999).
Won et al., "Stabilizers Against Heat-Induced Aggregation of RPR 114849, An Acidic Fibroblast Growth Factor (AFGF," Int. J. Pharm. 167:25-36 (1998).
Wright et al., "Recombinant Adeno-Associated Virus: Formulation Challenges and Strategies for a Gene Therapy Vector," Curr. Opin. Drug Disc. Dev. 6(2):174-178 (2003).

(Continued)

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(74) *Attorney, Agent, or Firm* — Roberta L. Robins; Robins Law Group

(57) **ABSTRACT**

Compositions and methods are provided for preparation of concentrated stock solutions of AAV virions without aggregation. Formulations for AAV preparation and storage are high ionic strength solutions (e.g. μ~500 mM) that are nonetheless isotonic with the intended target tissue. This combination of high ionic strength and modest osmolarity is achieved using salts of high valency, such as sodium citrate. AAV stock solutions up to 6.4×10^{13} vg/mL are possible using the formulations of the invention, with no aggregation being observed even after ten freeze-thaw cycles. The surfactant Pluronic® F68 may be added at 0.001% to prevent losses of virions to surfaces during handling. Virion preparations can also be treated with nucleases to eliminate small nucleic acid strands on virions surfaces that exacerbate aggregation.

6 Claims, 3 Drawing Sheets

(56) **References Cited**

OTHER PUBLICATIONS

Wright et al., "425. Formulation Development for AAV2 Vectors: Identification of Excipients That Inhibit Vector Aggregation," Mol. Ther. [Online] 9(S1):S163-S163 (2004).

Wright et al., "Identification of Factors That Contribute to Recombinant AAV2 Particle Aggregation and Methods to Prevent Its Occurrence During Vector Purification and Formulation," Mol. Ther. 12(1):171-178 (2005).

Xie et al., "Large-Scale Production, Purification and Crystallization of Wild-Type Adeno-Associated Virus-2," J. Virol. Methods 122(1):17-27 (2004).

Xie et al., "The Atomic Structure of Adeno-Associated Virus (AAV-2), A Vector for Human Gene Therapy," Proc. Natl. Acad. Sci. U.S.A. 99(16):10405-10410 (2002).

Zhen et al., "Infectious Titer Assay for Adeno-Associated Virus Vectors With Sufficient to Detect Single Infectious Events," Human Gene Ther. 15:709-715 (2004).

The Term "Purify" and "About", Merriam-Webster Online Dictionary, at the web-http://www.m-w.com, p. 1 and p. 2, Accessed on Nov. 30, 2006, Dec. 1, 2006.

The Definition of the Term "Ionic Strength", Answer.com, at the web-http://www.answers.com, p. 1, Accessed on Nov. 30, 2006.

* cited by examiner

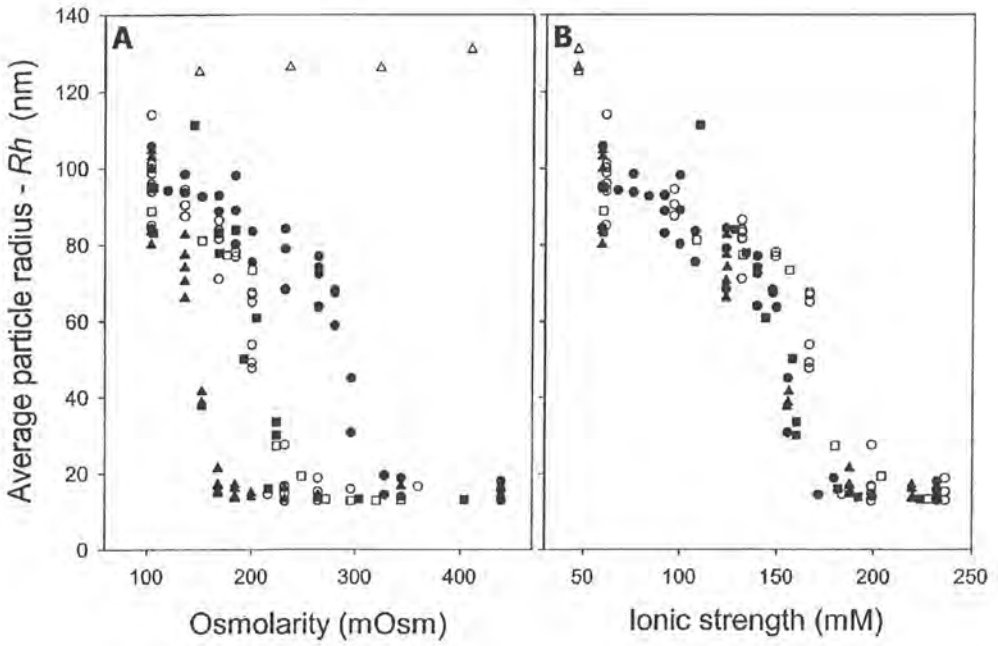

A
B
Average particle radius - Rh (nm)
0
20
40
60
80
100
120
140
100
200
300
400
Osmolarity (mOsm)
50
100
150
200
250
Ionic strength (mM)

FIGURE 1

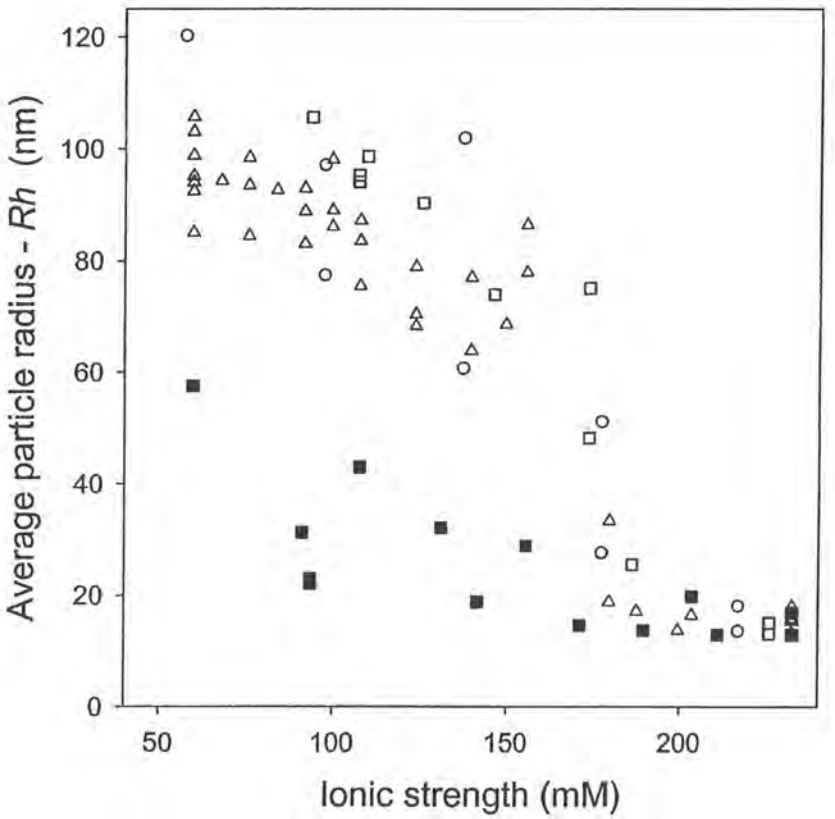

Average particle radius - Rh (nm)
120
100
80
60
40
20
0
50
100
150
200
Ionic strength (mM)

FIGURE 2

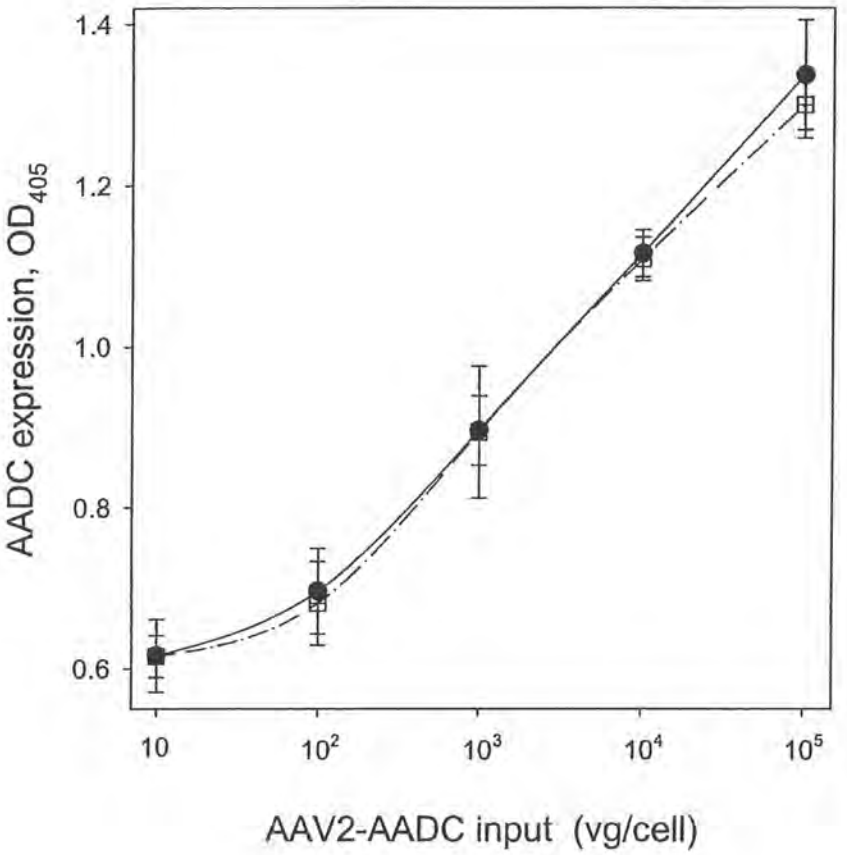

AADC expression, OD_{405}
1.4
1.2
1.0
0.8
0.6
10
10^2
10^3
10^4
10^5
AAV2-AADC input (vg/cell)

FIGURE 3

COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. application Ser. No. 11/141,996, now U.S. Pat. No. 7,704,721, from which application priority is claimed pursuant to 35 U.S.C. §120; which application claims the benefit under 35 U.S.C. §119(e) of provisional applications 60/575,997 filed Jun. 1, 2004 and 60/639,222 filed Dec. 22, 2004. The foregoing applications are hereby incorporated by reference in their entireties.

FIELD OF THE INVENTION

The present invention relates to compositions and methods of preparing and storing AAV virions that prevent aggregation.

BACKGROUND

Recombinant adeno-associated virus (rAAV) is a promising vector for human gene transfer. Grimm, D., and Kleinschmidt, J. A. (1999) *Hum Gene Ther.* 10: 2445-2450; High, K. A. (2001) *Ann. N.Y. Acad. Sci.* 953: 64-67; Pfeifer, A., and Verma, I. M. (2001) *Ann. Rev. Genomics Hum. Genet.* 2: 177-211. AAV is a member of the Dependovirus genus of the parvoviruses. AAV serotype 2 (AAV2) is composed of a single-strand DNA molecule of 4680 nucleotides encoding replication (rep) and encapsidation (cap) genes flanked by inverted terminal repeat (ITR) sequences. Berns, K. I. (1996) in *Fields Virology* (B. N. Fields et. al. Eds.), pp. 2173-2197. Lippincott-Raven Publishers, Philadelphia. The genome is packaged by three capsid proteins (VP1, VP2 and VP3), which are amino-terminal variants of the cap gene product. The resulting icosahedral virus particle has a diameter of ~26 nm. A high resolution crystal structure of AAV2 has been reported. Xie, Q. et al. (2002) *Proc. Natl. Acad. Sci. USA.* 99: 10405-10410.

The solubility of purified AAV2 virus particles is limited, and aggregation of AAV2 particles has been described as a problem. Croyle, M. A. et al. (2001) *Gene Therapy* 8: 1281-1290; Huang, J. et al. (2000) *Mol. Therapy* 1: 5286; Wright, J. F. et al. (2003) *Curr. Opin. Drug Disc. Dev.* 6: 174-178; Xie, Q. et al. (2004) *J. Virol. Methods* 122: 17-27. In commonly used buffered-saline solutions, significant aggregation occurs at concentrations of 10^{13} particles/mL, and aggregation increases at higher concentrations. Huang and co-workers reported that AAV vectors undergo concentration-dependent aggregation. Huang, J. et al. (2000) *Mol. Therapy* 1: S286. Xie and coworkers (Xie, Q. et al. (2004) *J. Virol. Methods* 122: 17-27) similarly reported that at concentrations exceeding 0.1 mg/mL, AAV2 vectors require elevated concentrations of salt to prevent aggregation. Aggregation of AAV2 vectors occurs at particle concentrations exceeding 10^{13} particles/mL in commonly used neutral-buffered solutions such as phosphate- and Tris-buffered saline. This corresponds to a protein concentration of ~0.06 mg/mL, and emphasizes the low solubility of AAV2 under these conditions. The effective vector concentration limit may be even lower for vectors purified using column chromatography techniques because excess empty capsids are co-purified and contribute to particle concentration.

Particle aggregation is a significant and not fully resolved issue for adenovirus vectors as well. Stability of a recently established adenovirus reference material (ARM) was recently reported. Adadevoh, K. et al. (2002) *BioProcessing* 1(2): 62-69. Aggregation of the reference material, formulated in 20 mM Tris, 25 mM NaCl, and 2.5% glycerol at pH 8.0, was assessed by dynamic light scattering, photon correlation spectroscopy and visual appearance. A variable level of vector aggregation following either freeze-thaw cycling or non-frozen storage was observed, resulting in restrictive protocols for the use of the ARM.

Aggregation can lead to losses during purification and inconsistencies in testing of purified vector preparations. The in vivo administration of AAV2 vectors to certain sites, such as the central nervous system, may require small volumes of highly concentrated vector, and the maximum achievable dose may be limited by low vector solubility.

Vector aggregation is also likely to influence biodistribution following in vivo administration, and cause adverse immune responses to vectors following their administration. As has been reported for proteins (Braun, A. et al. (1997) *Pharm. Res.* 14: 1472-1478), aggregation of vector may increase immunogenicity by targeting the vector to antigen presenting cells, and inducing enhanced immune responses to the capsid proteins and transgene product. The reports of immune responses to AAV vectors in pre-clinical (Chenuaud, P. et al. (2004) *Blood* 103: 3303-3304; Flotte, T. R. (2004) *Human Gene Ther.* 15: 716-717; Gao, G. et al. (2004) *Blood* 103: 3300-3302) and clinical (High, K. A. et al. (2004) *Blood* 104: 121a) studies illustrate the need to address all factors that may contribute to vector immunogenicity.

Testing protocols to characterize purified vectors are also likely to be affected by vector aggregation. Determination of the infectivity titer of vector was reported to be highly sensitive to vector aggregation. Zhen, Z. et al. (2004) *Human Gene Ther.* 15: 709-715. An important concern is that vector aggregates may have deleterious consequences following their in vivo administration because their transduction efficiency, biodistribution and immunogenicity may differ from monomeric particles. For example, intravascular delivery of AAV vectors to hepatocytes requires that the vectors pass through the fenestrated endothelial cell lining of hepatic sinusoids. These fenestrations have a radius ranging from 50 to 150 nm (Meijer, K. D. F., and Molema, G. (1995) *Sem. Liver Dis.* 15: 206) that is predicted to allow the passage of monomeric AAV vectors (diameter ~26 nm), but prevent the passage of larger vector aggregates. In biodistribution studies in mice, aggregated AAV2 vectors labeled with the fluorescent molecule Cy3 were sequestered in liver macrophages following vascular delivery. Huang, J. et al. (2000) *Mol. Therapy* 1: S286.

Formulation development for virus-based gene transfer vectors is a relatively recent area of investigation, and only a few studies have been reported describing systematic efforts to optimize AAV vector formulation and stability. Croyle, M. A. et al. (2001) *Gene Therapy* 8: 1281-1290; Wright, J. F. et al. (2003) *Curr. Opin. Drug Disc. Dev.* 6: 174-178; Xie, Q. et al. (2004) *J. Virol. Methods* 122: 17-27. Defining formulations compatible with pre-clinical and clinical applications that minimize changes in vector preparations is an important requirement to achieve consistently high vector safety and functional characteristics. As is well established for protein therapeutics (Chen, B. et al. (1994) *J. Pharm. Sci.* 83: 1657-1661; Shire, S. J. et al. (2004) *J. Pharm. Sci.* 93: 1390-1402; Wang, W. (1999) *Int. J. Pharm.* 185: 129-188; Won, C. M. et al. (1998) *Int. J. Pharm.* 167: 25-36), an important aspect of vector stability is solubility during preparation and storage, and vector aggregation is a problem that needs to be fully addressed. Vector aggregation leads to losses during vector purification, and while aggregates can be removed by filtration, the loss in yield results in higher costs and capacity

limitations when producing vector for pre-clinical and clinical studies. Even after filtration to remove aggregates, new aggregates can form in concentrated preparations of AAV2 vector in buffered-saline solutions.

The need exists for improved formulations and methods for purification and storage of AAV vectors, such as rAAV2, that prevent aggregation of virus particles.

SUMMARY OF THE INVENTION

These and other needs in the art are met by the present invention, which provides high ionic strength solutions for use in preparing and storing AAV vectors that maintain high infectivity titer and transduction efficiency, even after freeze-thaw cycles.

In one aspect the invention relates to methods of preventing aggregation of virions in a preparation of virions by adding excipients to achieve an ionic strength high enough to prevent aggregation. In another aspect the invention relates to compositions of virions having an ionic strength high enough to prevent aggregation.

In some embodiments of the invention, the ionic strength is at least about 150 mM, 200 mM, 250 mM, 300 mM, 350 mM, 400 mM, 450 mM, 500 mM, 600 mM, 700 mM or more. In some embodiments this ionic strength is accomplished using excipients comprising one or more multivalent ions, for example citrate, sulfate, magnesium or phosphate.

In additional embodiments, the osmolarity of the preparation of virions is maintained at near isotonic levels, for example 200 mOsm, 250 mOsm, 280 mOsm, 300 mOsm, 350 mOsm or 400 mOsm, even though the ionic strength is high enough to prevent virion aggregation.

In some embodiments the virions are adeno-associated virus (AAV) virions, for example AAV-2.

In other embodiments of the methods of the present invention preparations of virions are treated with a nuclease, for example Benzonase®. In further embodiments, nuclease treatment is combined with addition of excipients that achieve an ionic strength high enough to prevent aggregation.

In some embodiments of the present invention, the surfactant Pluronic® F68 is added to a preparation of virions, for example to 0.001%. In one embodiment, the composition comprises purified virus particles, 10 mM Tris pH 8.0, 100 mM sodium citrate and 0.001% Pluronic® F68.

In one embodiment, AAV vectors can be stored as compositions of the present invention at concentrations exceeding 1×10^{13} vg/mL, for example 2×10^{13}, 3×10^{13}, 4×10^{13}, 5×10^{13} and up to 6.4×10^{13} vg/mL, without significant aggregation. In some embodiments, AAV vectors stored using the methods and compositions of the invention do not exhibit significant aggregation when stored at 4° C. for five days. In other embodiments, AAV vectors that are stored as such compositions do not exhibit significant aggregation after one, five, ten or more freeze-thaw cycles at −20° C. or at −80° C.

In some embodiments, preparations of virions stored according to the methods and compositions of the invention exhibit an average particle radius (Rh), as measured by dynamic light scattering, indicating that no significant aggregation of virions has taken place. In some embodiments, preparations of virions stored according to the methods and compositions of the invention exhibit an average particle radius (Rh) greater than about 15 nm, 20 nm, or 30 nm.

In some embodiments, recovery of virions from preparations of virions stored according to the methods and compositions of the invention is greater than about 85%, 90% or 95% following filtration through a 0.22 μm filter.

In yet another aspect, the invention relates to kits comprising the high ionic strength formulations of the invention. In one embodiment the kit comprises a pre-mixed solution of excipients. In another embodiment the kit comprises two or more separate components of a high ionic strength composition of the present invention to be mixed by a user. In some embodiments the kit comprises sodium citrate, Tris® and Pluronic® F68. In other embodiments, the kit further comprises instructions for making a composition or performing a method of the present invention.

DESCRIPTION OF THE DRAWINGS

FIGS. 1A and 1B present data showing aggregation of AAV2-FIX particles as a function of osmolarity (FIG. 1A) or ionic strength (FIG. 1B) for various buffer compositions. AAV2-FIX vectors are prepared by Method 2 of Example 1. Average particle radius is measured by dynamic light scattering (DLS) following vector dilution in varying concentrations of excipients buffered with 10 mM sodium phosphate at pH 7.5. Excipients include sodium chloride (●), sodium citrate (○), sodium phosphate (■), sodium sulfate (□), magnesium sulfate (▲), and glycerol (Δ).

FIG. 2 presents data on AAV2-FIX aggregation as a function of the method of purification. The average particle radius is measured by DLS following vector dilution in varying concentrations of sodium chloride buffered with 10 mM sodium phosphate at pH 7.5. Vectors are purified by Method 1 (double CsCl gradient) (○); Method 2 (cation exchange chromatography) (□); Method 2 plus nuclease digestion (■); or Method 3 (chromatography plus one CsCl gradient) (Δ). Purification Methods 1-3 are described in Example 1.

FIG. 3 presents data on transgene expression from D7/4 cells transduced with rAAV2-AADC virions prepared and stored in high ionic strength formulation (□) or in a control formulation (●). The concentration of AADC was measured by ELISA (in triplicate for each data point) 72 hours post-transduction. Error bars represent standard deviations.

DETAILED DESCRIPTION OF THE INVENTION

AAV2 vector aggregation is frequently observed in concentrated preparations of vectors and can affect purification recovery, and in vivo potency and safety. Hence, an important objective for the development AAV2 vectors is to identify methods and formulations that prevent aggregation of vectors when concentrated stocks are prepared.

Unless otherwise indicated, the term "vector" as used herein refers to a recombinant AAV virion, or virus particle, regardless of the frequent use of "vector" to also refer to non-viral DNA molecules, such as plasmids, in other contexts.

The present invention is based in part on the observation that solution ionic strength is an important parameter in AAV vector aggregation, implicating the involvement of ionic interactions between virus particles in the aggregation process. The observation that elevated ionic strength increases AAV2 vector solubility regardless of the identity of the charged excipient supports the hypothesis that ionic strength of solution per se, rather than interactions involving a specific ionic species, is the relevant physico-chemical parameter. A threshold ionic strength of at least 200 mM is required to prevent aggregation at vector particle concentrations examined herein.

Of practical concern, commonly used buffered saline solutions have insufficient ionic strength to prevent AAV2 vector aggregation at concentrations exceeding 10^{13} particles/mL. It

is known that high salt concentrations increase AAV2 vector solubility (e.g. highly concentrated AAV2 vectors recovered from gradients generally remain soluble in concentrated CsCl). However, optimal formulations for pre-clinical and clinical studies should be close to isotonic (280-400 mOsm), especially for in vivo administration of vector to sites where dilution of hypertonic solutions may be slow. In embodiments of the present invention the exponential relationship of ionic strength with charge valency is used to develop isotonic formulations with high ionic strengths. Salt species with multiple charge valencies (e.g. salts of sulfate, citrate, and phosphate) that are commonly used as excipients in human parenteral formulations can provide the level of ionic strength needed to prevent AAV2 vector aggregation when used at isotonic concentrations. While isotonic (150 mM) sodium chloride has an ionic strength of 150 mM, a value insufficient to maintain AAV2 solubility at high vector concentrations, isotonic sodium citrate, with an ionic strength of ~500 mM, can support AAV2 vector concentrations of at least 6.4×10^{13} vg/mL without aggregation.

Without intending to be limited by theory, the low solubility of AAV2 particles may be caused by their highly symmetrical nature in conjunction with the stabilizing effect of complementary charged regions between neighbouring particles in aggregates. The surface charge density based on the crystal structure of AAV2 (Xie, Q. et al. (2002) *Proc. Natl. Acad. Sci. U.S.A.* 99: 10405-10410) reveals a pattern of positive and negative charges on the virus surface. Previous reports have shown that AAV2 vector aggregation is pH dependent, and hypothesized that amino acids with charged side groups are involved in inter-particle binding. Qu, G. et al. (2003) *Mol. Therapy* 7: S238. These reports hypothesized that if charged amino acid side chains are involved in vector aggregation, high concentrations of free amino acids could block vector particle interactions. However, we have found that amino acids with charged side chains are not effective in preventing AAV2 vector aggregation beyond their contribution to ionic strength.

Vector aggregation at low ionic strength was also found to be reduced but not prevented by efficient nuclease treatment of purified vector particles. Digestion at an earlier stage of the purification process (clarified HEK cell lysate) did not reduce aggregation following vector purification. It is likely that digestion of already purified virions is more efficient because of a higher enzyme to nucleic acid substrate ratio. One mechanism to explain these results is that residual nucleic acid impurities (e.g. host cell and plasmid DNA) bound to the vector surface can bridge to binding sites on neighbouring virus particles and thus cause aggregation. Purified AAV2 vectors (empty capsid free) have been reported to contain approximately 1% non-vector DNA. Smith, P. et al. (2003) *Mol. Therapy* 7: 5348. While >50% of this non-vector DNA was reported to be nuclease resistant and was packaged within capsid particles, some impurity DNA was nuclease resistant and appeared to be associated with the surface of purified vector particles. The observation that efficient nuclease treatment can reduce vector aggregation suggests that nucleic acids associated with the vector surface at an average level not greater than ~25 nucleotides per vector particle can contribute to AAV vector aggregation.

In summary, the use of high ionic strength solutions during AAV2 vector purification and final formulation, and efficient removal of residual vector surface DNA are two effective strategies to achieve highly concentrated solutions of AAV2 vectors for use in pre-clinical and clinical studies. High ionic strength solutions and nuclease treatment can be used in combination or separately. Although data were obtained using AAV2 vectors, the composition and methods of the present invention may also be useful with other AAV serotypes/variants, or other viral vectors such as adenoviruses, lentiviruses and retroviruses.

AAV Aggregation as a Function of Excipient Concentration

Initial screening experiments are performed to elucidate the mechanism of AAV vector aggregation and to identify classes of excipients that can reduce/prevent aggregation. Vector aggregation can be caused by dilution (5-fold) of vector in neutral-buffered saline with low concentration buffer (20 mM sodium phosphate, pH 7.2). Excipients are screened using this "dilution-stress" method to identify excipients that are able to prevent vector aggregation when included in the diluent. For screening, aggregation is measured by dynamic light scattering (DLS). Classes of excipients examined included selected inorganic salts, amino acids, uncharged carbohydrates, and surfactants. Results are presented in Table 1.

TABLE 1

SCREENING FOR EXCIPIENTS THAT PREVENT AAV2 VECTOR AGGREGATION USING DILUTION-STRESS METHOD

Excipient	Osm required to prevent aggregation (max tested)
Magnesium sulfate	180 mOsm
Sodium citrate	220 mOsm
Sodium chloride	320 mOsm
Sodium phosphate	220 mOsm
Sodium sulfate	220 mOsm
Arginine	NIA (200 mOsm)
Aspartic acid	320 mOsm
Glutamic acid	320 mOsm
Glycine	NIA (200 mOsm)
Histidine	NIA (200 mOsm)
Lysine	300 mOsm
Glycerol	NIA (5% w/v, 543 mOsm)
Iodixanol	NIA (5% w/v, 32 mOsm)
Mannitol	NIA (5% w/v, 275 mOsm)
Sorbitol	NIA (5% w/v, 275 mOsm)
Sucrose	NIA (5% w/v, 146 mOsm)
Trehalose	NIA (5% w/v, 146 mOsm)
Pluronic® F68	NIA (10% w/v, 12 mOsm)
Polysorbate 80	NIA (1% w/v)

NIA: No inhibition of aggregation

As illustrated in Table 1, charged excipients (inorganic salts and amino acids) prevent aggregation when present at sufficient concentrations. However, salt concentrations required to prevent vector aggregation vary, ranging from 180 mOsm for magnesium sulfate, to 320 mOsm for sodium chloride. The amino acids arginine, aspartic acid, glutamic acid, glycine, histidine, and lysine do not prevent aggregation at 200 mOsm, but lysine, aspartic acid, and glutamic acid prevent aggregation at 300-320 mOsm. Arginine, glycine and histidine were not tested at concentrations other than 200 mOsm. Selected carbohydrates have no effect on vector particle aggregation when present at concentrations up to 5% w/v. For example, 5% w/v glycerol (543 mOsm) does not prevent aggregation. The surfactants Polysorbate80 (1% w/v) and Pluronic® F68 (10% w/v) similarly have no effect on aggregation using the "dilution-stress" method.

AAV Aggregation as a Function of Osmolarity and Ionic Strength

FIGS. 1A and 1B show the results of a more detailed analysis of vector aggregation as a function of the concentration of various salts. FIG. 1A shows vector aggregation as a function of the osmolarity of selected excipients. For charged species a concentration-dependent inhibition of AAV2 vector

aggregation is observed. Salts with multivalent ions achieve a similar degree of inhibition of aggregation at lower concentrations than monovalent sodium chloride. For example, magnesium sulfate prevents aggregation at >200 mOsm whereas sodium chloride requires ≥350 mOsm to achieve a similar effect. Sodium citrate, sodium sulfate, and sodium phosphate are intermediate in their potency to prevent vector aggregation.

Although the results in FIG. **1**A and Table 1 show no effect of glycerol and certain sugars at concentrations up to 5% on AAV2 vector aggregation induced by low ionic strength, the data cannot rule out improvement of AAV2 solubility at glycerol concentrations above 5%. For example, Xie and coworkers reported that 25% (w/v) glycerol enabled concentration of AAV2 to very high concentrations (4.4 to 18×10^{14} particles/ml) in low ionic strength solutions. Xie, Q. et al. (2004) *J. Virol. Methods* 122: 17-27.

FIG. **1**B shows the data of FIG. **1**A plotted as a function of the calculated ionic strength, rather than osmolarity, for each excipient. FIG. **1**B demonstrates that vector aggregation is prevented when ionic strength is ~200 mM or greater regardless of which salt is used. These data suggested that the ionic strength (μ) of a solution, a parameter that depends on both solute concentration and charge valency, is the primary factor affecting aggregation.

Ionic strengths useful to prevent aggregation in embodiments of the present invention include, for example, 250 mM, 300 mM, 350 mM, 400 mM, 450 mM, 500 mM, 600 mM, 700 mM or higher ionic strengths. Multivalent ions are preferred to achieve these ionic strengths in methods and formulations of the present invention, such as divalent, trivalent, tetravalent, pentavalent ions and ions of even higher valency. The pH buffer in solutions and formulations of the present invention may be phosphate, Tris, or HEPES (or other Good's buffers), but any other suitable pH buffer may be used. In preferred embodiments, the multivalent ions and buffer are selected to be compatible with the target tissue for the vector being prepared.

Use of multivalent ions in the methods and compositions of the invention makes it possible to create compositions of high ionic strength but relatively low osmolarity. High ionic strength compositions of the present invention may be nearly isotonic, and may be, for example, about 200 mOsm, 250 mOsm, 280 mOsm, 300 mOsm, 350 mOsm or 400 mOsm, although other osmolarities may be acceptable for some uses of the compositions.

AAV Aggregation as a Function of the Method of AAV Purification

Recombinant AAV2 purified using different methods (e.g. density gradient purification versus ion-exchange chromatography) would be expected to have different impurity profiles. FIG. **2** shows vector aggregation as a function of ionic strength for several preparations of AAV differing in the purification method. Purification methods are described in Example 1. Sodium chloride is used to vary the ionic strength. AAV2-FIX vectors purified by double cesium chloride gradient ultracentrifugation (Method 1), by cation exchange column chromatography (Method 2), or by combined column and cesium chloride gradient ultracentrifugation (Method 3) each demonstrate similar aggregation responses as ionic strength is decreased. In contrast, AAV2-FIX purified by the column method and then subjected to a nuclease digestion step (Method 2+nuclease) shows reduced aggregation at low ionic strength.

AAV Aggregation at Preparative Scale

The data in Table 1 and FIGS. **1**A, **1**B and **2** involve vector aggregation at an analytical scale, employing DLS to measure aggregation. Table 2, in contrast, shows the effects of elevated ionic strength and nuclease treatment on AAV2 vector aggregation at a larger scale, using methods to induce and quantify vector aggregation that are relevant to preparative scale vector purification. Experimental details are provided in Example 2. Purified AAV vectors are diafiltered into solutions of various ionic strengths, the volume is reduced to achieve high vector concentrations, and aggregation is then assessed by measuring vector recovery after filtration through a 0.22 μm filter. Aliquots from a single pool of AAV2-AADC vector purified by Method 1 through the second CsCl gradient centrifugation step (1.8×10^{15} vg in 91 mL, 1.8×10^{13} vg/mL, in ~3M CsCl) are used as starting material in the diafiltration experiments. Tangential flow filtration using hollow fibers is used for diafiltration because it is scalable and yet it still enables preparation of volumes (min. 1.4 mL), and thus AAV concentrations, at which aggregation would be expected in neutral buffered saline.

In Experiment 1, three hollow fiber units are used to diafilter AAV2-AADC vector in formulations CF, TF1, or TF2, and the volume is reduced to a target of 2.5×10^{13} vg/mL. See Example 2. The samples are then filtered through a 0.22 μm filter. Results are shown in Table 2. Vector recovery ("Yield %") for both elevated ionic strength formulations TF1 (95±7.4%) and TF2 (93±7.4%) are significantly higher than the recovery using the control formulation CF (77±6.6%).

TABLE 2

AAV VECTOR RECOVERY AT PROCESS SCALE

Experiment	Formulation	μ (mM)	Target (vg/mL)	Actual (vg/mL)	Yield % (RSD)
1	CF	160	2.5E13	1.93E13	77 (6.6)
1	TF1	310	2.5E13	2.38E13	95 (7.4)
1	TF2	510	2.5E13	2.33E13	93 (7.4)
2	CF	160	6.7E13	3.98E13	59 (6.0)
2	TF2	510	6.7E13	6.42E13	96 (4.4)
3	CF (−Bz)	160	3.6E13	2.46E13	68 (11)
3	CF (+Bz)	160	3.6E13	3.29E13	91 (12)

In Experiment 2, AAV2-AADC is concentrated to a higher target value (6.7×10^{1} vg/mL) in CF or TF2. Vector recovery using TF2 (96±4.4%) is again significantly higher than recovery using CF (59±6.0%). Within the variability of the assays used, vector was recovered fully at both target concentrations using TF2, indicating that aggregation was prevented. In contrast, significant aggregation was observed at both target concentrations using CF, and the extent of aggregation (i.e. loss following 0.22 μm filtration) was higher at the higher target vector concentration. In an additional experiment (not shown), 50 μL samples of AAV2 vector are taken following concentration but prior to the 0.22 μm filtration step of Experiment 2, and examined by light microscopy. Vector concentrated in CF contains obvious amounts of visible material (not shown), while no such material is seen in vector concentrated in TF2.

Experiment 3 examines the effect of prior nuclease digestion of purified vector on aggregation. In the absence of nuclease digestion recovery of AAV2-AADC in CF is 68±11%, similar to the recoveries in Experiments 1 and 2. In contrast, purified vector treated with nuclease and then concentrated in CF gives higher recovery (91±12%). These prep scale results reflect the same effect of nuclease digestion shown in FIG. **2** using the "dilution-stress" (analytical scale) method.

The results presented in Table 2 demonstrate that the methods and compositions of the present invention increase the

recovery of AAV vector recovery. For example, in various embodiments of the present invention, recovery is improved from less than about 80% to at least about 85%, 90%, 95% or more.

AAV Stability and Activity Following Storage or Freeze-Thaw Cycling

Croyle and coworkers reported a significant loss of titer of AAV and adenovirus following multiple freeze-thaw cycling in sodium phosphate buffer, and demonstrated that the better pH buffering provided by potassium phosphate during freeze-thaw cycling prevented titer loss. Croyle, M. A. et al. (2001) *Gene Therapy* 8: 1281-1290. Results of our freeze-thaw stability study using sodium phosphate support these findings. We find that while 150 mM sodium phosphate provides sufficient ionic strength to prevent aggregation during preparation and non-frozen storage of concentrated AAV2-AADC vector, even a single freeze-thaw cycle at −20 or −80° C. results in aggregation.

AAV stability after storage or freeze-thaw (F/T) cycling is assessed in buffers of the present invention as follows. The concentrated vectors prepared in CF, TF1, and TF2 (Table 2, Experiment 1) are subjected to a short stability study to investigate whether aggregation will occur during refrigerated storage, or following multiple freeze-thaw (F/T) cycles. Aggregation is assessed by DLS using undiluted samples, and Rh values >20 nm are deemed to indicate the occurrence of some level of aggregation.

TABLE 3

STABILITY OF AAV2 VECTORS

	Particle radius - Rh (nm)							
Formulation	Pre	4° C. 5 d	−20° C. 1 F/T	−20° C. 5 F/T	−20° C. 10 F/T	−80° C. 1 F/T	−80° C. 5 F/T	−80° C. 10 F/T
CF	14.5	27.0	22.4	56.1	94.5	20.6	57.5	141
TF1	13.8	16.3	TH	TH	TH	TH	TH	TH
TF2	13.8	14.4	14.2	14.0	14.1	13.8	21.3	50.9

Pre: DLS radius measured immediately following 0.2 μm filtration.
Vector concentrations (vg/mL): CF: 1.93E13, TF1: 2.38E13, TF2: 2.33E13.
TH: signal intensity is too high to measure because of extensive aggregation.

As shown in Table 3, AAV2-AADC vector prepared in CF shows some aggregation after 5 days of storage at 4° C., as well as following one or more F/T cycles at −20 or −80° C. For vector prepared in TF1, no aggregation occurs after 5 days at 4° C., but aggregation occurs following a single F/T cycle at −20 or −80° C. as indicated by a DLS signal intensity that is too high to measure. Visual inspection of these samples reveals slight cloudiness, which is consistent with aggregation. For vector prepared in TF2, no aggregation is observed at 4° C., or following up to 10 F/T cycles at −20° C. Some aggregation is observed following 5 and 10 F/T cycles at −80° C.

AAV activity after storage or F/T cycling in TF2 is assessed as follows. As described above, the high ionic strength, isotonic formulation TF2 effectively prevents vector aggregation during concentration and storage, and therefore represents a promising candidate for further study. An important question is whether preparation and storage of the vector in high ionic strength TF2 would adversely affect its functional activity. To assess this, assays are performed to measure the infectious titer and the transduction efficiency of vectors prepared and stored for an extended period of time in TF2.

For infectivity, a highly sensitive infectivity assay capable of detecting single infectious events is used. Zhen, Z. et al. (2004) *Human Gene Ther.* 15: 709-715. AAV2-AADC is prepared in TF2 at a concentration of 6.4×10^{13} vg/mL. After being stored for 45 days at 4° C. the preparation has a vector genome to infectious unit ratio (vg/IU) of 13, compared to a value of 16 vg/IU for the reference vector. This difference is not significant given the reported variability of this assay (RSD ~50%).

Transduction efficiency is assessed by measuring the expression of AADC protein by ELISA following transduction of D7/4 cells. FIG. 3 shows no significant difference between vector prepared in TF2 and the reference control for vector input ranging from 10 to 10^5 vg/cell. Together, these data indicate that preparation and storage of AAV2 vectors in high ionic strength TF2 does not have a deleterious effect on vector infectivity or transduction efficiency.

CONCLUSION

The effect of ionic strength (μ) on virus particle interactions is determined to elucidate the mechanism of vector aggregation. The ionic strength of neutral-buffered isotonic saline (μ=150 mM) is insufficient to prevent aggregation of AAV2 vectors purified by gradient ultracentrifugation or by cation exchange chromatography at concentrations exceeding ~10^{13} particles/mL. Inclusion of sugars (sorbitol, sucrose, mannitol, trehalose, glycerol) at concentrations up to 5% (w/v) or of surfactants Tween80® (1%) or Pluronic® F68 (10%) does not prevent aggregation of vector particles.

In contrast, vector particles remain soluble when elevated ionic strength solutions (μ>200 mM) are used during purification and for final vector formulation. Elevated ionic strength solutions using isotonic excipient concentrations for in vivo administration are prepared with salts of multivalent ions, including sodium citrate, sodium phosphate, and magnesium sulfate. An isotonic formulation containing 10 mM Tris, 100 mM sodium citrate, 0.001% Pluronic® F68, pH 8.0 (μ~500 mM) enables concentration of AAV2-AADC vectors to 6.4×10^{13} vg/mL with no aggregation observed during preparation and following ten freeze-thaw cycles at −20° C. See Table 3, below, and accompanying discussion. AAV2-AADC vectors prepared and stored for an extended period in elevated ionic strength formulation retain high infectivity titer (13 IU/vg) and transduction efficiency.

Nuclease treatment of purified AAV2 vectors reduces the degree of vector aggregation, implicating vector surface nucleic acid impurities in inter-particle interactions. Hence, purification methods to efficiently remove vector surface residual nucleic acids, coupled with the use of elevated ionic strength isotonic formulations, are useful methods to prevent AAV2 vector aggregation.

Example 1

AAV Purification Methods

AAV2 vectors expressing human coagulation factor IX (FIX) or human amino acid decarboxylase (AADC) are produced by triple transfection of HEK293 cells as previously described (Matsushita, T. et al. (1998) *Gene Therapy* 5: 938-945), with modifications. For the large scale preparations, cells are cultured and transfected in 850 mm^2 roller bottles (Corning). Vectors are purified by one of three methods.

In purification Method 1, modified from Matsushita, transfected HEK293 cells in roller bottles are collected by centrifugation (1000 g, 15 min), resuspended in 10 mM sodium phosphate, 500 mM sodium chloride, pH 7.2, and lysed by three freeze/thaw cycles (alternating an ethanol/dry ice bath

and a 37° C. water bath). The cell lysate is clarified by centrifugation (8,000 g, 15 min). The supernatant is then diluted to 200 mM NaCl by addition of 10 mM sodium phosphate, pH 7.2, and digested with Benzonase® (Merck, Purity Grade 1; 200 U/mL, 1 h, 37° C.). The lysate is adjusted to 25 mM $CaCl_2$ using a 1M stock solution, and incubated at 4° C. for one hour.

The mixture is centrifuged (8,000 g, 15 min), and the supernatant containing vector is collected. To precipitate virus from the clarified cell lysate, polyethylene glycol (PEG8000) is added to a final concentration of 8%, the mixture incubated at 4° C. for three hours, and then centrifuged (8,000 g, 15 min). The pellets containing vector are re-suspended with mixing in 0.15M NaCl, 50 mM Hepes, 25 mM EDTA, pH 8.0 and incubated at 4° C. for 16 hours. The resuspended material is pooled, and solid cesium chloride is added to a final density of 1.40 gm/ml. Vector is then banded by ultracentrifugation (SW28, 27,000 rpm, 24 h, 20° C.) using a Beckman model LE-80 centrifuge. The centrifugation tubes are fractionated, and densities from 1.38 to 1.42 gm/mL containing vector are pooled. This material is banded a second time by ultracentrifugation (NVT65 rotor, 65,000 rpm, 16 h, 20° C.), and fractions containing purified AAV2 vectors are pooled. To concentrate vector and to perform buffer exchange, vectors in concentrated cesium chloride solution are subjected to ultrafiltration/diafiltration (UF/DF) by tangential flow filtration as described below (Example 2).

In purification Method 2, cell harvests containing AAV are microfluidized and filtered sequentially through 0.65 and 0.22 μm filters (Sartorius). Virus is purified from the clarified cell lysates by cation exchange chromatography using Poros HS50 resin as previously described. U.S. Pat. No. 6,593,123. For the nuclease digestion described in FIG. **2**, column-purified vectors are incubated (4 h, RT) with 100 U/mL Benzonase and 10 U/mL DNAse I (RNAse free, Roche Diagnostics, Indianapolis, Ind.).

For purification Method 3, AAV2 vectors purified by cation exchange chromatography are subjected to an additional cesium chloride gradient ultracentrifugation step (SW28, 27,000 rpm, 20 h) to remove empty capsids prior to UF/DF.

Real time quantitative PCR (Q-PCR) is used to quantify AAV preparations as previously described. Sommer, J. M. et al. (2003) *Mol. Therapy* 7: 122-128. Vectors purified by each of the three methods are analyzed by SDS-PAGE/silver staining analysis, and in all cases VP1, VP2 and VP3 are present in the expected ratios, with the capsid proteins representing >95% of total proteins as determined by scanning densitometry. However, unlike gradient-purified AAV2 vectors purified using Methods 1 and 3, vectors purified by Method 2 (column chromatography) contain empty capsids, ranging from 3-10 empty capsids per vector genome.

Example 2

Ultrafiltration and Diafiltration to Detect AAV Aggregation

Disposable hollow fiber tangential flow filtration devices (Amersham BioSciences 8" Midgee, 100 kDa nominal pore size) are used to concentrate and diafilter AAV2 vectors purified by the methods described above, and for the UF/DF experiments described in Table 2. For all UF/DF procedures a volume of diafiltration buffer corresponding to 10× the product volume is used, and it is added in ~1 mL increments to approximate continuous diafiltration. Using this method, the calculated residual CsCl after diafiltration is <0.5 mM.

The following three formulations were used for UF/DF: Control Formulation (CF: 140 mM sodium chloride, 10 mM sodium phosphate, 5% sorbitol, pH 7.3); Test Formulation 1 (TF1: 150 mM sodium phosphate, pH7.5); and Test Formulation 2 (TF2: 100 mM sodium citrate, 10 mM Tris, pH8.0). For Experiment 1 shown in Table 2, diafiltration is performed at a volume corresponding to a vector concentration of 1×10^{13} vg/mL, and following diafiltration the volume is reduced to a value corresponding to 2.5×10^{13} vg/mL (assuming no vector loss).

For Experiment 2, diafiltration is performed at a volume corresponding to a 2×10^{13} vg/mL, and the volume is then reduced to a value corresponding to 6.7×10^{13} vg/mL.

For Experiment 3 (CF±Bz), AAV2-AADC (approximately 1.2×10^{14} vg) is first diafiltered into TF1 (a formulation compatible with nuclease activity) and then passed through a 0.22 μm filter. The titer of this material is determined, and the volume is adjusted to correspond to a concentration of 1×10^{13} vg/mL. To 10 mL of this material, $MgCl_2$ is added to a concentration of 2 mM, and then divided into two equal aliquots. One aliquot is incubated with Benzonase (200 U/mL, 4 h, RT), and the second is mock-incubated. Each aliquot is then diafiltered at a volume corresponding to a vector concentration 2×10^{13} vg/mL, and then concentrated to a 3.6×10^{13} vg/mL target. Following all UF/DF protocols, Pluronic® F-68 (BASF Corp., Mount Olive, N.J.) from a 1% stock is added to the vector product to a final concentration of 0.001%, and the solution is passed through a 0.22 μm syringe filter (Sartorius). All UF/DF procedures are performed in a laminar flow cabinet.

Example 3

Measurement of Vector Aggregation by Dynamic Light Scattering

Purified vectors are analyzed for aggregation by dynamic light scattering (DLS) using a Protein Solutions DynaPro 99 (λ=825.4 nm). Primary data (particle radius—Rh, average value measured over 30 cycles, 10 cycles/min) are used for all analyses reported. A "dilution-stress" method is used to assess the effect of varying excipients on vector aggregation. In this method, 80 μL of test diluent is added to 20 μL of vector solution with mixing in the actual cuvette used for DLS measurement, and data collection is initiated within 10 seconds of mixing. Prior to addition of test diluents, the Rh value for AAV2 vector preparations is measured and confirmed to be <15 nm to ensure that the starting material is monomeric. Samples that are not 100% monomeric are passed through a 0.22 μm syringe disc filter (Sartorius, low protein binding) to remove aggregates.

The osmolarity and ionic strength values given in FIGS. **1** and **2** are calculated using all excipients present in the mixture (i.e. weighted: test diluent (80%) and starting vector formulation (20%)). Osmolarity is calculated according to the equation: Osmolarity=Σc_i, where c_i is the molar concentration of each solute species. The ionic strength (μ) is calculated according to the equation: $\mu=\frac{1}{2}\Sigma c_i z_i^2$, where z_i is the charge on each species. In conditions that resulted in vector aggregation (e.g. low μ) a progressive increase in Rh is observed over the course of data collection. To validate the use of the average Rh measured over the 3 minute interval following dilution as a reliable measure of aggregation, the average rate of increase of Rh ($\Delta Rh/\Delta t$) over the same time interval is also determined (not shown). Analysis of $\Delta Rh/\Delta t$ gives results concordant with those obtained using the average Rh value reported in FIGS. **1** and **2**.

Example 4

AAV Virion Infectivity

Infectivity of AAV2-AADC vectors is determined using a highly sensitive assay as previously described. Zhen, Z. et al. (2004) *Human Gene Ther.* 15: 709-715. Briefly, samples are serially diluted (10-fold dilutions, 10 replicates/dilution) and added to D7/4 cells (modified HeLa cells expressing AAV rep and cap) grown in 96 well tissue culture plates (Falcon, cat. #353227) in DMEM medium containing 10% FBS. Adenovirus (Ad-5, 100 vp/cell) is added to each well to provide helper functions. After 48 h, replication of AAV vector in each well is quantified by Q-PCR using transgene-specific primers and probes, and the frequency of infection at limiting dilution is analyzed by the Karber method to calculate the infectivity titer. The test sample is run concurrently with an AAV2-AADC reference previously prepared in CF and stored at −80° C.

The transduction efficiency of AAV2 vectors is quantified by a whole cell ELISA. D7/4 cells grown in 96 well plates are infected with 10-fold serial dilutions of the test sample and reference vector, corresponding to 10 to 10^5 vg/cell input (5 replicates/dilution). After 48 h, the culture medium is removed, and cells are washed twice with 200 μL PBS (10 mM sodium phosphate, 140 mM sodium chloride, pH 7.2). Cells are then permeabilized and fixed by addition of 100 μL of PBS containing 0.5% Triton X-100 and 4% paraformaldehyde to each well (15 min). The fixing solution is removed, and the cells are washed twice with PBS containing 0.5% Triton X-100. Non-specific sites are blocked by adding PBS containing 3% bovine serum albumin (BSA) and 0.5% Triton X-100 (60 min).

After washing, cells are incubated for one hour with rabbit anti-AADC IgG antibody (Chemicon, AB136), and washed. Cells are then incubated for one hour with alkaline phosphatase-conjugated goat anti-rabbit IgG, and washed. Antibodies are diluted 1:1000 in PBS containing 1% BSA, 0.5% Triton X-100. Substrate (PNPP, Pierce, cat. #34047) is then added (1 mg/mL in 1× diethanolamine substrate buffer, Pierce, cat. #34064), and after incubation for 30 min the concentration of cleaved substrate is measured spectrophotometrically (λ=405 nm). Human AADC expression as a function of vector input is fitted using a spline curve (SigmaPlot). The AAV2-AADC reference vector is measured concurrently with the test sample.

While preferred illustrative embodiments of the present invention are described, it will be apparent to one skilled in the art that various changes and modifications may be made therein without departing from the invention, and it is intended in the appended claims to cover all such changes and modifications that fall within the true spirit and scope of the invention.

All publications, patents and patent applications referred to herein are hereby incorporated by reference in their entireties.

We claim:

1. A composition for the storage of purified, recombinant adeno-associated virus (AAV) vector particles, comprising:

purified, recombinant AAV vector particles at a concentration exceeding 1×10^{13} vg/ml up to 6.4×10^{13} vg/ml;

a pH buffer, wherein the pH of the composition is between 7.5 and 8.0; and

excipients comprising one or more multivalent ions selected from the group consisting of citrate, sulfate, magnesium, and phosphate; wherein the ionic strength of the composition is greater than 200 mM, and wherein the purified AAV vector particles are stored in the composition without significant aggregation.

2. The composition of claim 1, further comprising ethylene oxide/propylene oxide block copolymer Pluronic® F68.

3. The composition of claim 2, wherein the Pluronic® F68 is present at a concentration of 0.001% (w/v).

4. The composition of claim 1, wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate.

5. The composition of claim 1, wherein the purified, recombinant AAV vector particles have an average particle radius (Rh) of less than about 20 nm as measured by dynamic light scattering.

6. The composition of claim 1, wherein recovery of the purified, recombinant virus particles is at least about 90% following filtration of the composition of said AAV vector particles through a 0.22 μm filter.

* * * * *

U.S. DEPARTMENT OF COMMERCE
PATENT & TRADEMARK OFFICE

PTO-1683
(Rev. 7-96)

Exhibit 2

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Transcript of John Fraser Wright

Date: June 16, 2023
Case: Genzyme Corporation, et al. -v- Novartis Gene Therapies, Inc., et al.

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IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

GENZYME CORPORATION and AVENTIS INC., Plaintiffs, vs. NOVARTIS GENE THERAPIES, INC. and NOVARTIS PHARMACEUTICALS CORPORATION, Defendants. : C.A. No. 21-1736 (RGA)

HIGHLY CONFIDENTIAL

VIDEOTAPED DEPOSITION OF JOHN FRASER WRIGHT

Palo Alto, California

Friday, June 16, 2023

9:30 A.M.

Job No.: 496641
Pages: 1-379
Reported by: Crystal Hereford, RPR

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Videotaped deposition of JOHN FRASER WRIGHT, conducted at the law offices of DECHERT, LLC, 3000 El Camino Real, Five Palo Alto Square, Suite 650, Palo Alto, California 94306.

Pursuant to notice, before CRYSTAL HEREFORD, Registered Professional Reporter, Certificate No. 30819; California Certified Reporter, Certificate No. 14416; and Arizona Certified Shorthand Reporter, Certificate No. 50893.

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P R O C E E D I N G S

THE VIDEOGRAPHER: Here begins Media Number 1 in the videotaped deposition of Dr. John Fraser Wright in the matter of Genzyme Corporation, et al., versus Novartis Gene Therapies, Inc., et al., in the United States District Court, for the District of Delaware, Case Number 21-1736 (RGA).

Today's date is June 16, 2023. The time on the video monitor is 9:30 a.m. Pacific Standard time.

The videographer today is Joe Ramirez, representing Planet Depos.

This video deposition is taking place at Dechert LLP, Palo Alto.

Would counsel please voice-identify themselves and state whom they represent.

MR. WEINGARTEN: David Weingarten from Finnegan, Henderson, Farabow, Garrett & Dunner, representing Defendant Novartis entities, and joined with me today is Christopher Weber, also of Finnegan.

MS. ANTONS: Amanda Antons of Dechert LLP, representing Plaintiffs and the witness.

THE VIDEOGRAPHER: The court reporter today is Crystal Hereford, representing Planet Depos.

The witness will now be sworn.

JOHN FRASER WRIGHT,

a witness herein, having been first duly sworn by the certified reporter to speak the truth and nothing but the truth, was examined and testified as follows:

EXAMINATION

BY MR. WEINGARTEN:

Q. Good morning.

A. Good morning.

Q. Could you please state your full name for the record?

A. John Fraser Wright.

Q. And where do you currently reside, Dr. Wright?

A. [redacted]

Q. And, Dr. Wright, you understand that you're being deposed here today?

A. Yes.

Q. And we're going to go over some ground rules. Have you been deposed before?

A. No.

Q. So the first time deposed, so we'll go over the rules probably in a little bit more detail.

A. Thank you.

Q. But I think the key issue here is, is that I'm going to ask you some questions. You're going to answer those questions under oath.

We have a court reporter here that's going to take down what you say.

A. Uh-huh.

Q. If you don't understand the question that I ask, then just ask for clarity. You know, I can repeat the question or I can rephrase the question.

A. Uh-huh.

Q. If you answer the question, I'm going to assume you understood it.

A. Uh-huh.

Q. When I ask you a question, your counsel might object.

A. Uh-huh.

Q. If she objects, unless she instructs you specifically not to answer it, you still have to answer it. We'll deal with the objections later.

A. Uh-huh.

Q. With regard to the questions that are being asked, there are going to be a number of questions being asked, and there might be a point in time that, you know, I lose track of time, and you might need to take a break --

A. Yeah.

Q. -- for whatever reason. Or counsel might ask for a break as well. And we'll certainly accommodate you.

But if we're in the middle of a question or if we're in the middle of a series of questions related to a document, we might ask you to just wait a few minutes, but we will be mindful of the break, and we'll certainly give it to you.

A. Could I ask for a clarification?

So a series of questions. So that would be within a few minutes. Is that correct?

Q. Well, presumably it would be. But, you know, I guess the short answer is, is that if you ask for a break, I'll do my best to give it to you right away.

A. Okay. Thank you.

Q. No problem.

And, you know, when I ask you questions, the court reporter is trying to jot down or put down what you've said and also what I've said.

A. Uh-huh.

Q. I tend to speak quickly, so she might have

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to tell me a number of times to slow down. And she might do the same for you. I don't know.

But ultimately, when you answer the question, she's unable to really put down the "ums" and "yeah" and shaking of heads. So try the best you can to provide verbal answers, you know, such as "yes," "no," whatever the answer is to the question rather than motions and so forth.

A. Sure. Understood. Uh-huh.

Q. Is there any reason that you're unable to provide, you know, honest and truthful testimony here today?

A. No. There's no reason. I'm not sure if it'll come to this, but I would just like to state that I'm -- you know, I'm 65. And, you know, I wear bifocal glasses, and, you know, these days I quite often ask "Can you repeat the question?" when I'm speaking with my family. So just understand that I have probably what's typical somewhat reduced hearing and vision at this stage.

Q. I appreciate that. I can understand that and have similar issues myself. So I will do my best to be mindful.

A. Thank you.

Q. But if you need time to review something,

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you know, take your time. We're not under a rush here. We have the day. We certainly will do what it takes to make sure that you have the ability to, you know, review whatever is in front of you.

A. Thank you. I appreciate it. Uh-huh.

Q. Now, you said this is the first time you're being deposed. How did you learn that you were going to be deposed?

A. I learned from a reach-out from Dechert, I believe, or the preceding legal firm. Another point is I'm not great with name recollection, especially in the area that I'm not familiar with. And I think the deposition information came about a month or six weeks ago, something like that.

MR. WEINGARTEN: So I'm going to ask the court reporter to mark this as -- I guess we'll go with Wright Exhibit 1.

(Exhibit Number 1 marked.)

A. Great.

Q. And, Dr. Wright, what's been handed to you has been now marked as Wright Exhibit 1.

A. Uh-huh.

Q. And it has at the top the -- what we'll refer to as the case caption, Genzyme Corporation and Aventis v. the Novartis Gene Therapy entities.

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Do you see that?

A. I do. Yes.

Q. Okay. And do you see right underneath that, it refers to the defendant's notice of deposition of John Fraser Wright? Do you see that?

A. I do. Yes.

Q. And take a second to look at the document -- it's only a few pages -- and tell me if you've seen this document before.

A. Yes. I have seen the document, and I've looked at the three pages. This is a document that I am familiar with. Yes. Uh-huh.

Q. All right. And so you understand that you're here to provide your testimony in your personal capacity and to answer the questions the best you can remember?

A. Uh-huh. Yes. Uh-huh. Yep.

Q. What did you do prepare for your deposition today, Dr. Wright?

MS. ANTONS: I'll just caution the witness to not disclose conversations with counsel. You can answer questions generally.

THE WITNESS: Okay. So specifics of conversations.

MS. ANTONS: Yes. Our conversations are

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privileged, so you cannot share -- disclose what we spoke about.

THE WITNESS: I understand. Yeah.

A. So I have met with Dechert counsel in preparation yesterday, for example, for much of the day. That's the primary preparation.

I have also reviewed, myself, relevant documents. I have relevant publications, at least a couple, you know, in the peer-reviewed literature.

And I've had a copy of the patent, of course.

And most recently, I've been able to view some additional documents, including lab notebooks from that time and also a provisional patent version that was prepared in 2004.

Q. So let's just -- the first thing you said was that you met with Dechert yesterday. Could you -- without going into specifics, do you recall -- I know you said you're not good with names, but do you recall who you met with?

A. Amanda.

Q. Was there anybody else present during that time?

A. Yes.

Q. And who was present?

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A. Uh-huh.
Q. Now, let's return to the Wright Exhibit 11.
A. Yes.
Q. In addition to the '721 patent that I'll refer to --
A. Yeah.
Q. -- underneath "Related U.S. application Data," it also refers to provisional applications. Do you see it says "Provisional Application Number," and it's number 60/575,997 --
A. Uh-huh.
Q. -- filed on June 1, 2004?
A. Uh-huh.
Q. And then there's another one listed, Provisional Number 60/639,222 filed on December 22, 2004.
A. Uh-huh.
Q. Now, you indicated that you reviewed a provisional application in preparation for your deposition. Do you recall if you reviewed both of these provisional applications?
A. Well, it seemed -- my first reaction is it's the same provisional. I don't know. I mean, again, it's complex to me how these documents

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evolved. We did some scientific studies. We wrote up our results. And, you know, the legal department brought in a lawyer and wrote up a patent, and we reviewed it. So I don't know -- I prepared one provisional, as far as I remember.
Q. Yeah. My question was a little different.
A. Okay.
Q. In preparation for your deposition --
A. Oh, I see.
Q. -- you indicated that you reviewed a provisional application.
A. Yes.
Q. And I'm -- what I'd like to know is, did you review one provisional application or two provisional applications?
A. One. And it wasn't in any type of format of, you know, looked like a provisional. It was just -- it was a document. Yeah.
Q. And you indicated that you recall you prepared an application.
A. Uh-huh.
Q. Was it the one you prepared?
A. I believe so. I believe so. I can tell in the recollection and the way I do my dates. I have a particular way of doing dates which not very

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many people do, so -- uh-huh.
Q. We'll come back to that, and maybe we'll figure out which one it is in a few minutes.
A. Uh-huh. Yeah.
Q. That's no problem.
A. Uh-huh.
MS. ANTONS: Counsel, does it make sense to break for lunch?
MR. WEINGARTEN: Give me five minutes.
MS. ANTONS: Yep.
MR. WEINGARTEN: And then I think it'll be a good time.
MS. ANTONS: Okay.
MR. WEINGARTEN: Thank you, though. I don't miss many meals, so I don't think...
Will you please mark that as Wright Exhibit 13.
(Exhibit Number 13 marked.)
A. Uh-huh.
Q. Doctor, you should have in front of you Wright Exhibit 13, bearing Bates Number GENZ_NOV_00003911, and the second page is 3915. So what I'd represent to you is that this is an excerpt of a bigger document, but the page I'm referring to is a single page of the document --

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A. Yeah, the cover page and a single page of the larger --
Q. And then this --
A. Yeah -- or cover --
Q. And the --
A. Yeah. Yes.
Q. And the declaration, which is a declaration that relates to the patent application. We're going to talk about that.
A. Uh-huh. Uh-huh.
Q. So, Doctor, if you look at the declaration, this is on page Bates number ending in 3915.
A. Yes.
Q. This is a declaration, and the title -- it describes the title of the invention as "Compositions and Methods to Prevent AAV Vector Aggregation." That's the title of the patent that we've been talking about, right?
A. Yes. Uh-huh.
Q. '542.
A. Uh-huh.
Q. And then it says -- below, it says "This declaration is directed to," and it has an application number, "Application Number 11/141,996,

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have a deleterious effect without testing. Is that right?
MS. ANTONS: Objection; form.
A. Well, I could make a good prediction, but I think in this type of work, you have to validate your predictions. So there would be an expectation with any change. Even going to low ionic strengths, you'd have to verify that you have efficacy and safety of your investigational product. And when you go to low ionic strength, of course, you get aggregation, and that is not a good thing, based on our experience, you know, through this series of studies.
MR. WEINGARTEN: Where are we on the time for the last --
THE VIDEOGRAPHER: For this session?
MR. WEINGARTEN: Yes.
THE VIDEOGRAPHER: 45 minutes.
MR. WEINGARTEN: Okay. Good.
(Exhibit Number 17 marked.)
MR. WEINGARTEN: I'm going to ask the court reporter to mark as Wright Exhibit 17 --
THE WITNESS: Thanks.
MR. WEINGARTEN: -- the document bearing Bates Number NVS-ZOL-00138971 --

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A. Uh-huh. Yes.
MR. WEINGARTEN: -- through 8988.
A. I've got 8989, which is just a -- yeah. Yeah. My last page is 8989.
Q. 8989. I stand corrected. Thank you.
A. Yeah.
Q. And, Dr. Wright, this is a provisional application, including the cover sheet.
A. Uh-huh.
Q. Do you see that on the top of the page, the first page, "Provisional Application for Patent Cover Sheet"?
A. I do. Yes.
Q. And this is a cover sheet that -- if you look at the title of the invention, three lines down, four lines down, it's "Characterization of AAV2 Vector Aggregation."
A. Sir, I'm chuckling because I see the evolution of the title.
But yes. I see that. I'm sorry. Pardon me. Uh-huh.
Q. And then right above that, you're listed as the inventor of this.
A. Yeah. Okay. Uh-huh.
Q. And this is -- we're going to talk about

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this. This is one of the two provisional applications that we talked about earlier, and this is -- let me just pull up the -- this is the provisional application that is cited on the face of the patent.
A. Right.
Q. Let's just go back to Wright Exhibit 11. And this is the application that was filed -- provisional application that was filed in December of 2004.
A. The one you gave me says "Date, 01 June 2004." June 1, 2004. Is that correct?
Q. Yes. I'm sorry. You're right.
A. Yeah.
Q. This is the 60/575,997, filed June 1, 2004.
A. Right. Uh-huh.
Q. That's right.
A. Uh-huh.
Q. So that is the second or the most recent of the two provisionals by date.
A. Uh-huh.
Q. And so take a look at this for a second, and tell me if you recognize this.
A. Not the first two pages, but yes. This is

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a document that I actually reviewed and was very helpful, and I recognize this as my writing style.
Q. This is a document that you reviewed with counsel yesterday?
A. Yes. Yeah. Uh-huh.
Q. And so this -- and so I want to introduce one other provisional.
A. Uh-huh.
Q. Which is Exhibit -- we're going to call it Wright Exhibit 18. So we'll have both of them at hand.
(Exhibit Number 18 marked.)
Q. And so this is a document bearing Bates Number GENZ_NOV_00149038 to 072. Do you see that?
A. Correct. I do. Uh-huh. Uh-huh.
Q. And so these are the two provisional applications that are cited on the face of the patent. One, as you pointed out, Exhibit 17, is June 1, 2004.
A. Yes.
Q. And the other is December 22, 2004.
A. So we're later in that same year. Yes.
Q. So let's start on Wright Exhibit 18, if we can. So you can put 17 aside for now.
A. 17 aside. Okay.

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Q. And so this application was the application -- turn to the first -- well, the third page, which is Bates Number 040.
A. Yes.
Q. And this is the first page of -- substantively the first page of the application. Do you see that?
A. Yes. Uh-huh.
Q. And do you recall seeing this application?
A. Yes, I think so. I haven't seen it recently, but I think at the time this looks familiar. Again, there's back and forth over a period of time where I had a lot of things going on. And this looks like this is my writing, and this looks like a lawyer has put this together.
Q. Now, just turning -- so before we get into the substance of this, turning back to the front page.
A. Yes.
Q. At the bottom of the front page, there is the name -- signature and name of Gregory R. Bellomy. Do you see that?
A. Yes.
Q. Have you interacted with Mr. Bellomy?
A. Possibly. Not closely -- not closely, but

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he's not -- I think he must have been somebody that came in -- if he was at Avigen, it must have been somebody that came in later in my tenure. Uh-huh.
Q. And so it's possible that you interacted with him, but maybe -- maybe not.
A. Maybe not. Yeah. Yeah.
Q. Were you involved in the preparation of this particular provisional application? And what I mean by that -- by preparation, I mean as to the drafting of the application, you know, the disclosure.
A. Well, I'd go back to Exhibit 17. I wrote -- I think I substantially wrote Exhibit 17, submitted it in June.
And I would imagine -- and I haven't read through this -- I have read through this, I'm certain, but not for a long time, so I'd have to read through and just see. But I would expect that this was presented to me and I was asked for my review -- for review of it and comments, and we probably had some discussions at the time too. And it could have been with Gregory. I just -- I don't know Gregory well. For example, I know, like, Christine Thompson well. I know her. Yeah.
Q. And Christine Thompson was the person who

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you worked with --
A. At Avigen.
Q. -- at Avigen for the first provisional application?
A. Well, it's just a senior legal person at Avigen that I knew well, and she was there most of the time that I was there. Greg might have come in the last 6 or 12 months. I really don't know. I can't remember -- I'm sorry. I can't remember. Uh-huh.
I'm sorry. I went quickly there. Did I answer your question?
Q. No, you did. I'm just...
A. Uh-huh. I don't recall.
Q. So I want to turn to page 14 of the application, which is Bates Number 053. Do you see that?
A. Yes.
Q. And the first full paragraph on that page in the context of "AAV Aggregation at Preparative Scale" says "The data in Table 1 and Figures 1A, 1B, and 2 involve vector aggregation at an analytical scale, comma, employing a method to measure aggregation, DLS, that is difficult to quantify reliably." Do you see that?

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A. Yes.
Q. Okay. We can put that to the side.
A. Uh-huh.
Q. Let's return a moment now to Wright Exhibit 11, which is the patent.
A. Yes.
Q. The '542 patent.
And so what I'd like to do is just get a little bit of a better understanding of what everybody's contributions were, scientific contributions, if you will, who did what, so to speak.
A. Sure.
Q. So what were your contributions to the experiment and disclosure of the '542 patent?
MS. ANTONS: Objection; form.
A. So as the director of development and at that latter stage the clinical manufacturer manufacturing at Avigen, I was in the position to certainly recognize the problem acutely. Acutely. When it's in your department and there's a failure of a manufacturing run because of aggregation, it elevates it substantially, appropriately. It does in any case, but nevertheless, it's immediate.
And I was able to, I think, form and

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assemble a team that could do the series of experiments progressive, from screening experiments to, you know, the stability studies that we did as a function of storage and freeze-thaw, defined storage and freeze-thaw, to the prep- -- what I call "preparative scale" that was highly relevant to the actual manufacturing process, tying in three evolving man- -- production -- or purification processes.

Q. So in addition --

A. That's part of -- okay. So if I could just say. So really hands-on. I was actually quite hands-on on the dilution experiments. I was quite -- I was -- I got into them.

And Tannie, who -- you've seen Tannie Le -- I recall was probably not a Ph.D. She might have been, but I think she would have been a technician or a junior scientist.

I think she was probably recognized as an organized person. And me being a little bit not necessarily organized -- I don't think it's one of my strengths, frankly -- is to -- she -- I think she was able to be my right-hand person as we set up these experiments and implemented them. For example, Tannie can prepare these things for

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experiment tomorrow. I would design an experiment, perhaps discuss it with Guang or Jurg and others and things like that, so -- okay. I'll stop.

Q. Yeah. Did Tannie design any experiments as well?

A. No. No. I'd say no.

Q. What about Jurg?

A. I would say no. I would say that we would pressure test experiments with Jurg, but I would say no, generally. I mean, I greatly respect Jurg, and I don't think that there was any problem about inventorship on this at the time. But Jurg was a senior scientific person in my general group that I spoke to a lot.

Q. Yeah. I didn't mention inventorship. I'm just asking contributions.

A. Contributions. Okay. Okay.

Yeah. No, he contributed. You know, he contributed.

Q. And my question, did he contribute to the design of some of these experiments?

A. Right. The design...

So I would say, to a degree, in discussion, yes. But most importantly, Jurg was the head of the analytical group. So we had to be able

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to measure things. So when you have a bunch of samples and you have to get the titer and et cetera, et cetera, we needed expert help. And, you know, Peter Smith, for example, is the person in Jurg's group, I believe, who did all our qPCR titers.

And I'll let you call out other author -- or other persons as you wish. Yeah.

Q. Okay. And so with regard to the experiments that are -- I think you said that you were pretty hands-on with the dilution experiments.

A. Quite hands-on.

Q. Those are the experiments in Figures 1A, 1B --

A. Yeah.

Q. -- and Figure 2?

A. Yep. Uh-huh. Yes.

Q. What about the vector recovery experiments that are reported in Column 8? Who was responsible or contributed to those experiments?

A. Okay. 8. So -- okay. Column 8. This is at scale, right? At process scale?

We would have had more people there, so this is not a one-person or a two-person. I suspect this is more like a two- or three-person.

And I may have been in there. This is not

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in a GMP environment. This is in a process development environment. So it wouldn't be surprising that I would be there. I had been through so many of these runs and seen this fogginess occur as you come down to the level that -- you know, it was kind of the eureka moment if it didn't happen, you know, when you got to the end of the diafiltration.

But I think other people that might have been there might have been Joseph. Joseph Prado was probably in as a right-hand man, I think, to help out there.

Jennifer Bahr-Davidson may have been in there. I can't remember.

I think Hoc wasn't there at the time, or he wouldn't have been in. I do notice that Hoc was an author on that abstract.

So I can't quite -- those folks are all kind of in that GMP side of the operations.

Q. So it's possible they contributed to some of the work that's ascribed in here.

A. Yeah. And provision -- for example, the provision of the starting material that got divvied up into thirds to run these three different lead test formulations, that was prepared in the

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manufacturing group, I believe. It's a large-scale operation. It's rather precious material. It was a big effort to make that much material. Uh-huh.

Q. Now, what about the experiments that are described about the stability studies with dynamic light scattering? Who was the -- involved with those experiments that are described in Table 3?

A. That's a good question.

I was pretty involved with this overall, so I suspect -- because I like to be in the lab, and this was a bit of a pet project, if I could say. We needed solutions here -- I was probably present. I don't think I'm the person that would have been putting them in and taking them out, but I was probably present at least for part of the time.

But to take those things out at, say, 10 o'clock -- the same time on sequential -- subsequent days and that type of thing, it could have been Tannie. It could have been Tannie. Yeah. I can't recall.

MR. WEINGARTEN: I think we've been going for about an hour, so maybe it's a good time to take a break.

THE WITNESS: Sure.

THE VIDEOGRAPHER: Going off the record.

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The time is 4:22 p.m.

(Off the record.)

THE VIDEOGRAPHER: Back on the record.

The time is 4:32 p.m.

Q. Welcome back, Dr. Wright.

A. Thank you.

Q. I didn't ask you after the last lunch break. I'll ask you now. During either of these last two breaks, did you discuss the testimony with counsel?

A. No.

Q. I figured not, but -- Amanda is a very seasoned attorney. I don't think that would happen.

All right. So let's now turn back to Wright Exhibit 17. If you could put that --

A. Yes.

Q. -- in front of you.

And this is the first provisional, and I think you said -- correct me if I'm wrong -- this is the one that you wrote.

A. Yeah. This really looks like something that I would write, the formatting. Yep. And as I read through it -- it was very satisfying for me to read through this, frankly. It presents in the way -- the way I think a scientist, or at least me, would present the whole story. Uh-huh.

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Q. And on the front of this document, this provisional application, it has a name Mary Christina Thompson. Do you see that?

A. Yes.

Q. And do you know who she is?

A. Yes. I know Christina Thompson. Uh-huh.

Q. And can you describe what her role was at Avigen?

A. Well, she was hired in -- I think she may have been the second in the hierarchy in the legal department of Avigen when Ken Chahine was the head of the legal department -- whatever the title was. I'm not sure what the title was. But when Ken became, I think, the CEO of the company after reorganization that occurred, I think Christina was probably the head of the legal department. I don't know if that's the chief legal officer or whatever the title might be. But yeah. That was my understanding. She was a senior legal person at Avigen.

Q. And when you came up with possible new patent applications, would she be somebody that you would work with?

A. I think so. Yeah. I think that's right,

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Christina and -- but there were probably other persons underneath her -- another person underneath her who might handle these submitted in here, so...

Q. Do you recall what their name might have been?

A. No, I don't. And it's possible that Gregory was one of them, and I just -- I lost track. Yeah.

Q. And you're referring to Gregory Bellomy?

A. Yes, who is on this other one. Again, I just can't place that -- the name right now. Uh-huh.

Q. So if we turn to the third page of the document, it's actually page 1 of the application --

A. Yes.

Q. -- Bates Number 973, this refers to the -- I'm reading the title here. "Characterization of AAV2 Vector Aggregation and Recommended Excipients/Formulations to Achieve Vector Concentrations of 2E13 vg/mL or Greater." Do you see that?

A. Yes.

Q. And there's a summary that follows.

A. Yes.

Q. In the summary, Point Number 3 refers to

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Q. -- in Appendix E, that appears to be the same as the patent formulation -- Test Formulation 2. Take a look at that, and please confirm that's the same.
A. Yeah. pH 8, the citrate, and the -- and it's pH 8. Yeah.
Q. Okay.
A. It reads the same again, with the same caveat with the formulation, the addition of the poloxamer 188, the Pluronic F-68. Uh-huh.
But I do understand, given the caveat -- there is really a wording issue here. So when we're testing formulations, during the process, we don't have poloxamer, but it's -- really, poloxamer is included typically, and it was here, you know, for example, prior to the filtration step. Yeah. Uh-huh.
Q. So let's focus on the -- we'll get to the -- we'll get to the data.
A. Okay.
Q. But let's just look at the formulation itself, just test formulation.
A. Uh-huh.
Q. You agree that the control formulations are identical.

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A. Yes.
Q. You agree that Test Formulation 1 of the patent is the FTF Formulation 1 of Appendix E of the first provisional.
A. Yes.
Q. And that the Test Formulation 2 of the patent is identical to the FTF3 of the first provisional.
A. I do. And, again, I'd just restate the comment that, you know, you have to also look at the note, including the subtext on the Pluronic F-68. But yes. The recipes are the same.
Q. I'm looking for a yes or no answer here, because, you know, this -- I'm asking if the formulations are identical, not when you use them, what happens to them, what you add to them.
A. Uh-huh.
Q. So I'm going to ask you again.
A. Uh-huh. Uh-huh.
Q. Formulation of the patent TF -- Test Formulation 2 is identical to the test formulation FTF3 in the first provisional application as the formulation exists prior to use, if you want to go there.
A. Yeah. I think that's right. During the Example 2 where we did this large-scale material, these formulations are perfect matches aside from the fact that we used these different numbers. The FTF1 and FTF3 --

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Q. That's right.
A. -- correspond to TF1 and TF2.
Q. Exactly.
A. Yeah. Yeah. Uh-huh.
Q. So the one thing that I don't see in the patent --
A. Yeah.
Q. -- in Wright Exhibit 11 --
A. Yes.
Q. -- that is in the provisional application, Exhibit 17, is formulation FTF2.
A. Right.
Q. Is there a reason that you excluded that from the patent?
A. Well, you know, the glycerol -- you know, it's hard for me to remember right now. But we had a bit of a philosophy of what is -- what are the minimal set of excipients that were required to meet the specifications that we expected, the stability specifications, preventing aggregation, ensuring functional activity.

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So unless there was data supporting the use of something like glycerol or sorbitol -- keep in mind that the original control formulation had sorbitol in it too. We couldn't see any benefit to sorbitol based on these series of experiments. So hence, the test future formulations and control contained sorbitol.
I think the glycerol was included in one of the formulations at this stage, because there was a fairly prominent literature out there that glycerols and sorbitols and sucrose and things like this -- which are kind of related in many regards -- are cryoprotectants and may be relevant in the freeze-thaw situation, which is a relevant situation.
But I think that we felt that the data was compelling, so -- probably when we put it all together. That's my recollection.
Q. It wasn't compelling, in that it didn't demonstrate --
A. An advantage.
Q. An advantage over what?
A. Over a formulation that didn't contain the glycerol or the sorbitols. You know, that goes back to the screening.

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Q. Does your invention exclude glycerol in the formulation?
MS. ANTONS: Objection; form.
A. I don't think it excludes it. No. You can add whatever you said.
Q. Okay.
A. As far as I know. I don't know the legal consideration.
Q. Yeah.
A. But, you know, if people like to put it in glycerol, that's --
Q. Your intent was not to exclude glycerol --
A. No. No --
Q. -- from the formulation.
A. -- it wasn't. No. We were trying to be a bit ideal- -- not idealist, but with the objective what is the simplest formulation that works.
Q. And in the control formulation, you have sorbitol, though, right?
A. Control, yeah. That was a historic -- that was what had been -- that was operational at Avigen, I mean, essentially when I arrived, except, in fact, the -- the Pluronic F-68 had -- we had replaced something that was called Tween with Pluronic F-68.

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Q. So what I want to make sure I'm clear on -- because I don't want to misunderstand you.
A. Yeah.
Q. -- is you purposely did not include the Test Formulation 2 in the application that would lead to the issued patent because you didn't think it was beneficial.
A. I think --
MS. ANTONS: Objection; form.
A. I think that, as I mentioned before, it was very expensive to do this large-scale demonstration experiment. And, you know, you have to make a decision. Are we going to try one, our best formulation based on all of our theoretical insights that we had gained? Are we going to do two? Are we going to do three? Are we going to do five? Are we going to spend another six months and make a lot more material?
So I think the decision at that time -- my recollection is that we picked these two, a mid-high and a high ionic strength, didn't think it was compelling enough to include the glycerol-containing material.
Q. Looking down on this page --
A. Yep.

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Q. -- on Exhibit 17 -- right? Exhibit 17.
A. Uh-huh.
Q. You have data for this experiment. So I'm a little unclear about the cost and the scale up and all this stuff. You've done the experiments.
A. Ah. Right.
Q. Right? So --
A. Why didn't we use --
Q. -- you did the experiment. So the question is: Why did you not include those experiments --
A. The three -- correct. Yeah. Okay.
So I want -- I'm sorry. So I take -- you know, again, this is my recollection.
Q. Yeah.
A. Right. So there must have been a specific reason that we wouldn't include the 1 percent glycerol.
I can't remember. I can't remember going forward. Maybe there's some other patent somewhere. I don't know. Yeah.
Q. And so the reason you gave a few minutes ago is -- was without the benefit of seeing that you actually had the data.
A. Yeah. I'm sorry. I'm sorry. I think it

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is important to emphasize that the screening conditions used small amounts of vector. In aggregate, we looked at, you know, dozens, if not hundreds, of permutations and combinations; derived theoretical insights from it, what we thought were supported by the data; and then made the best decision. And I acknowledge that we took -- you're right -- three test formulations into this large-scale stuff. So we did it.
Q. And you report data here. So looking down at the bottom here, it says E25.
A. Yeah.
Q. Do you see where it says "E25"?
A. E25 -- yeah. Oh --
(Simultaneous speaking.)
A. -- yeah, yeah. That's right. Yeah. Uh-huh.
Q. So I'm guessing -- and you can tell me -- E stands for "Experiment 25"?
A. Yeah.
Q. Okay.
A. That's correct. That's -- I believe that's correct.
Q. Okay.
A. Yeah. Yeah. Uh-huh.

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Q. I mean, can we go with that for purposes of today? E25 is Experiment --
A. Yeah. Yeah. I -- yeah. Uh-huh.
Q. And it describes those experiments being done, obviously, in time for this provisional application, but they were being done and recorded in Notebook 670. Do you see that?
A. Yes. That's correct. Uh-huh.
Q. And if you look down in that table, there is control formulation.
A. Uh-huh.
Q. And it's talking about a post-filter. Now, I want to make sure I understand. This is a -- this is talking about percent recovery, right? This is -- these are the --
A. Yeah.
Q. -- discussions that we had regarding --
A. Yeah.
Q. -- you filter --
A. Uh-huh.
Q. -- and you see how much recovery you get.
A. Right. So this is the same data, I believe, that we looked at, except minus the FTF2 -- TF2. It's funny. There's some inconsistencies in my grammar here. You know, you can see I've gone to

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TF here, but I believe this is probably the same data.
Q. Yeah. And, in fact, if you look at "CF post-filter, 2-day" --
A. 2-day and 7-day. Right.
Q. That's right. But let's look at the 2-day.
A. Yeah. Uh-huh.
Q. Okay.
A. Uh-huh.
Q. And there is a difference between --
A. Right.
Q. -- 2-day versus 7-day.
A. Yes.
Q. Do you agree?
A. Yes, there is. Yeah. Uh-huh.
Q. But if you look at -- and we're talking about, you know, Wright -- just so we're clear, Wright Exhibit 17, Bates Number 984.
If you look at the "CF post-filter 2-day experiment" --
A. Yes.
Q. -- and you compare it to the patent, which is Wright Exhibit 11 --
A. Yes.

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Q. -- and you look at Table 2, Column 8 --
A. Yeah.
Q. -- Experiment 1, that is the 1.93 E to the 13th and the 77 percent.
A. If you give me a moment --
Q. Yes.
A. Because I am curious myself.
Q. Yeah.
A. So we had 2-day data and 7-day data. So I can see that the 77 percent was, in fact, the better data for the control formulation. You can see that if that had been left for a week --
Q. Well, my question is different, Doctor.
A. Okay. Yeah.
Q. Okay?
A. Uh-huh.
Q. All I'm asking right now --
A. Yep. Uh-huh.
Q. -- is that if you look at the 2-day data, the vg/mL that are reported there --
A. Yep.
Q. -- the target and the recovery --
A. Yeah.
Q. -- match what's in Experiment 1, Table 2, of the patent.

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A. That is correct. Yep. Uh-huh.
Q. And if we go down and we look at the third row of Appendix E of Wright Exhibit 17, it has a "TF1 post-filter, 2-day."
A. Yep.
Q. And that matches -- take a look at that -- the patent, Experiment 1, TF1.
A. Uh-huh. Yes.
Q. And then you have E25, Exhibit 17.
A. Yeah.
Q. And if you look down, it has "TF2 post-filter, 2-day," and that's the experiment that's not reported in the patent, correct?
A. Right. The TF2 in this provision --
Q. That's right.
A. -- correct? Uh-huh.
Q. So go down to the TF3 in the provisional.
A. Uh-huh.
Q. And that's the data that is reported for Experiment 1, TF2, at the 510.
A. Correct.
Q. So the data that was excluded from Table 2 --
A. Uh-huh.
Q. -- is the data for TF2, which is the 210

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ionic strength. Do you agree?
A. Yes.
Q. Okay.
A. I'd like to make one more comment, if I can, about the glycerol.
Q. Well, we'll come back to that --
A. Okay. I'd like to make a comment, though. Yeah.
Q. Because I'm just asking you right now --
A. Yeah. Uh-huh.
Q. -- what data -- I mean, you agree -- glycerol aside --
A. Yep.
Q. -- you agree that these experiments match up to the table --
A. Yes.
Q. -- in the patent.
A. Yeah, they do. Yeah.
Q. Okay.
A. Uh-huh. Uh-huh.
Q. And now, this -- these recoveries here are based upon the target value, right? Whether you look at the patent or -- you know, Wright Exhibit 11, Table 2, or you look at Wright Exhibit 17, page 12 of 16, or Bates Number 984.

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A. Yes.
Q. The recovery that's reported is based upon the vg/mL compared to the target. And we talked about that earlier.
A. Correct.
Q. Right?
A. Uh-huh.
Q. Now, if you look at the provisional application --
A. Uh-huh.
Q. -- it includes an additional column on the right.
A. Right.
Q. And it has the DLS data.
A. Right.
Q. But that's DLS post-filtration.
A. Right.
Q. Which basically was your attempt to get it below 15 before you did something else with it, presumably.
A. Yeah. This is just verification post-filtration that we have material that -- yeah. I mean, it's as we would predict after -- post-filtration.
But it does come to the question when

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post-filtration. This, I suspect, would have been rather immediate post-filtration, probably that day when the experiment was done. Yeah. But I don't really remember. But looking at this data, that's...
Can I comment on the glycerol?
Q. Yes. Go ahead.
A. So I think the problem with glycerol is that -- my interpretation, my impression, was that glycerol is not really an acceptable human parenteral. So glycerol has been used a lot in biochemistry to stabilize proteins when you're doing research, and I think it had been used at the research level with AAV vectors. And I think we've seen that people trying to get high concentrations for crystallography studies use glycerol.
But it's not something that I would formulate a drug with and put it -- and have it go into a human. So I think that glycerol gets disqualified.
Now, this is my impression right now. But when I think of glycerol, it's just not the type of thing -- unlike sorbitol, you know, which might be fine to go into a formulation that goes into a human. But -- so I want to just put that on the

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table. That's always been my opinion without doing a deep research on it.
Q. But, Doctor, you agree that you tested glycerol in the list of candidate excipients.
A. I did. Yes. Uh-huh. We did.
Q. And you included it as one of the three test formulations that you chose to bring forward and to do these purification filtration studies?
A. Yeah. Uh-huh, uh-huh, uh-huh.
Q. Okay. All right. So let's move on from this, then.
(Exhibit Number 19 marked.)
Q. And, Doctor, you should have just been handed what has been labeled as "Wright Exhibit 19."
A. Yes.
Q. And this is a -- we'll get to it. It's a notebook. But it bears Bates Number GENZ_NOV_00012079 --
A. 079. Oh, yes. Yes.
Q. Yeah, through 115. Do you see that?
A. Yes, I do. Uh-huh.
[redacted]
A. Right.
Q. Do you recognize this document, Doctor?

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Q.

A. Yes. Uh-huh.

Q.

.

330

A. Oh, yes.

Q.

A. I'm sorry. Yeah. Uh-huh. Yeah.

Q.

A. Right, right, right. Uh-huh.

Q.

331

Q. So why don't you take a quick look through this notebook. We didn't see anything in this notebook -- it looks like the last entry by you --

A. Uh-huh.

A. Yeah. Yeah.

332

A. I'm just -- I'll finish it here.

Q. Yes. Definitely look through it, please.

A. Yeah. Yeah.

353

that's correct. Yeah. Uh-huh.
Q. Okay. You can --
A. 77 percent is in the patent, and it is in the provisional. We were just looking at it. Yes.
Q. Okay.
A. Yeah. And I think we've -- and we haven't found that number specifically in here yet.
Q. That's right.
And so -- but that's a recovery percent compared to the target.
A. Yes.
Q. Which you said was a number that, you know, you sort of strive to get during the reduction in volume. You know, so as you put it through diafiltration --
A. Right.
Q. -- then you -- after you do that and you get the right buffer --
A. Right.
Q. -- you can then reduce the volume to a --
A. Yes.
Q. -- target --
A. Right.
Q. -- and that's that target you had.
A. Right. It is. So it's a measure of loss.

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I mean, 100 minus that number is a measure of loss.
Q. Well, that's a target, though. This is not actual. Okay? So this --
A. But the target is calculated on input data. So it's a real number to the degree that those input numbers are accurate. But that applies to everything here.
Q. So let's look at the notebook, Wright 20.
A. Uh-huh.
Q. And let's look at what the input numbers are.
A. Okay.
Q. Okay? So Bates Number 676.
A. 676. Okay. Uh-huh.

355

A. Uh-huh.
A. Uh-huh.
A. Uh-huh.
A. Uh-huh.
Q. Right.
A. Again, I don't know the context of this table. I see this. Uh-huh. Uh-huh.
Q. Well, but the context that I'd like to focus on --
A. Uh-huh.
A. Right. Uh-huh.

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A. Uh-huh. Uh-huh. Yeah.
Q. Well, let's look at it.
A. Yeah.
Q. Okay?
A. Uh-huh.
Q. These results are reported on this
A. Uh-huh.
Q.
A. Uh-huh.
Q.

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That's true.
Q. Right?
[redacted]
A. Uh-huh. Uh-huh.
[redacted]
A. Uh-huh.
[redacted]
A. Yeah. I'm just trying to go through this.
Q. Yeah.
A. Yeah. Let's just see what we have here. Again, I haven't seen this --
Q. We have time.
A. -- in 20 years. Yeah. I haven't seen this in 20 years, so I just need to digest it.
Q. I just want to make sure I understand the --
A. Yeah. Yeah.

358

[redacted]
Q. That's right.
A. Yeah. Uh-huh.
[redacted]
A. Uh-huh.
A. Uh-huh.
[redacted]
A. Yeah.
[redacted]
A. Yeah, yeah, yeah.
Q. And so that number matches.
A. Right.
Q. Right?
A. Right.
[redacted]

359

A. Yep. Uh-huh.
[redacted]
A. Right. Right.
[redacted]
A. Right. Right.
[redacted]
Q. No, I understand.
[redacted]

360

A. Yeah.
[redacted]
A. Yeah.
[redacted]
A. Yeah.
Q. Okay?
A. Uh-huh.
[redacted]
A. Yeah.
[redacted]
A. Yeah.
[redacted]

361

Q. That's what's reported.
A. Yeah. Uh-huh.
[redacted]
A. Correct.
[redacted]
A. Uh-huh. Uh-huh.
[redacted]
A. Correct. Uh-huh. Et cetera.
[redacted]
A. Uh-huh.
[redacted]
A. Uh-huh.
[redacted]
A. Right.
[redacted]
A. Correct. Uh-huh.
Q. Okay. That's all I wanted to know on that.
A. Okay. Okay. Fair enough.

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Q. All right. Now, let's move forward.
[redacted]
Q. These are the numbers.
A. Yeah, they are.
Q. All right. So --
A. Correct. Right.
Q. So let's now talk about -- so let's go back to Wright Exhibit 17, Bates Number 984.
A. Yeah.
[redacted]
A. Yes.
[redacted]
A. Yes.
Q. Okay. Now, I want you to compare that to the notebook. This is Wright Exhibit 20.
A. Uh-huh.
Q. On Bates Number 679.

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A. 679. Okay.
[redacted]
A. Uh-huh.
[redacted]
A. Great.
Q. Nothing special here. I'm just --
[redacted]
A. Uh-huh.
Q. Right?
A. Uh-huh.
[redacted]
A. Uh-huh.
[redacted]
A. Okay. Could you repeat that? I was --
Q. Yes.
A. -- just digesting. I'm sorry.
Q. Okay. No problem.
A. Uh-huh.
Q. So I'll rephrase it, because I don't remember exactly the wording.
A. Okay.

364

Q. In the laboratory notebook, Wright Exhibit 20 --
A. Yes.
Q. -- on Bates Number 679 --
A. Yes.
[redacted]
A. Yeah.
[redacted]
A. Uh-huh.
[redacted]
A. Yeah, here.
Q. Okay?
A. Yes. Uh-huh.
Q. All right.
A. Correct. I agree.
[redacted]
A. Uh-huh.
[redacted]
A. Yes.

365

[redacted]

A. Well, I'm not quite sure if I understand that question. Could you rephrase? Yeah.

Q. Yeah. You know, let me -- instead of rephrasing it, I'll take you to data.

A. Okay.

Q. It's easier that way.

A. Okay. Uh-huh.

Q. Let's move forward to page 139 in the notebook, Bates Number 684.

A. Uh-huh. 139. Great.

Q. Okay?

[redacted]

Q. Yes.

A. Again, I'm just catching -- I can't -- I'm a little bit cut off on the left side, but I think I can understand --

Q. Are you looking on page 684?

366

A. 685. So 684 is better.

Q. Okay.

A. Fair enough. Fair enough. Yeah. Uh-huh.

Q. All right.

A. Okay. That looks better. Uh-huh.

[redacted]

A. Yes.

[redacted]

A. Yes.

[redacted]

A. Yes.

[redacted]

A. Uh-huh.

[redacted]

A. Yes.

[redacted]

A. Yes.

[redacted]

A. Uh-huh.

[redacted]

367

[redacted]

Q. Okay.

A. Uh-huh.

[redacted]

A. Yeah. Uh-huh.

[redacted]

A. Yes.

[redacted]

A. Yes.

[redacted]

A. Yeah.

[redacted]

A. Uh-huh.

[redacted]1.

A. Yeah.

[redacted].

A. Uh-huh. So we're talking about...

Q. Now, let me stop you there, Doctor.

A. Uh-huh.

368

[redacted]

Q. Right.

A. Uh-huh. Yeah.

[redacted]

A. Uh-huh.

[redacted]

A. Yes.

[redacted]

A. Right.

[redacted]

A. Uh-huh.

[redacted]

A. Uh-huh.

[redacted]

369

[redacted]

A. Yeah.

[redacted]

A. Uh-huh.

[redacted]

A. Uh-huh.

[redacted]

Q. That's right. But you did the experiment.

[redacted]

ht?

A. No. No. But I --

Q. Okay. And --

A. I don't want to put stuff out there that I would -- people would follow.

370

Q. Okay.

[redacted]

A. Uh-huh.

[redacted]

A. Uh-huh.

[redacted]

A. Yeah. That's right. Yeah.

[redacted]

A. Yeah. Interesting.

Q. Okay. Now, let's go forward in your notebook.

[redacted]

Q. Yeah.

A. Maybe.

[redacted]

A. Uh-huh. Uh-huh.

[redacted]

A. Yeah. Okay.

Q. Okay. And then let's move forward to -- I want to make sure I give you one that doesn't cut

371

off.

A. Uh-huh.

Q. Let's go to page 141 of the notebook, Bates 687.

A. 687. Uh-huh. Uh-huh.

[redacted]

A. 687. Right. Okay. Yeah.

[redacted]

.

A. Uh-huh.

[redacted]

Q. Okay.

[redacted]

372

[redacted]

A. Okay.

[redacted]

Q. Okay.

A. Uh-huh. Uh-huh.

Q. And let's go on to the next experiment.

A. Uh-huh.

Q. Let's go to Bates Number 691[redacted]

[redacted].

A. Okay.

Q. Do you see that?

[redacted]

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Q. Middle of the page.
A. Uh-huh.
Q. It says --
[redacted]
Q. Right.
A. Yes.
[redacted]
[redacted]t.
A. Uh-huh. Uh-huh.
[redacted]
[redacted]
A. Uh-huh.
Q. Do you agree?
[redacted]
Q. So that's right.
[redacted]
Q. That's right.
[redacted]
Q. Okay.
[redacted]
[redacted]
Q. That's right.
A. Yeah.
Q. And so that's the same data, right?
A. Uh-huh, uh-huh, uh-huh.

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[redacted]
Q. Okay.
[redacted]
Q. And then let's go -- let's go to...
[redacted]
Q. That's right.
A. -- all match --
Q. That's right. That's right.
A. -- just for clarity. Yeah.
Q. No, I agree.
A. Yeah.
[redacted]
A. Yeah.

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Q. And you go to 693, [redacted]
A. Uh-huh.
[redacted]
A. Uh-huh.
Q. -- in Wright Exhibit 20 --
A. Uh-huh.
[redacted]
Q. Okay.
A. Uh-huh --
[redacted]
MR. WEINGARTEN: I think it's a good time to take a break. I think we've been going for an hour.
THE WITNESS: Uh-huh.
MR. WEINGARTEN: So let's go off the record.
THE WITNESS: Uh-huh.

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THE VIDEOGRAPHER: We're going off the record. The time is 5:40 p.m.
(Off the record.)
(Mr. Loeb joins the proceedings.)
THE VIDEOGRAPHER: We're back on the record. The time is 5:56 p.m.
MR. WEINGARTEN: Thank you, Dr. Wright.
THE WITNESS: Yes.
MR. WEINGARTEN: Defendants have no additional questions at this time.
MS. ANTONS: Okay. I have just a couple questions.
MR. WEINGARTEN: We will reserve the right to respond to any questions.
But let's go ahead and put the newcomer on the record, if we're going to do that.
MR. LOEB: Sure. I am Jonathan Loeb of Dechert, representing Genzyme.
EXAMINATION
BY MS. ANTONS:
Q. Can you just put Exhibit 11 and Exhibit 17 in front of you.
A. Sure. Yes.
Q. And if you could turn in Exhibit 17 to the page ending in 984.

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A. Yes, I have it.
Q. Okay. And do you recall earlier when counsel was asking about the Test Formulation 2 in Exhibit 17 that was not included in the '542 patent?
A. Yes, I do.
Q. Okay. And Test Formulation 2 in Exhibit 17 contains glycerol. Is that right?
A. Correct.
Q. Okay. And can you turn to Table 1 in Exhibit 11.
A. Table 1. Yes. Uh-huh.
Q. And in Table 1, do you see glycerol listed there?
A. Yes.
Q. And this Table 1 is a list of excipients that were tested to prevent aggregation. Is that fair?
A. Yes. They were tested for their ability to prevent aggregation.
Q. Okay. In the right-hand column, next to glycerol in Table 1 --
A. Uh-huh.
Q. -- what do you see there?
A. The osmolality required to prevent aggregation. So I think no inhibition of

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aggregation was observed with glycerol.
Q. Okay.
A. Uh-huh.
MS. ANTONS: I have no further questions.
MR. WEINGARTEN: Okay. We have no further questions as well.
We'd like to thank you for your time. Appreciate it.
THE WITNESS: Uh-huh.
MR. WEINGARTEN: And go enjoy dinner.
THE WITNESS: Okay. Thank you.
THE VIDEOGRAPHER: This marks the end of the deposition of Dr. John Fraser Wright. We are going off the record at 5:59 p.m.
(Proceedings concluded.)

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CERTIFICATE OF SHORTHAND REPORTER

I, CRYSTAL HEREFORD, the officer before whom the foregoing deposition was taken, do hereby certify that the foregoing transcript is a true and correct record of the testimony given; that said testimony was taken by me stenographically and thereafter reduced to typewriting under my direction; that reading and signing was not waived; and that I am neither counsel for, related to, nor employed by any of the parties to this case and have no interest, financial or otherwise, in its outcome.
IN WITNESS WHEREOF, I have hereunto set my hand and affixed my signature this 23rd day of June 2023.

Crystal Hereford

Exhibit 3

8217198

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

March 3, 2022

THIS IS TO CERTIFY THAT ANNEXED IS A TRUE COPY FROM THE RECORDS OF THIS OFFICE OF THE FILE WRAPPER AND CONTENTS OF:

APPLICATION NUMBER: *12/661,553*
FILING DATE: *March 19, 2010*
PATENT NUMBER: *9,051,542*
ISSUE DATE: *June 9, 2015*

By Authority of the
Under Secretary of Commerce for Intellectual Property
and Director of the United States Patent and Trademark Office

Trudie Wallace
Trudie Wallace
Certifying Officer

U.S. PTO
12/661553
03/19/2010

02570 U.S. PTO 031910

PTO/SB/05 (09-04)

UTILITY PATENT APPLICATION TRANSMITTAL

Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	0800-0045.01
First Inventor	Wright et al.
Title	COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION
Express Mail Label No.	EV 514 190 691 US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. ☒ **Fee Transmittal Form** (e.g., PTO/SB/17) *(Submit an original and a duplicate for fee processing)*
2. ☐ **Applicant claims small entity status.** See 37 CFR 1.27.
3. ☒ **Specification** [*Total Pages* 29] Both the claims and abstract must start on a new page *(For information on the preferred arrangement, see MPEP 608.01(a))*
4. ☒ **Drawing(s)** *(35 U.S.C.113)* [*Total Sheets* 3]
5. **Oath or Declaration** [*Total Sheets* 2]
   a. ☐ Newly executed (original or copy)
   b. ☒ A copy from a prior application (37 CFR 1.63 (d)) *(for a continuation/divisional with Box 18 completed)*
   i. ☐ DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).
6. ☒ **Application Data Sheet.** See 37 CFR 1.76 (4 pages)
7. ☐ **CD-ROM** or **CD-R** in duplicate, large table or Computer Program *(Appendix)*
   ☐ Landscape Table on CD
8. **Nucleotide and/or Amino Acid Sequence Submission** *(if applicable, items a. - c. are required)*
   a. ☐ Computer Readable Form (CRF)
   b. Specification Sequence Listing on:
      i. ☐ CD-ROM or CD-R (2 copies); or
      ii. ☐ Paper
   c. ☐ Statements verifying identity of above copies

ADDRESS TO:

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Alexandria, VA 22313-1450

ACCOMPANYING APPLICATION PARTS

9. ☐ **Assignment Papers** (cover sheet & document(s))
   Name of Assignee _____
10. ☐ **37 CFR 3.73(b) Statement** *(when there is an assignee)* ☐ **Power of Attorney**
11. ☐ **English Translation Document** *(if applicable)*
12. ☐ **Information Disclosure Statement** (PTO/SB/08 or PTO-1449)
    ☐ Copies of citations attached
13. ☐ **Preliminary Amendment**
14. ☒ **Return Receipt Postcard** (MPEP 503) (1 page) *(Should be specifically itemized)*
15. ☐ **Certified Copy of Priority Document(s)** *(if foreign priority is claimed)*
16. ☐ **Nonpublication Request** under 35 U.S.C. 122 (b)(2)(B)(i). Applicant must attach form PTO/SB/35 or its equivalent.
17. ☒ Other: Check for $1090.00

18. If a CONTINUING APPLICATION, *check appropriate box, and supply the requisite information below and in the first sentence of the specification following the title, or in an Application Data Sheet under 37 CFR 1.76:*

☒ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No 11/141,996

Prior application information: *Examiner* S.K. Singh *Art Unit:* 1657

19. CORRESPONDENCE ADDRESS

☒ The address associated with Customer Number: **20855** *OR* ☐ Correspondence address below

Name					
Address					
City		State		Zip Code	
Country		Telephone		Fax	

Signature	Dahna Pasternak	Date	3/19/10
Name (Print/Type)	Dahna S. Pasternak	Registration No. (Attorney/Agent)	41,411

PTO/SB/17 (12-04)

Effective on 12/08/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL
For FY 2010

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT	($)1090.00

Complete if Known	
Application Number	Not Asssigned
Filing Date	Herewith
First Named Inventor	Wright et al.
Examiner Name	Not Assigned
Art Unit	Not Assigned
Attorney Docket No.	0800-0045.01

METHOD OF PAYMENT (check all that apply)

☒ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify): _____

☒ Deposit Account Deposit Account Number: 18-1648 Deposit Account Name: Robins & Pasternak LLP

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☐ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, **except for the filing fee**

☒ Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17 ☒ Credit any overpayments

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FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

	FILING FEES		SEARCH FEES		EXAMINATION FEES		
		Small Entity		Small Entity		Small Entity	
Application Type	Fee ($)	Fee ($)	Fee ($)	Fee ($)	Fee ($)	Fee ($)	Fees Paid ($)
Utility	330	165	540	270	220	110	1090
Design	220	110	100	50	140	70	
Plant	220	110	330	165	170	85	
Reissue	330	165	540	270	650	325	
Provisional	220	110	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 or, for Reissues, each claim over 20 and more than in the original patent	52	26
Each independent claim over 3 or, for Reissues, each independent claim more than in the original patent	220	110
Multiple dependent claims	390	195

Total Claims		Extra Claims	Fee ($)		Fee Paid ($)
11	-20 or HP =	0	0	=	0

HP = highest number of total claims paid for, if greater than 20

Indep. Claims		Extra Claims	Fee ($)		Fee Paid ($)
2	-3 or HP =	0	0	=	

HP = highest number of independent claims paid for, if greater than 3

Multiple Dependent Claims	
Fee ($)	Fee Paid ($)

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper, the application size fee due is $270 ($135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets		Extra Sheets		Number of each additional 50 or fraction thereof	Fee ($)		Fee Paid ($)
32	- 100 =	0	/ 50 =		0	=	

(round up to a whole number) x

4. OTHER FEE(S)0

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	
Other: _____	

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COPY

Attorney Docket No.: 0800-0045
Client Ref. No.:

PTO/SB/01A (09-04)

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

As the below named inventor(s), I/we declare that:

This declaration is directed to:

☐ The attached application, or

☒ Application No. 11/141,996, filed on June 1, 2005,

☐ as amended on _____ (if applicable);

I/we believe that I/we am/are the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought;

I/we have reviewed and understand the contents of the above-identified application, including the claims, as amended by any amendment specifically referred to above;

I/we acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me/us to be material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT International filing date of the continuation-in-part application.

All statements made herein of my/our own knowledge are true, all statements made herein on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and may jeopardize the validity of the application or any patent issuing thereon.

FULL NAME OF INVENTOR(S)

Inventor one: John Fraser Wright — Date: _____
Signature: _____ — Citizen of: Canada

Inventor two: Guang Qu — Date: 9/27/2005
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Inventor four: _____ — Date: _____
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☐ Additional inventors or a legal representative are being named on _____ additional form(s) attached hereto.

COPY

Attorney Docket No.: 0800-0045
Client Ref. No.:

PTO/SB/01A (09-04)

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

As the below named inventor(s), I/we declare that:

This declaration is directed to:

☐ The attached application, or

☒ Application No. 11/141,996, filed on June 1, 2005,

☐ as amended on _____ (if applicable);

I/we believe that I/we am/are the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought;

I/we have reviewed and understand the contents of the above-identified application, including the claims, as amended by any amendment specifically referred to above;

I/we acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me/us to be material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT International filing date of the continuation-in-part application.

All statements made herein of my/our own knowledge are true, all statements made herein on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and may jeopardize the validity of the application or any patent issuing thereon.

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☐ Additional inventors or a legal representative are being named on _____ additional form(s) attached hereto.

Application Data Sheet

Application Information

Application Number::	
Filing Date::	
Application Type::	Regular
Subject Matter::	Utility
Suggested classification::	
Suggested Group Art Unit::	
CD-ROM or CD-R?::	None
Number of CD disks::	
Number of copies of CDs::	
Sequence submission?::	
Computer Readable Form (CRF)?::	No
Number of copies of CRF::	
Title::	COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION
Attorney Docket Number::	0800-0045.01
Request for Early Publication?::	No
Request for Non-Publication?::	No
Suggested Drawing Figure::	
Total Drawings Sheets::	3
Small Entity?::	No
Latin Name::	
Variety denomination name::	
Petition included?::	No
Petition Type::	
Licensed US Govt. Agency::	
Contract or Grant Numbers::	
Secrecy Order in Parent Appl.?::	No

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DOMESTIC PRIORITY INFORMATION

Application::	Continuity Type::	Parent Application::	Parent Filing Date::
This Application	Claims the benefit under 35 USC 120 of	11/141,996	June 1, 2005
and	Claims the benefit under 35 USC 119(e) of	60/575,997	June 1, 2004
and	Claims the benefit under 35 USC 119(e) of	60/639,222	December 22, 2004

FOREIGN PRIORITY INFORMATION

Country::	Application Number::	Filing Date::	Priority Claimed::

ASSIGNEE INFORMATION

Assignee name::

Street of mailing address::

City of mailing address::

State of mailing address::

Country of mailing address::

Zip Code of mailing address::

0800-0045.01
PATENT

UTILITY PATENT APPLICATION

COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

Inventor: John Fraser Wright

Guang Qu

Entity: Large

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0800-0045
PATENT

COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. application serial no. 11/141,996, from which application priority is claimed pursuant to 35 U.S.C. §120; which application claims the benefit under 35 U.S.C. § 119(e) of provisional applications 60/575,997 filed June 1, 2004 and 60/639,222 filed December 22, 2004. The foregoing applications are hereby incorporated by reference in their entireties.

FIELD OF THE INVENTION

The present invention relates to compositions and methods of preparing and storing AAV virions that prevent aggregation.

BACKGROUND

Recombinant adeno-associated virus (rAAV) is a promising vector for human gene transfer. Grimm, D., and Kleinschmidt, J. A. (1999) *Hum Gene Ther.* 10: 2445-2450; High, K. A. (2001) *Ann. N. Y. Acad. Sci.* 953: 64-67; Pfeifer, A., and Verma, I. M. (2001) *Ann. Rev. Genomics Hum. Genet.* **2**: 177-211. AAV is a member of the Dependovirus genus of the parvoviruses. AAV serotype 2 (AAV2) is composed of a single-strand DNA molecule of 4680 nucleotides encoding replication (*rep*) and encapsidation (*cap*) genes flanked by inverted terminal repeat (ITR) sequences. Berns, K. I. (1996) in *Fields Virology* (B. N. Fields *et. al.* Eds.), pp. 2173-2197. Lippincott-Raven Publishers, Philadelphia. The genome is packaged by three capsid proteins (VP1, VP2 and VP3), which are amino-terminal variants of the *cap* gene

product. The resulting icosahedral virus particle has a diameter of ~26 nm. A high resolution crystal structure of AAV2 has been reported. Xie, Q. *et al.* (2002) *Proc. Natl. Acad. Sci. U.S.A.* **99**: 10405-10410.

The solubility of purified AAV2 virus particles is limited, and aggregation of AAV2 particles has been described as a problem. Croyle, M. A. *et al.* (2001) *Gene Therapy* 8: 1281-1290; Huang, J. *et al.* (2000) *Mol. Therapy* 1: S286; Wright, J. F. *et al.* (2003) *Curr. Opin. Drug Disc. Dev.* 6: 174-178; Xie, Q. *et al.* (2004) *J. Virol. Methods* 122: 17-27. In commonly used buffered-saline solutions, significant aggregation occurs at concentrations of 10^{13} particles/mL, and aggregation increases at higher concentrations. Huang and co-workers reported that AAV vectors undergo concentration-dependent aggregation. Huang, J. *et al.* (2000) *Mol. Therapy* 1: S286. Xie and coworkers (Xie, Q. *et al.* (2004) *J. Virol. Methods* 122: 17-27) similarly reported that at concentrations exceeding 0.1mg/mL, AAV2 vectors require elevated concentrations of salt to prevent aggregation. Aggregation of AAV2 vectors occurs at particle concentrations exceeding 10^{13} particles/mL in commonly used neutral-buffered solutions such as phosphate- and Tris-buffered saline. This corresponds to a protein concentration of ~0.06 mg/mL, and emphasizes the low solubility of AAV2 under these conditions. The effective vector concentration limit may be even lower for vectors purified using column chromatography techniques because excess empty capsids are co-purified and contribute to particle concentration.

Particle aggregation is a significant and not fully resolved issue for adenovirus vectors as well. Stability of a recently established adenovirus reference material (ARM) was recently reported. Adadevoh, K. *et al.* (2002) *BioProcessing* 1(2): 62-69. Aggregation of the reference material, formulated in 20mM Tris, 25 mM NaCl, and 2.5% glycerol at pH 8.0, was assessed by dynamic light scattering, photon correlation spectroscopy and visual appearance. A variable

level of vector aggregation following either freeze-thaw cycling or non-frozen storage was observed, resulting in restrictive protocols for the use of the ARM.

Aggregation can lead to losses during purification and inconsistencies in testing of purified vector preparations. The *in vivo* administration of AAV2 vectors to certain sites, such as the central nervous system, may require small volumes of highly concentrated vector, and the maximum achievable dose may be limited by low vector solubility.

Vector aggregation is also likely to influence biodistribution following *in vivo* administration, and cause adverse immune responses to vectors following their administration. As has been reported for proteins (Braun, A. *et al.* (1997) *Pharm. Res.* 14: 1472-1478), aggregation of vector may increase immunogenicity by targeting the vector to antigen presenting cells, and inducing enhanced immune responses to the capsid proteins and transgene product. The reports of immune responses to AAV vectors in pre-clinical (Chenuaud, P. *et al.* (2004) *Blood* 103: 3303-3304; Flotte, T. R. (2004) *Human Gene Ther.* 15: 716-717; Gao, G. *et al.* (2004) *Blood* 103: 3300-3302) and clinical (High, K. A. *et al.* (2004) *Blood* 104: 121a) studies illustrate the need to address all factors that may contribute to vector immunogenicity.

Testing protocols to characterize purified vectors are also likely to be affected by vector aggregation. Determination of the infectivity titer of vector was reported to be highly sensitive to vector aggregation. Zhen, Z. *et al.* (2004) *Human Gene Ther.* 15: 709-715. An important concern is that vector aggregates may have deleterious consequences following their *in vivo* administration because their transduction efficiency, biodistribution and immunogenicity may differ from monomeric particles. For example, intravascular delivery of AAV vectors to hepatocytes requires that the vectors pass through the fenestrated endothelial cell lining of hepatic sinusoids. These fenestrations have a radius ranging from 50 to 150 nm (Meijer, K. D.

F., and Molema, G. (1995) *Sem. Liver Dis.* 15: 206) that is predicted to allow the passage of monomeric AAV vectors (diameter ~26 nm), but prevent the passage of larger vector aggregates. In biodistribution studies in mice, aggregated AAV2 vectors labeled with the fluorescent molecule Cy3 were sequestered in liver macrophages following vascular delivery. Huang, J. *et al.* (2000) *Mol. Therapy* 1: S286.

Formulation development for virus-based gene transfer vectors is a relatively recent area of investigation, and only a few studies have been reported describing systematic efforts to optimize AAV vector formulation and stability. Croyle, M. A. *et al.* (2001) *Gene Therapy* 8: 1281-1290; Wright, J. F. *et al.* (2003) *Curr. Opin. Drug Disc. Dev.* 6: 174-178; Xie, Q. *et al.* (2004) *J. Virol. Methods* 122: 17-27. Defining formulations compatible with pre-clinical and clinical applications that minimize changes in vector preparations is an important requirement to achieve consistently high vector safety and functional characteristics. As is well established for protein therapeutics (Chen, B. *et al.* (1994) *J. Pharm. Sci.* 83: 1657-1661; Shire, S. J. *et al.* (2004) *J. Pharm. Sci.* 93: 1390-1402; Wang, W. (1999) *Int. J. Pharm.* 185: 129-188; Won, C. M. *et al.* (1998) *Int. J. Pharm.* 167: 25-36), an important aspect of vector stability is solubility during preparation and storage, and vector aggregation is a problem that needs to be fully addressed. Vector aggregation leads to losses during vector purification, and while aggregates can be removed by filtration, the loss in yield results in higher costs and capacity limitations when producing vector for pre-clinical and clinical studies. Even after filtration to remove aggregates, new aggregates can form in concentrated preparations of AAV2 vector in buffered-saline solutions.

The need exists for improved formulations and methods for purification and storage of AAV vectors, such as rAAV2, that prevent aggregation of virus particles.

SUMMARY OF THE INVENTION

These and other needs in the art are met by the present invention, which provides high ionic strength solutions for use in preparing and storing AAV vectors that maintain high infectivity titer and transduction efficiency, even after freeze-thaw cycles.

In one aspect the invention relates to methods of preventing aggregation of virions in a preparation of virions by adding excipients to achieve an ionic strength high enough to prevent aggregation. In another aspect the invention relates to compositions of virions having an ionic strength high enough to prevent aggregation.

In some embodiments of the invention, the ionic strength is at least about 150mM, 200mM, 250mM, 300mM, 350mM, 400mM, 450mM, 500mM, 600mM, 700mM or more. In some embodiments this ionic strength is accomplished using excipients comprising one or more multivalent ions, for example citrate, sulfate, magnesium or phosphate.

In additional embodiments, the osmolarity of the preparation of virions is maintained at near isotonic levels, for example 200mOsm, 250mOsm, 280mOsm, 300mOsm, 350mOsm or 400mOsm, even though the ionic strength is high enough to prevent virion aggregation.

In some embodiments the virions are adeno-associated virus (AAV) virions, for example AAV-2.

In other embodiments of the methods of the present invention preparations of virions are treated with a nuclease, for example Benzonase®. In further embodiments, nuclease treatment is combined with addition of excipients that achieve an ionic strength high enough to prevent aggregation.

In some embodiments of the present invention, the surfactant Pluronic® F68 is added to a preparation of virions, for example to 0.001%. In one embodiment, the composition comprises purified virus particles, 10 mM Tris pH 8.0, 100mM sodium citrate and 0.001% Pluronic® F68.

In one embodiment, AAV vectors can be stored as compositions of the present invention at concentrations exceeding $1x10^{13}$ vg/mL, for example $2x10^{13}$, $3x10^{13}$, $4x10^{13}$, $5x10^{13}$ and up to $6.4x10^{13}$ vg/mL, without significant aggregation. In some embodiments, AAV vectors stored using the methods and compositions of the invention do not exhibit significant aggregation when stored at 4°C for five days. In other embodiments, AAV vectors that are stored as such compositions do not exhibit significant aggregation after one, five, ten or more freeze-thaw cycles at -20°C or at -80°C.

In some embodiments, preparations of virions stored according to the methods and compositions of the invention exhibit an average particle radius (*Rh*), as measured by dynamic light scattering, indicating that no significant aggregation of virions has taken place. In some embodiments, preparations of virions stored according to the methods and compositions of the invention exhibit an average particle radius (*Rh*) greater than about 15nm, 20nm, or 30nm.

In some embodiments, recovery of virions from preparations of virions stored according to the methods and compositions of the invention is greater than about 85%, 90% or 95% following filtration through a 0.22μm filter.

In yet another aspect, the invention relates to kits comprising the high ionic strength formulations of the invention. In one embodiment the kit comprises a pre-mixed solution of excipients. In another embodiment the kit comprises two or more separate components of a high ionic strength composition of the present invention to be mixed by a user. In some embodiments the kit comprises sodium citrate, Tris® and Pluronic® F68. In other embodiments, the kit further

comprises instructions for making a composition or performing a method of the present invention.

DESCRIPTION OF THE DRAWINGS

FIGS. 1A and 1B present data showing aggregation of AAV2-FIX particles as a function of osmolarity (FIG. 1A) or ionic strength (FIG. 1B) for various buffer compositions. AAV2-FIX vectors are prepared by Method 2 of Example 1. Average particle radius is measured by dynamic light scattering (DLS) following vector dilution in varying concentrations of excipients buffered with 10 mM sodium phosphate at pH 7.5. Excipients include sodium chloride (●), sodium citrate (○), sodium phosphate (■), sodium sulfate (□), magnesium sulfate (▲), and glycerol (Δ).

FIG. 2 presents data on AAV2-FIX aggregation as a function of the method of purification. The average particle radius is measured by DLS following vector dilution in varying concentrations of sodium chloride buffered with10mM sodium phosphate at pH 7.5. Vectors are purified by Method 1 (double CsCl gradient) (○); Method 2 (cation exchange chromatography) (□); Method 2 plus nuclease digestion (■); or Method 3 (chromatography plus one CsCl gradient) (Δ). Purification Methods 1-3 are described in Example 1.

FIG. 3 presents data on transgene expression from D7/4 cells transduced with rAAV2-AADC virions prepared and stored in high ionic strength formulation (□) or in a control formulation (●). The concentration of AADC was measured by ELISA (in triplicate for each data point) 72 hours post-transduction. Error bars represent standard deviations.

DETAILED DESCRIPTION OF THE INVENTION

AAV2 vector aggregation is frequently observed in concentrated preparations of vectors and can affect purification recovery, and *in vivo* potency and safety. Hence, an important objective for the development AAV2 vectors is to identify methods and formulations that prevent aggregation of vectors when concentrated stocks are prepared.

Unless otherwise indicated, the term "vector" as used herein refers to a recombinant AAV virion, or virus particle, regardless of the frequent use of "vector" to also refer to non-viral DNA molecules, such as plasmids, in other contexts.

The present invention is based in part on the observation that solution ionic strength is an important parameter in AAV vector aggregation, implicating the involvement of ionic interactions between virus particles in the aggregation process. The observation that elevated ionic strength increases AAV2 vector solubility regardless of the identity of the charged excipient supports the hypothesis that ionic strength of solution *per se*, rather than interactions involving a specific ionic species, is the relevant physico-chemical parameter. A threshold ionic strength of at least 200mM is required to prevent aggregation at vector particle concentrations examined herein.

Of practical concern, commonly used buffered saline solutions have insufficient ionic strength to prevent AAV2 vector aggregation at concentrations exceeding 10^{13} particles/mL. It is known that high salt concentrations increase AAV2 vector solubility (e.g. highly concentrated AAV2 vectors recovered from gradients generally remain soluble in concentrated CsCl). However, optimal formulations for pre-clinical and clinical studies should be close to isotonic (280-400 mOsm), especially for *in vivo* administration of vector to sites where dilution of hypertonic solutions may be slow. In embodiments of the present invention the exponential

relationship of ionic strength with charge valency is used to develop isotonic formulations with high ionic strengths. Salt species with multiple charge valencies (e.g. salts of sulfate, citrate, and phosphate) that are commonly used as excipients in human parenteral formulations can provide the level of ionic strength needed to prevent AAV2 vector aggregation when used at isotonic concentrations. While isotonic (150mM) sodium chloride has an ionic strength of 150mM, a value insufficient to maintain AAV2 solubility at high vector concentrations, isotonic sodium citrate, with an ionic strength of ~500mM, can support AAV2 vector concentrations of at least 6.4×10^{13} vg/mL without aggregation.

Without intending to be limited by theory, the low solubility of AAV2 particles may be caused by their highly symmetrical nature in conjunction with the stabilizing effect of complementary charged regions between neighbouring particles in aggregates. The surface charge density based on the crystal structure of AAV2 (Xie, Q. *et al.* (2002) *Proc. Natl. Acad. Sci. U.S.A.* 99: 10405-10410) reveals a pattern of positive and negative charges on the virus surface. Previous reports have shown that AAV2 vector aggregation is pH dependent, and hypothesized that amino acids with charged side groups are involved in inter-particle binding. Qu, G. *et al.* (2003) *Mol. Therapy* 7: S238. These reports hypothesized that if charged amino acid side chains are involved in vector aggregation, high concentrations of free amino acids could block vector particle interactions. However, we have found that amino acids with charged side chains are not effective in preventing AAV2 vector aggregation beyond their contribution to ionic strength.

Vector aggregation at low ionic strength was also found to be reduced but not prevented by efficient nuclease treatment of purified vector particles. Digestion at an earlier stage of the purification process (clarified HEK cell lysate) did not reduce aggregation following vector

purification. It is likely that digestion of already purified virions is more efficient because of a higher enzyme to nucleic acid substrate ratio. One mechanism to explain these results is that residual nucleic acid impurities (e.g. host cell and plasmid DNA) bound to the vector surface can bridge to binding sites on neighbouring virus particles and thus cause aggregation. Purified AAV2 vectors (empty capsid free) have been reported to contain approximately 1% non-vector DNA. Smith, P. *et al.* (2003) *Mol. Therapy* 7: S348. While >50% of this non-vector DNA was reported to be nuclease resistant and was packaged within capsid particles, some impurity DNA was nuclease resistant and appeared to be associated with the surface of purified vector particles. The observation that efficient nuclease treatment can reduce vector aggregation suggests that nucleic acids associated with the vector surface at an average level not greater than ~25 nucleotides per vector particle can contribute to AAV vector aggregation.

In summary, the use of high ionic strength solutions during AAV2 vector purification and final formulation, and efficient removal of residual vector surface DNA are two effective strategies to achieve highly concentrated solutions of AAV2 vectors for use in pre-clinical and clinical studies. High ionic strength solutions and nuclease treatment can be used in combination or separately. Although data were obtained using AAV2 vectors, the composition and methods of the present invention may also be useful with other AAV serotypes / variants, or other viral vectors such as adenoviruses, lentiviruses and retroviruses.

AAV Aggregation as a Function of Excipient Concentration

Initial screening experiments are performed to elucidate the mechanism of AAV vector aggregation and to identify classes of excipients that can reduce / prevent aggregation. Vector aggregation can be caused by dilution (5-fold) of vector in neutral-buffered saline with low

concentration buffer (20mM sodium phosphate, pH 7.2). Excipients are screened using this "dilution-stress" method to identify excipients that are able to prevent vector aggregation when included in the diluent. For screening, aggregation is measured by dynamic light scattering (DLS). Classes of excipients examined included selected inorganic salts, amino acids, uncharged carbohydrates, and surfactants. Results are presented in Table 1.

TABLE 1

SCREENING FOR EXCIPIENTS THAT PREVENT AAV2 VECTOR AGGREGATION USING DILUTION-STRESS METHOD

Excipient	Osm required to prevent aggregation (max tested)
Magnesium sulfate	180 mOsm
Sodium citrate	220 mOsm
Sodium chloride	320 mOsm
Sodium phosphate	220 mOsm
Sodium sulfate	220 mOsm
Arginine	NIA (200 mOsm)
Aspartic acid	320 mOsm
Glutamic acid	320 mOsm
Glycine	NIA (200 mOsm)
Histidine	NIA (200 mOsm)
Lysine	300 mOsm
Glycerol	NIA (5% w/v, 543 mOsm)
Iodixanol	NIA (5% w/v, 32 mOsm)
Mannitol	NIA (5% w/v, 275 mOsm)
Sorbitol	NIA (5% w/v, 275 mOsm)
Sucrose	NIA (5% w/v, 146 mOsm)
Trehalose	NIA (5% w/v, 146 mOsm)
Pluronic® F68	NIA (10% w/v, 12 mOsm)
Polysorbate 80	NIA (1% w/v)

NIA: No inhibition of aggregation

As illustrated in Table 1, charged excipients (inorganic salts and amino acids) prevent aggregation when present at sufficient concentrations. However, salt concentrations required to prevent vector aggregation vary, ranging from 180 mOsm for magnesium sulfate, to 320 mOsm for sodium chloride. The amino acids arginine, aspartic acid, glutamic acid, glycine, histidine, and lysine do not prevent aggregation at 200 mOsm, but lysine, aspartic acid, and glutamic acid prevent aggregation at 300-320 mOsm. Arginine, glycine and histidine were not tested at concentrations other than 200 mOsm. Selected carbohydrates have no effect on vector particle aggregation when present at concentrations up to 5% w/v. For example, 5% w/v glycerol (543 mOsm) does not prevent aggregation. The surfactants Polysorbate80 (1% w/v) and Pluronic® F68 (10% w/v) similarly have no effect on aggregation using the "dilution-stress" method.

AAV Aggregation as a Function of Osmolarity and Ionic Strength

FIGS. 1A and 1B show the results of a more detailed analysis of vector aggregation as a function of the concentration of various salts. FIG. 1A shows vector aggregation as a function of the osmolarity of selected excipients. For charged species a concentration-dependent inhibition of AAV2 vector aggregation is observed. Salts with multivalent ions achieve a similar degree of inhibition of aggregation at lower concentrations than monovalent sodium chloride. For example, magnesium sulfate prevents aggregation at $\geq$ 200 mOsm whereas sodium chloride requires $\geq$ 350 mOsm to achieve a similar effect. Sodium citrate, sodium sulfate, and sodium phosphate are intermediate in their potency to prevent vector aggregation.

Although the results in FIG. 1A and Table 1 show no effect of glycerol and certain sugars at concentrations up to 5% on AAV2 vector aggregation induced by low ionic strength, the data

cannot rule out improvement of AAV2 solubility at glycerol concentrations above 5%. For example, Xie and co-workers reported that 25% (w/v) glycerol enabled concentration of AAV2 to very high concentrations (4.4 to 18 x 10^{14} particles/ml) in low ionic strength solutions. Xie, Q. *et al.* (2004) *J. Virol. Methods* 122: 17-27.

FIG. 1B shows the data of FIG. 1A plotted as a function of the calculated ionic strength, rather than osmolarity, for each excipient. FIG. 1B demonstrates that vector aggregation is prevented when ionic strength is ~200 mM or greater regardless of which salt is used. These data suggested that the ionic strength (μ) of a solution, a parameter that depends on both solute concentration and charge valency, is the primary factor affecting aggregation.

Ionic strengths useful to prevent aggregation in embodiments of the present invention include, for example, 250 mM, 300 mM, 350 mM, 400 mM, 450 mM, 500 mM, 600 mM, 700 mM or higher ionic strengths. Multivalent ions are preferred to achieve these ionic strengths in methods and formulations of the present invention, such as divalent, trivalent, tetravalent, pentavalent ions and ions of even higher valency. The pH buffer in solutions and formulations of the present invention may be phosphate, Tris, or HEPES (or other Good's buffers), but any other suitable pH buffer may be used. In preferred embodiments, the multivalent ions and buffer are selected to be compatible with the target tissue for the vector being prepared.

Use of multivalent ions in the methods and compositions of the invention makes it possible to create compositions of high ionic strength but relatively low osmolarity. High ionic strength compositions of the present invention may be nearly isotonic, and may be, for example, about 200mOsm, 250mOsm, 280mOsm, 300mOsm, 350mOsm or 400mOsm, although other osmolarities may be acceptable for some uses of the compositions.

AAV Aggregation as a Function of the Method of AAV Purification

Recombinant AAV2 purified using different methods (e.g. density gradient purification versus ion-exchange chromatography) would be expected to have different impurity profiles. FIG. 2 shows vector aggregation as a function of ionic strength for several preparations of AAV differing in the purification method. Purification methods are described in Example 1. Sodium chloride is used to vary the ionic strength. AAV2-FIX vectors purified by double cesium chloride gradient ultracentrifugation (Method 1), by cation exchange column chromatography (Method 2), or by combined column and cesium chloride gradient ultracentrifugation (Method 3) each demonstrate similar aggregation responses as ionic strength is decreased. In contrast, AAV2-FIX purified by the column method and then subjected to a nuclease digestion step (Method 2 + nuclease) shows reduced aggregation at low ionic strength.

AAV Aggregation at Preparative Scale

The data in Table 1 and FIGS. 1A, 1B and 2 involve vector aggregation *at an analytical scale*, employing DLS to measure aggregation. Table 2, in contrast, shows the effects of elevated ionic strength and nuclease treatment on AAV2 vector aggregation at a larger scale, using methods to induce and quantify vector aggregation that are relevant to *preparative scale* vector purification. Experimental details are provided in Example 2. Purified AAV vectors are diafiltered into solutions of various ionic strengths, the volume is reduced to achieve high vector concentrations, and aggregation is then assessed by measuring vector recovery after filtration through a 0.22μm filter. Aliquots from a single pool of AAV2-AADC vector purified by Method 1 through the second CsCl gradient centrifugation step ($1.8x10^{15}$ vg in 91mL, $1.8x10^{13}$ vg/mL, in ~3M CsCl) are used as starting material in the diafiltration experiments. Tangential

flow filtration using hollow fibers is used for diafiltration because it is scalable and yet it still enables preparation of volumes (min. 1.4mL), and thus AAV concentrations, at which aggregation would be expected in neutral buffered saline.

In Experiment 1, three hollow fiber units are used to diafilter AAV2-AADC vector in formulations CF, TF1, or TF2, and the volume is reduced to a target of $2.5x10^{13}$ vg/mL. *See* Example 2. The samples are then filtered through a 0.22µm filter. Results are shown in Table 2. Vector recovery ("Yield %") for both elevated ionic strength formulations TF1 (95 ± 7.4%) and TF2 (93 ± 7.4%) are significantly higher than the recovery using the control formulation CF (77 ± 6.6%).

TABLE 2
AAV VECTOR RECOVERY AT PROCESS SCALE

Experiment	Formulation	µ (mM)	Target (vg/mL)	Actual (vg/mL)	Yield % (RSD)
1	CF	160	2.5E13	1.93E13	77 (6.6)
1	TF1	310	2.5E13	2.38E13	95 (7.4)
1	TF2	510	2.5E13	2.33E13	93 (7.4)
2	CF	160	6.7E13	3.98E13	59 (6.0)
2	TF2	510	6.7E13	6.42E13	96 (4.4)
3	CF (-Bz)	160	3.6E13	2.46E13	68 (11)
3	CF (+Bz)	160	3.6E13	3.29E13	91 (12)

In Experiment 2, AAV2-AADC is concentrated to a higher target value ($6.7x10^{13}$ vg/mL) in CF or TF2. Vector recovery using TF2 (96 ± 4.4%) is again significantly higher than recovery using CF (59 ± 6.0%). Within the variability of the assays used, vector was recovered fully at both target concentrations using TF2, indicating that aggregation was prevented. In contrast, significant aggregation was observed at both target concentrations using CF, and the extent of

aggregation (i.e. loss following 0.22μm filtration) was higher at the higher target vector concentration. In an additional experiment (not shown), 50 μL samples of AAV2 vector are taken following concentration but prior to the 0.22μm filtration step of Experiment 2, and examined by light microscopy. Vector concentrated in CF contains obvious amounts of visible material (not shown), while no such material is seen in vector concentrated in TF2.

Experiment 3 examines the effect of prior nuclease digestion of purified vector on aggregation. In the absence of nuclease digestion recovery of AAV2-AADC in CF is 68 ± 11%, similar to the recoveries in Experiments 1 and 2. In contrast, purified vector treated with nuclease and then concentrated in CF gives higher recovery (91 ± 12%). These prep scale results reflect the same effect of nuclease digestion shown in FIG. 2 using the "dilution-stress" (analytical scale) method.

The results presented in Table 2 demonstrate that the methods and compositions of the present invention increase the recovery of AAV vector recovery. For example, in various embodiments of the present invention, recovery is improved from less than about 80% to at least about 85%, 90%, 95% or more.

AAV Stability and Activity Following Storage or Freeze-Thaw Cycling

Croyle and coworkers reported a significant loss of titer of AAV and adenovirus following multiple freeze-thaw cycling in sodium phosphate buffer, and demonstrated that the better pH buffering provided by potassium phosphate during freeze-thaw cycling prevented titer loss. Croyle, M. A. *et al.* (2001) *Gene Therapy* 8: 1281-1290. Results of our freeze-thaw stability study using sodium phosphate support these findings. We find that while 150mM sodium phosphate provides sufficient ionic strength to prevent aggregation during preparation

and non-frozen storage of concentrated AAV2-AADC vector, even a single freeze-thaw cycle at -20 or -80 °C results in aggregation.

AAV stability after storage or freeze-thaw (F/T) cycling is assessed in buffers of the present invention as follows. The concentrated vectors prepared in CF, TF1, and TF2 (Table 2, Experiment 1) are subjected to a short stability study to investigate whether aggregation will occur during refrigerated storage, or following multiple freeze-thaw (F/T) cycles. Aggregation is assessed by DLS using undiluted samples, and *Rh* values >20nm are deemed to indicate the occurrence of some level of aggregation.

TABLE 3

STABILITY OF AAV2 VECTORS

	Particle radius – Rh (nm)							
Formulation	Pre	4 °C	- 20 °C			- 80 °C		
		5d	1 F/T	5 F/T	10 F/T	1 F/T	5 F/T	10 F/T
CF	14.5	27.0	22.4	56.1	94.5	20.6	57.5	141
TF1	13.8	16.3	TH	TH	TH	TH	TH	TH
TF2	13.8	14.4	14.2	14.0	14.1	13.8	21.3	50.9

Pre: DLS radius measured immediately following 0.2μm filtration.

Vector concentrations (vg/mL): CF: 1.93E13, TF1: 2.38E13, TF2: 2.33E13.

TH: signal intensity is too high to measure because of extensive aggregation.

As shown in Table 3, AAV2-AADC vector prepared in CF shows some aggregation after 5 days of storage at 4 °C, as well as following one or more F/T cycles at -20 or -80°C. For vector prepared in TF1, no aggregation occurs after 5 days at 4°C, but aggregation occurs following a single F/T cycle at -20 or -80 °C as indicated by a DLS signal intensity that is too

high to measure. Visual inspection of these samples reveals slight cloudiness, which is consistent with aggregation. For vector prepared in TF2, no aggregation is observed at 4 °C, or following up to 10 F/T cycles at -20 °C. Some aggregation is observed following 5 and 10 F/T cycles at -80 °C.

AAV activity after storage or F/T cycling in TF2 is assessed as follows. As described above, the high ionic strength, isotonic formulation TF2 effectively prevents vector aggregation during concentration and storage, and therefore represents a promising candidate for further study. An important question is whether preparation and storage of the vector in high ionic strength TF2 would adversely affect its functional activity. To assess this, assays are performed to measure the infectious titer and the transduction efficiency of vectors prepared and stored for an extended period of time in TF2.

For infectivity, a highly sensitive infectivity assay capable of detecting single infectious events is used. Zhen, Z. *et al.* (2004) *Human Gene Ther.* 15: 709-715. AAV2-AADC is prepared in TF2 at a concentration of $6.4x10^{13}$ vg/mL. After being stored for 45 days at 4 °C the preparation has a vector genome to infectious unit ratio (vg/IU) of 13, compared to a value of 16 vg/IU for the reference vector. This difference is not significant given the reported variability of this assay (RSD ~50%).

Transduction efficiency is assessed by measuring the expression of AADC protein by ELISA following transduction of D7/4 cells. FIG. 3 shows no significant difference between vector prepared in TF2 and the reference control for vector input ranging from 10 to 10^5 vg/cell. Together, these data indicate that preparation and storage of AAV2 vectors in high ionic strength TF2 does not have a deleterious effect on vector infectivity or transduction efficiency.

Conclusion

The effect of ionic strength (μ) on virus particle interactions is determined to elucidate the mechanism of vector aggregation. The ionic strength of neutral-buffered isotonic saline (μ = 150mM) is insufficient to prevent aggregation of AAV2 vectors purified by gradient ultracentrifugation or by cation exchange chromatography at concentrations exceeding ~10^{13} particles/mL. Inclusion of sugars (sorbitol, sucrose, mannitol, trehalose, glycerol) at concentrations up to 5% (w/v) or of surfactants Tween80® (1%) or Pluronic® F68 (10%) does not prevent aggregation of vector particles.

In contrast, vector particles remain soluble when elevated ionic strength solutions (μ > 200mM) are used during purification and for final vector formulation. Elevated ionic strength solutions using isotonic excipient concentrations for *in vivo* administration are prepared with salts of multivalent ions, including sodium citrate, sodium phosphate, and magnesium sulfate. An isotonic formulation containing 10mM Tris, 100mM sodium citrate, 0.001% Pluronic® F68, pH 8.0 (μ ~500mM) enables concentration of AAV2-AADC vectors to 6.4x10^{13} vg/mL with no aggregation observed during preparation and following ten freeze-thaw cycles at -20 °C. *See* Table 3, below, and accompanying discussion. AAV2-AADC vectors prepared and stored for an extended period in elevated ionic strength formulation retain high infectivity titer (13 IU/vg) and transduction efficiency.

Nuclease treatment of purified AAV2 vectors reduces the degree of vector aggregation, implicating vector surface nucleic acid impurities in inter-particle interactions. Hence, purification methods to efficiently remove vector surface residual nucleic acids, coupled with the use of elevated ionic strength isotonic formulations, are useful methods to prevent AAV2 vector aggregation.

EXAMPLE 1

AAV PURIFICATION METHODS

AAV2 vectors expressing human coagulation factor IX (FIX) or human amino acid decarboxylase (AADC) are produced by triple transfection of HEK293 cells as previously described (Matsushita, T. *et al.* (1998) *Gene Therapy* **5**: 938-945), with modifications. For the large scale preparations, cells are cultured and transfected in 850 mm^2 roller bottles (Corning). Vectors are purified by one of three methods.

In purification Method 1, modified from Matsushita, transfected HEK293 cells in roller bottles are collected by centrifugation (1000g, 15min), resuspended in 10mM sodium phosphate, 500mM sodium chloride, pH 7.2, and lysed by three freeze / thaw cycles (alternating an ethanol / dry ice bath and a 37°C water bath). The cell lysate is clarified by centrifugation (8,000g, 15 min). The supernatant is then diluted to 200mM NaCl by addition of 10mM sodium phosphate, pH 7.2, and digested with Benzonase® (Merck, Purity Grade 1; 200 U/mL, 1h, 37 °C). The lysate is adjusted to 25mM $CaCl_2$ using a 1M stock solution, and incubated at 4°C for one hour.

The mixture is centrifuged (8,000g, 15 min), and the supernatant containing vector is collected. To precipitate virus from the clarified cell lysate, polyethylene glycol (PEG8000) is added to a final concentration of 8%, the mixture incubated at 4°C for three hours, and then centrifuged (8,000g, 15 min). The pellets containing vector are re-suspended with mixing in 0.15M NaCl, 50mM Hepes, 25mM EDTA, pH 8.0 and incubated at 4°C for 16 hours. The resuspended material is pooled, and solid cesium chloride is added to a final density of 1.40

gm/ml. Vector is then banded by ultracentrifugation (SW28, 27,000rpm, 24h, 20°C) using a Beckman model LE-80 centrifuge. The centrifugation tubes are fractionated, and densities from 1.38 to 1.42 gm/mL containing vector are pooled. This material is banded a second time by ultracentrifugation (NVT65 rotor, 65,000 rpm, 16h, 20°C), and fractions containing purified AAV2 vectors are pooled. To concentrate vector and to perform buffer exchange, vectors in concentrated cesium chloride solution are subjected to ultrafiltration / diafiltration (UF/DF) by tangential flow filtration as described below (Example 2).

In purification Method 2, cell harvests containing AAV are microfluidized and filtered sequentially through 0.65 and 0.22 μm filters (Sartorius). Virus is purified from the clarified cell lysates by cation exchange chromatography using Poros HS50 resin as previously described. U.S. Pat. No. 6,593,123. For the nuclease digestion described in FIG. 2, column-purified vectors are incubated (4h, RT) with 100 U/mL Benzonase and 10 U/mL DNAse I (RNAse free, Roche Diagnostics, Indianapolis, Indiana).

For purification Method 3, AAV2 vectors purified by cation exchange chromatography are subjected to an additional cesium chloride gradient ultracentrifugation step (SW28, 27,000rpm, 20h) to remove empty capsids prior to UF/DF.

Real time quantitative PCR (Q-PCR) is used to quantify AAV preparations as previously described. Sommer, J. M. *et al.* (2003) *Mol. Therapy* **7**: 122-128. Vectors purified by each of the three methods are analyzed by SDS-PAGE / silver staining analysis, and in all cases VP1, VP2 and VP3 are present in the expected ratios, with the capsid proteins representing >95% of total proteins as determined by scanning densitometry. However, unlike gradient-purified AAV2 vectors purified using Methods 1 and 3, vectors purified by Method 2 (column chromatography) contain empty capsids, ranging from 3-10 empty capsids per vector genome.

EXAMPLE 2

ULTRAFILTRATION AND DIAFILTRATION TO DETECT AAV AGGREGATION

Disposable hollow fiber tangential flow filtration devices (Amersham BioSciences 8" Midgee, 100 kDa nominal pore size) are used to concentrate and diafilter AAV2 vectors purified by the methods described above, and for the UF/DF experiments described in Table 2. For all UF/DF procedures a volume of diafiltration buffer corresponding to 10x the product volume is used, and it is added in ~1mL increments to approximate continuous diafiltration. Using this method, the calculated residual CsCl after diafiltration is <0.5mM.

The following three formulations were used for UF/DF: Control Formulation (CF: 140mM sodium chloride, 10mM sodium phosphate, 5% sorbitol, pH 7.3); Test Formulation 1 (TF1: 150mM sodium phosphate, pH7.5); and Test Formulation 2 (TF2: 100 mM sodium citrate, 10mM Tris, pH8.0). For Experiment 1 shown in Table 2, diafiltration is performed at a volume corresponding to a vector concentration of $1x10^{13}$ vg/mL, and following diafiltration the volume is reduced to a value corresponding to $2.5x10^{13}$ vg/mL (assuming no vector loss).

For Experiment 2, diafiltration is performed at a volume corresponding to a $2x10^{13}$ vg/mL, and the volume is then reduced to a value corresponding to $6.7x10^{13}$ vg/mL.

For Experiment 3 (CF ± Bz), AAV2-AADC (approximately $1.2x10^{14}$ vg) is first diafiltered into TF1 (a formulation compatible with nuclease activity) and then passed through a 0.22 μm filter. The titer of this material is determined, and the volume is adjusted to correspond to a concentration of $1x10^{13}$ vg/mL. To 10 mL of this material, $MgCl_2$ is added to a concentration of 2 mM, and then divided into two equal aliquots. One aliquot is incubated with Benzonase (200 U/mL, 4h, RT), and the second is mock-incubated. Each aliquot is then

diafiltered at a volume corresponding to a vector concentration $2x10^{13}$ vg/mL, and then concentrated to a $3.6x10^{13}$ vg/mL target. Following all UF/DF protocols, Pluronic® F-68 (BASF Corp., Mount Olive, NJ) from a 1% stock is added to the vector product to a final concentration of 0.001%, and the solution is passed through a 0.22μm syringe filter (Sartorius). All UF/DF procedures are performed in a laminar flow cabinet.

EXAMPLE 3

MEASUREMENT OF VECTOR AGGREGATION BY DYNAMIC LIGHT SCATTERING

Purified vectors are analyzed for aggregation by dynamic light scattering (DLS) using a Protein Solutions *DynaPro 99* (λ=825.4 nm). Primary data (particle radius – *Rh*, average value measured over 30 cycles, 10 cycles/min) are used for all analyses reported. A "dilution-stress" method is used to assess the effect of varying excipients on vector aggregation. In this method, 80 μL of test diluent is added to 20 μL of vector solution with mixing in the actual cuvette used for DLS measurement, and data collection is initiated within 10 seconds of mixing. Prior to addition of test diluents, the *Rh* value for AAV2 vector preparations is measured and confirmed to be <15 nm to ensure that the starting material is monomeric. Samples that are not 100% monomeric are passed through a 0.22μm syringe disc filter (Sartorius, low protein binding) to remove aggregates.

The osmolarity and ionic strength values given in FIGS. 1 and 2 are calculated using all excipients present in the mixture (i.e. weighted: test diluent (80%) and starting vector formulation (20%)). Osmolarity is calculated according to the equation: Osmolarity = $\sum c_i$, where c_i is the molar concentration of each solute species. The ionic strength (μ) is calculated

according to the equation: $\mu = \frac{1}{2} \sum c_i z_i^2$, where z_i is the charge on each species. In conditions that resulted in vector aggregation (e.g. low μ) a progressive increase in *Rh* is observed over the course of data collection. To validate the use of the average *Rh* measured over the 3 minute interval following dilution as a reliable measure of aggregation, the average rate of increase of *Rh* (ΔRh / Δt) over the same time interval is also determined (not shown). Analysis of ΔRh / Δt gives results concordant with those obtained using the average *Rh* value reported in FIGS. 1 and 2.

EXAMPLE 4

AAV VIRION INFECTIVITY

Infectivity of AAV2-AADC vectors is determined using a highly sensitive assay as previously described. Zhen, Z. *et al.* (2004) *Human Gene Ther.* 15: 709-715. Briefly, samples are serially diluted (10-fold dilutions, 10 replicates / dilution) and added to D7/4 cells (modified HeLa cells expressing AAV *rep* and *cap*) grown in 96 well tissue culture plates (Falcon, cat. #353227) in DMEM medium containing 10% FBS. Adenovirus (Ad-5, 100 vp/cell) is added to each well to provide helper functions. After 48h, replication of AAV vector in each well is quantified by Q-PCR using transgene-specific primers and probes, and the frequency of infection at limiting dilution is analyzed by the Karber method to calculate the infectivity titer. The test sample is run concurrently with an AAV2-AADC reference previously prepared in CF and stored at -80 °C.

The transduction efficiency of AAV2 vectors is quantified by a whole cell ELISA. D7/4 cells grown in 96 well plates are infected with 10-fold serial dilutions of the test sample and reference vector, corresponding to 10 to 10^5 vg / cell input (5 replicates / dilution). After 48h,

the culture medium is removed, and cells are washed twice with 200 µL PBS (10 mM sodium phosphate, 140mM sodium chloride, pH 7.2). Cells are then permeabilized and fixed by addition of 100µL of PBS containing 0.5% Triton X-100 and 4% paraformaldehyde to each well (15 min). The fixing solution is removed, and the cells are washed twice with PBS containing 0.5% Triton X-100. Non-specific sites are blocked by adding PBS containing 3% bovine serum albumin (BSA) and 0.5% Triton X-100 (60min).

After washing, cells are incubated for one hour with rabbit anti-AADC IgG antibody (Chemicon, AB136), and washed. Cells are then incubated for one hour with alkaline phosphatase-conjugated goat anti-rabbit IgG, and washed. Antibodies are diluted 1:1000 in PBS containing 1% BSA, 0.5% Triton X-100. Substrate (PNPP, Pierce, cat. #34047) is then added (1 mg/mL in 1X diethanolamine substrate buffer, Pierce, cat. #34064), and after incubation for 30min the concentration of cleaved substrate is measured spectrophotometrically (λ=405nm). Human AADC expression as a function of vector input is fitted using a spline curve (SigmaPlot). The AAV2-AADC reference vector is measured concurrently with the test sample.

While preferred illustrative embodiments of the present invention are described, it will be apparent to one skilled in the art that various changes and modifications may be made therein without departing from the invention, and it is intended in the appended claims to cover all such changes and modifications that fall within the true spirit and scope of the invention.

All publications, patents and patent applications referred to herein are hereby incorporated by reference in their entireties.

We claim:

1. A composition for the storage of purified virus particles, comprising:

purified virus particles;

a pH buffer; and

excipients comprising one or more multivalent ions; wherein the ionic strength of the composition is greater than about 200 mM.

2. The composition of claim 1, wherein the purified virus particles are AAV virus particles.

3. The composition of claim 1, wherein one of the one or more multivalent ions is citrate.

4. The composition of claim 1, further comprising Pluronic® F68.

5. The composition of claim 4, wherein the Pluronic® F68 is present at 0.001%.

6. The composition of claim 1, wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate.

7. The composition of claim 1, wherein the average particle radius (*Rh*) of the purified virus particles is less than about 20nm as measured by dynamic light scattering.

8. The composition of claim 1, wherein recovery of the purified virus particles is at least about 90% following filtration of the composition of virions through a 0.22μm filter.

9. A method of preventing aggregation of virions in a preparation of virions, comprising treating said preparation of virions with Benzonase®.

10. The method of claim 9, wherein, after Benzonase® treatment, the average particle radius (*Rh*) of the virions in the preparation of virions is less than about 20nm as measured by dynamic light scattering.

11. The method of claim 9, wherein, after Benzonase® treatment, recovery of the virions is at least about 90% following filtration of the preparation of virions through a 0.22μm filter.

ABSTRACT

Compositions and methods are provided for preparation of concentrated stock solutions of AAV virions without aggregation. Formulations for AAV preparation and storage are high ionic strength solutions (e.g. μ ~500mM) that are nonetheless isotonic with the intended target tissue. This combination of high ionic strength and modest osmolarity is achieved using salts of high valency, such as sodium citrate. AAV stock solutions up to $6.4x10^{13}$ vg/mL are possible using the formulations of the invention, with no aggregation being observed even after ten freeze-thaw cycles. The surfactant Pluronic® F68 may be added at 0.001% to prevent losses of virions to surfaces during handling. Virion preparations can also be treated with nucleases to eliminate small nucleic acid strands on virions surfaces that exacerbate aggregation.

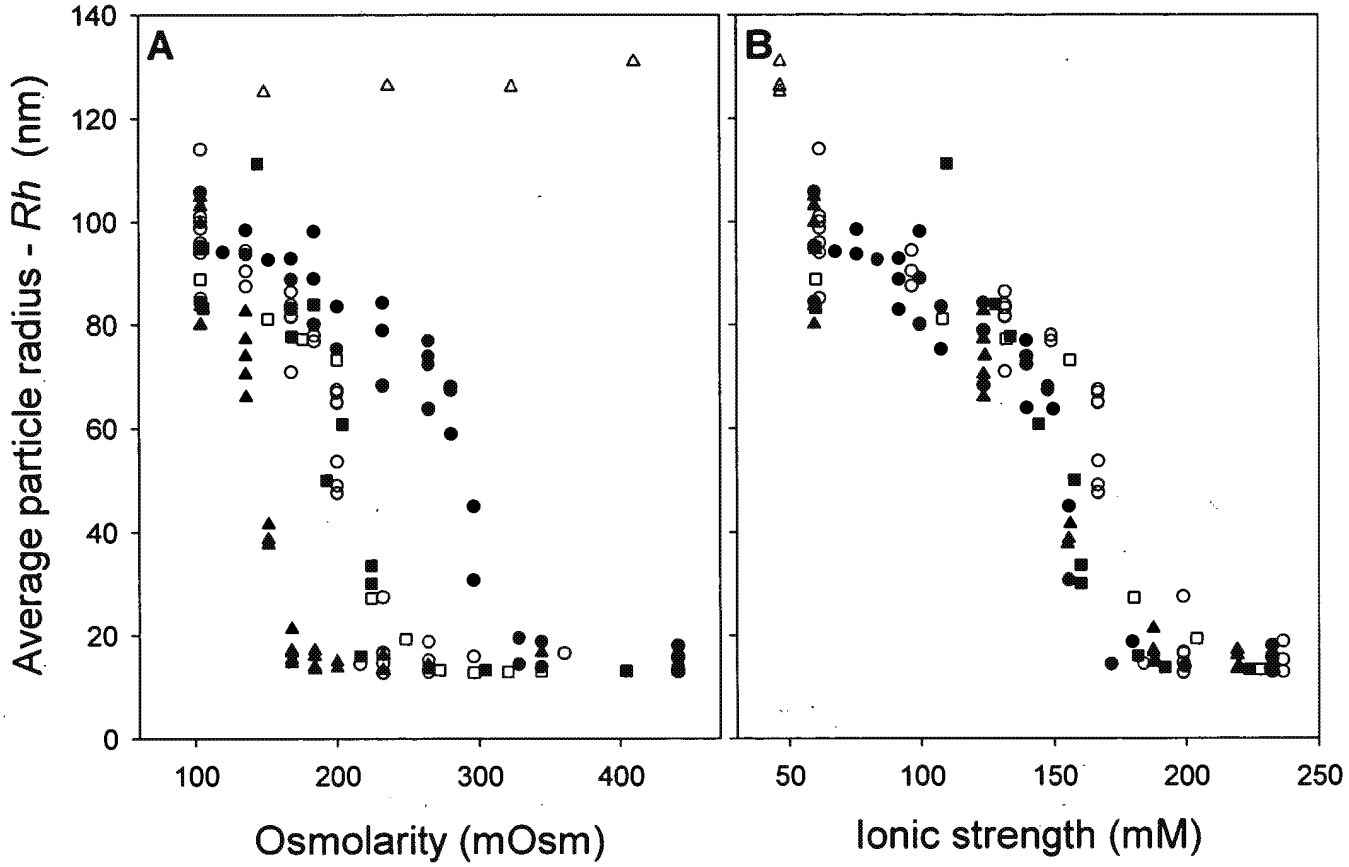

A
B
Average particle radius - Rh (nm)
140
120
100
80
60
40
20
0
100
200
300
400
Osmolarity (mOsm)
50
100
150
200
250
Ionic strength (mM)

FIGURE 1

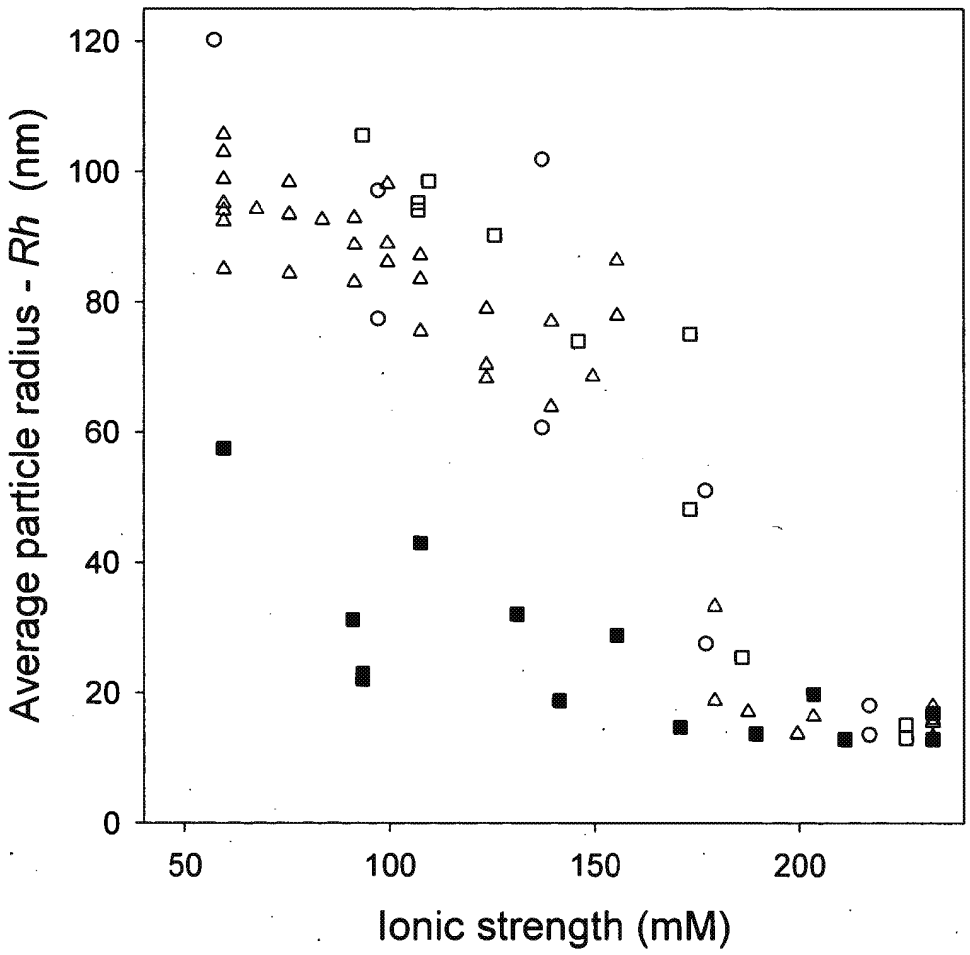

Average particle radius - Rh (nm)
120
100
80
60
40
20
0
50
100
150
200
Ionic strength (mM)

FIGURE 2

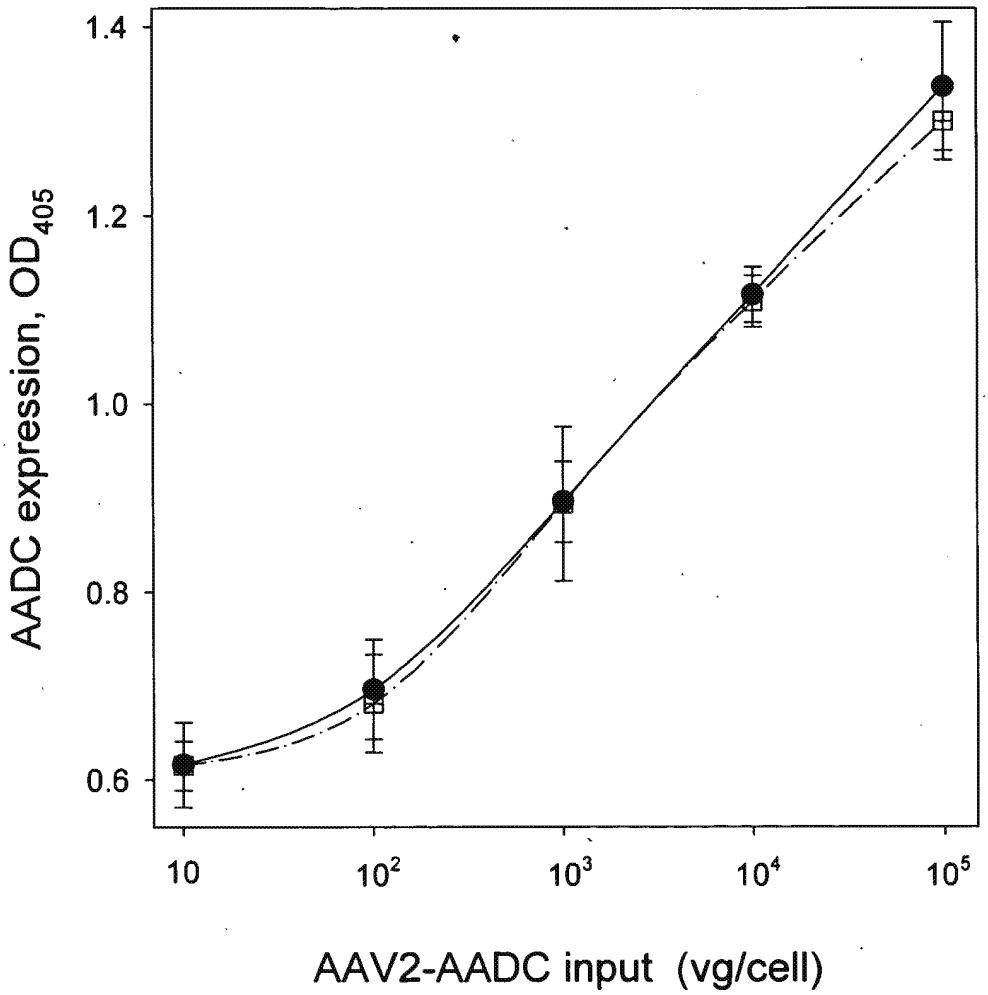

AADC expression, OD405
1.4
1.2
1.0
0.8
0.6
10
10^2
10^3
10^4
10^5
AAV2-AADC input (vg/cell)

FIGURE 3

PATENT APPLICATION SERIAL NO._____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

03/23/2010 HDESTA1 00000018 12661553

01 FC:1011 330.00 OP
02 FC:1111 540.00 OP
03 FC:1311 220.00 OP

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

12/661,553

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY FEE ($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))							330
SEARCH FEE (37 CFR 1.16(k), (i), or (m))							540
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))							220
TOTAL CLAIMS (37 CFR 1.16(i))	11 minus 20 =		X 26 =		OR	X 52 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	2 minus 3 =		X 110 =			X 220 =	
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $270 ($135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))			N/A			N/A	
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	1090

APPLICATION AS AMENDED – PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY ADDI-TIONAL FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY ADDI-TIONAL FEE ($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	X =		OR	X =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X =		OR	X =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))					N/A		OR	N/A	
						TOTAL ADD'T FEE		OR	TOTAL ADD'T FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDI-TIONAL FEE ($)	OR	RATE ($)	ADDI-TIONAL FEE ($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	X =		OR	X =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X =		OR	X =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))					N/A		OR	N/A	
						TOTAL ADD'T FEE		OR	TOTAL ADD'T FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
12/661,553	03/19/2010	1657	1090	0800-0045.01	11	2

CONFIRMATION NO. 4726

20855
ROBINS & PASTERNAK
1731 EMBARCADERO ROAD
SUITE 230
PALO ALTO, CA 94303

FILING RECEIPT

OC000000040932096

Date Mailed: 04/05/2010

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Applicant(s)

John Fraser Wright, Princeton, NJ;
Guang Qu, Alameda, CA;

Power of Attorney: None

Domestic Priority data as claimed by applicant

This application is a CON of 11/141,996 06/01/2005
which claims benefit of 60/575,997 06/01/2004
and claims benefit of 60/639,222 12/22/2004

Foreign Applications

If Required, Foreign Filing License Granted: 04/01/2010

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 12/661,553**

Projected Publication Date: To Be Determined - pending completion of Corrected Papers

Non-Publication Request: No

Early Publication Request: No

Title

Compositions and methods to prevent AAV vector aggregation

Preliminary Class

435

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as

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This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

Atty Dkt No: 0800-0045.01
PATENT

CERTIFICATE OF TRANSMISSION PURSUANT TO 37 CFR § 1.8

I hereby certify pursuant to 37 CFR §1.8 that this correspondence is being transmitted via EFS to the United States Patent and Trademark Office on the date shown below.

Date 5/4/12 Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

JOHN FRASER WRIGHT et al. Confirmation No.: 4726

Application No.: 12/661,553 Art Unit: 1653

Filing Date: March 19, 2010 Examiner: Satyendra K. Singh

Title: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

TRANSMITTAL

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Sir:

In response to the Notice Regarding Power of Attorney mailed April 27, 2012 (attached), please find attached a copy of the executed Assignment filed in parent case 11/141,996 and a copy of the documents filed on April 17, 2012 including Transmittal, Power of Attorney and 3.73(b) Statement. The attached documents were filed in parent application no. 11/141,996 (now U.S. Patent No. 7,704,721) from which the instant application claims priority. Please enter and use the attached Power of Attorney and Statement for the instant application no. 12/661,553, filed March 19, 2010.

Respectfully submitted,

Date: 5/4/2012 By:

Roberta L. Robins
Registration No. 33,208
Attorney for Applicant

ROBINS & PASTERNAK LLP
1731 Embarcadero Road, Suite 230
Palo Alto, CA 94030
Tel: (650) 493-3400, Fax: (650) 493-3440

COPY

Atty Dkt No. 0800-0045

ASSIGNMENT

JOINT

THIS ASSIGNMENT, by **John Fraser Wright** and **Guang Qu** (hereinafter referred to as the assignors), residing at Princeton, New Jersey and Alameda, California respectively, witnesseth:

WHEREAS, the said assignors have invented certain new and useful improvements in COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION set forth in an application for Letters Patent of the United States, bearing Application No. 11/141,996 and filed on June 1, 2005; and

WHEREAS, **Avigen, Inc.**, a corporation duly organized under and pursuant to the laws of Delaware, and having its principal place of business at 1301 Harbor Bay Parkway, Alameda, CA 94502 (hereinafter referred to as the assignee) is desirous of acquiring the entire right, title and interest in and to said inventions and said application for Letters Patent of the United States, and in and to any Letters Patent or Patents, United States or foreign, to be obtained therefor and thereon:

NOW THEREFORE, in consideration of One Dollar ($1.00) and other good and sufficient considerations, the receipt of which is hereby acknowledged, the said assignors have sold, assigned, transferred and set over, and by these presents do sell, assign, transfer and set over, unto the assignee, its successors, legal representatives and assigns, the entire right, title and interest in and to the above-mentioned inventions, application for Letters Patent, and any and all Letters Patent or Patents in the United States of America and all foreign countries which may be granted therefor and thereon, and in and to any and all divisions, continuations, and continuations-in-part of said application, or reissues or extensions of said Letters Patent or Patents, and all rights under the International Union for the Protection of Industrial Property, the same to be held and enjoyed by the said assignee, for its own use and behoof and the use and behoof of its successors, legal representatives and assigns, to the full end of the term or terms for which Letters Patent or Patents may be granted, as fully and entirely as the same would have been held and enjoyed by the assignors, had this sale and assignment not been made.

AND for the same consideration, the said assignors hereby covenant and agree to and with the said assignee, its successors, legal representatives and assigns, that, at the time of execution and delivery of these presents, the said assignors are the sole and lawful owners of the entire right, title and interest in and to the said inventions and the application for Letters Patent above-mentioned, and that the same are unencumbered and that the said assignors have good and full right and lawful authority to sell and convey the same in the manner herein set forth.

AND for the same consideration, the said assignors hereby covenant and agree to and with the said assignee, its successors, legal representatives and assigns, that the said assignors will, whenever counsel of the said assignee, or the counsel of its successors, legal representatives and assigns, shall advise that any

proceeding in connection with said inventions, or said application for Letters Patent, or any proceeding in connection with Letters Patent for said inventions in any country, including interference proceedings, is lawful and desirable, or that any division, continuation or continuation-in-part of any application for Letters Patent or any reissue or extension of any Letters Patent, to be obtained thereon, is lawful and desirable, sign all papers and documents, take all lawful oaths, and do all acts necessary or required to be done for the procurement, maintenance, enforcement and defense of Letters Patent for said inventions, without charge to said assignee, its successors, legal representatives and assigns, but at the cost and expense of the said assignee, its successors, legal representatives and assigns.

AND the said assignors hereby request the Commissioner of Patents to issue said Letters Patent of the United States to the said assignee as the assignee of said inventions and the Letters Patent to be issued thereon for the sole use and behoof of the said assignee, its successors, legal representatives and assigns.

Date: 04 04 05

John Fraser Wright

Date: _____

Guang Qu

COPY

Atty Dkt No. 0800-0045

ASSIGNMENT

JOINT

THIS ASSIGNMENT, by **John Fraser Wright** and **Guang Qu** (hereinafter referred to as the assignors), residing at Princeton, New Jersey and Alameda, California respectively, witnesseth:

WHEREAS, the said assignors have invented certain new and useful improvements in COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION set forth in an application for Letters Patent of the United States, bearing Application No. 11/141,996 and filed on June 1, 2005; and

WHEREAS, **Avigen, Inc.**, a corporation duly organized under and pursuant to the laws of Delaware, and having its principal place of business at 1301 Harbor Bay Parkway, Alameda, CA 94502 (hereinafter referred to as the assignee) is desirous of acquiring the entire right, title and interest in and to said inventions and said application for Letters Patent of the United States, and in and to any Letters Patent or Patents, United States or foreign, to be obtained therefor and thereon:

NOW THEREFORE, in consideration of One Dollar ($1.00) and other good and sufficient considerations, the receipt of which is hereby acknowledged, the said assignors have sold, assigned, transferred and set over, and by these presents do sell, assign, transfer and set over, unto the assignee, its successors, legal representatives and assigns, the entire right, title and interest in and to the above-mentioned inventions, application for Letters Patent, and any and all Letters Patent or Patents in the United States of America and all foreign countries which may be granted therefor and thereon, and in and to any and all divisions, continuations, and continuations-in-part of said application, or reissues or extensions of said Letters Patent or Patents, and all rights under the International Union for the Protection of Industrial Property, the same to be held and enjoyed by the said assignee, for its own use and behoof and the use and behoof of its successors, legal representatives and assigns, to the full end of the term or terms for which Letters Patent or Patents may be granted, as fully and entirely as the same would have been held and enjoyed by the assignors, had this sale and assignment not been made.

AND for the same consideration, the said assignors hereby covenant and agree to and with the said assignee, its successors, legal representatives and assigns, that, at the time of execution and delivery of these presents, the said assignors are the sole and lawful owners of the entire right, title and interest in and to the said inventions and the application for Letters Patent above-mentioned, and that the same are unencumbered and that the said assignors have good and full right and lawful authority to sell and convey the same in the manner herein set forth.

AND for the same consideration, the said assignors hereby covenant and agree to and with the said assignee, its successors, legal representatives and assigns, that the said assignors will, whenever counsel of the said assignee, or the counsel of its successors, legal representatives and assigns, shall advise that any

proceeding in connection with said inventions, or said application for Letters Patent, or any proceeding in connection with Letters Patent for said inventions in any country, including interference proceedings, is lawful and desirable, or that any division, continuation or continuation-in-part of any application for Letters Patent or any reissue or extension of any Letters Patent, to be obtained thereon, is lawful and desirable, sign all papers and documents, take all lawful oaths, and do all acts necessary or required to be done for the procurement, maintenance, enforcement and defense of Letters Patent for said inventions, without charge to said assignee, its successors, legal representatives and assigns, but at the cost and expense of the said assignee, its successors, legal representatives and assigns.

AND the said assignors hereby request the Commissioner of Patents to issue said Letters Patent of the United States to the said assignee as the assignee of said inventions and the Letters Patent to be issued thereon for the sole use and behoof of the said assignee, its successors, legal representatives and assigns.

Date: _____

_____
John Fraser Wright

Date: 9/27/2005

_____
Guang Qu

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/661,553	03/19/2010	John Fraser Wright	0800-0045.01	4726

20855 7590 07/17/2012

PASTERNAK PATENT LAW
1900 EMBARCADERO ROAD
SUITE 211
PALO ALTO, CA 94303

EXAMINER	
SINGH, SATYENDRA K	
ART UNIT	PAPER NUMBER
1657	

MAIL DATE	DELIVERY MODE
07/17/2012	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	12/661,553	WRIGHT ET AL.	
	Examiner	Art Unit	
	SATYENDRA SINGH	1657	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *09 May 2012*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *1 and 3-8* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1 and 3-8* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

10)☒ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

12)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's submission filed on 05/09/2012 is duly acknowledged.

Claims 2, and 9-11 (invention of group II) have been canceled by applicants.

Claims 1 and 3-8 (elected invention of group I), as currently amended, have been examined on their merits in this office action.

Objection to Specification

The disclosure is objected to for the following informalities:

Page 1, first paragraph of the instant disclosure should be amended to acknowledge the issued patent US 7,704,721 from application 11/141,996. Appropriate correction is required.

Claim Rejections - 35 USC § 112- withdrawn

In view of the current amendments presented in claims 4, 5 and claim 7, the 112-second rejections, as set forth in the previous office action, have been withdrawn by the examiner.

Claim Rejections - 35 USC § 102- withdrawn

In view of current amendments to claim 1, the rejection of claims 1, 3, 7 and 8 under 35 U.S.C. 102(b) over cited references of Vihinen-Ranta et al (1998) and Andersson et al (1979), as previously made by the examiner, have been withdrawn.

Claim Rejections - 35 USC § 102-maintained

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

1. **Claims 1, 7 and 8** (as currently amended) are/remain rejected under 35 U.S.C. 102(b) as being anticipated by **Zolotukhin et al** (2000; US 6,146,874; IDS).

Claims are directed to "a composition for the storage of purified adeno-associated virus (AAV) particles, comprising: purified AAV particles; a pH buffer; and excipients comprising one or more multivalent ions; wherein the ionic strength of the composition is greater than 200 mM, wherein aggregation of the purified AAV particles in the composition is prevented (claim 1); wherein the purified AAV particles have an average particle radius (*Rh*) of less than about 20 nm as measured by dynamic light scattering (instant claim 7 as amended); and wherein recovery of the purified virus particles is at least about 90% following filtration of the composition of virions through a 0.22 μm filter" (claim 8).

Zolotukhin et al (2000) disclose the composition comprising: purified recombinant AAV virus particles; a pH buffer such as **phosphate buffered-saline with magnesium chloride and potassium chloride** (PBS-MK); and excipients comprising one or more multivalent ions such as phosphate and magnesium; wherein the ionic strength of the composition is greater than 200 mM (see Zolotukhin et al, column 11, 4th paragraph, lines 35-40, in particular), wherein they elute the purified rAAV particles in **PBS-MK buffer having 1M NaCl**, i.e. elution buffer. The limitations of claims 7 and 8 (average particle radius and percent recovery following filtration) are also met by the prior art as these limitations are taken to be an inherent characteristic features of the purified viral composition as disclosed by Zolotukhin et al. Since, all the components including purified virus particles, pH buffer and multivalent ions, as recited in claims 1 and 7, or claims 1 and 8, are the same, as disclosed in the cited prior art, these features will necessarily follow from the composition disclosed in the art.

As per MPEP 2111.01, *during examination, the claims must be interpreted as broadly as their terms reasonably allow. In re American Academy of Science Tech Center, F.3d, 2004 WL 1067528 (Fed. Cir. May 13, 2004)(The USPTO uses a different standard for construing claims than that used by district courts; during examination the USPTO must give claims their broadest reasonable interpretation.). This means that the words of the claim must be given their plain meaning unless applicant has provided a clear definition in the specification. In re Zletz*, 893 F.2d 319, 321, 13 USPQ2d 1320, 1322 (Fed. Cir. 1989).

Response to 102(b) Arguments over Zolotukhin et al

Applicant's arguments filed on 5/9/2012 (as they pertain to the 102(b) rejection of claims 1, 2, 7 and 8) have been fully considered but they are not persuasive for the following reasons:

Applicants argument that "… *Zolotukhin does not teach a composition where the ionic strength of a purified preparation of rAAV virions is greater than 200 mM. Rather, in the passage cited by the Examiner to evidence the teaching of excipients greater than 200 mM ionic strength, the rAAV is* ***in the process of being purified****. See, for example, Figure 1.... considered "purified" in the disclosure*" (see remarks, page 6, 2nd and 3rd paragraphs), is duly noted and considered. However, it is noted that the term "purified" is not specifically defined in the instant disclosure, as being tied to any particular step and/or degree of purification of the AAV particles, or for that matter number of virions associated with such composition as claimed. The cited reference of Zolotukhin et al disclose, *albeit* during the process of purification the composition comprising purified rAAV virus particles, a pH buffer such as phosphate buffered-saline with magnesium chloride and potassium chloride (PBS-MK used as elution buffer containing 1M NaCl), and excipients comprising multivalent ions such phosphate and magnesium, wherein the ionic strength of said composition is greater than 200mM, and therefore anticipate the claimed invention as currently presented. The arguments regarding Lactate Ringer's composition and ionic strength as made by the applicants is not found to be persuasive because Zolotukhin et al

disclose all the elements of the claimed composition. In addition, since the components including purified AAV particles, pH buffer and multivalent ions, as recited in instant claim 1 are the same, as disclosed in the cited prior art, the features recited in claims 7 and 8 would necessarily follow from the composition disclosed in the art.

Claim Rejections - 35 USC § 103- maintained

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* v. *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

1. Claims **1 and 3-8** (as amended) are/remain rejected under 35 U.S.C. 103(a) as being unpatentable over Zolotukhin et al (2000; US 6,146,874; IDS) taken with Andersson et al (1979; US 4,138,287; [A]), Zhang et al (2001; US 6,194,191; IDS) and Chen et al (1994; IDS).

Claims are directed to "a composition (for the storage) of purified adeno-associated virus (AAV) particles, comprising: purified AAV particles; a pH buffer; and excipients comprising one or more multivalent ions; wherein the ionic strength of the composition is greater than 200

mM, wherein aggregation of the purified AAV particles in the composition is prevented (instant claim 1); wherein one of the one or more multivalent ions is citrate (claim 3); further comprising Pluronic® F68 (ethylene oxide/propylene oxide block copolymer) at 0.001% (w/v) (claims 4-5); wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate (claim 6); wherein the purified AAV particles have an average particle radius (*Rh*) of less than about 20 nm as measured by dynamic light scattering (instant claim 7 as amended); and wherein recovery of the purified virus particles is at least about 90% following filtration of the composition of virions through a 0.22 μm filter" (claim 8).

The detailed teachings of Zolotukhin et al have been discussed above, and are further relied upon in the same manner herein.

Andersson et al (1979) disclose the composition comprising purified hepatitis virus (HB_sAg) particles (see abstract, in particular), a pH buffer such as Tris-sodium citrate buffer, pH 7.5, and excipients comprising a multivalent ion such as **sodium citrate**, wherein the ionic strength of the composition is greater than about 200 mM (see elution of purified virus from column using 0.5 M NaCI in Tris-citrate buffer; see Andersson et al, example 2 and claim 5, in particular; meets the limitations of claims 1 and 3).

However, the composition, further comprising **Pluronic® F68** at 0.001% w/v (claims 4-5); and wherein the pH buffer is **10 mM Tris, pH 8.0** and the excipients comprise **100 mM sodium citrate** (claim 6), is not specifically disclosed by the inventions of Zolotukhin et al taken with Andersson et al.

Zhang et al (2001) disclose the use of a surfactant such as **Pluronic® F68** (0.1% in growth medium for adenovirus infection and viral production, etc.; see column 4, 2nd paragraph and columns 53-54, in particular) for production of adenoviral particles in serum-free suspension cultures using spinner flasks, and also use in the cryopreservation media (see column 53, last paragraph).

Chen et al (1994) disclose strategies to suppress aggregation of proteins (such as recombinant keratinocyte growth factor) during liquid formulation development (see Chen et al, abstract, table 1-2, in particular) by adding sulfated polysaccharides (such as heparin) and **citrate salts** (such as 0.1 to 0.5 M sodium citrate; see Chen et al, page 1661, left column, in particular) that were found to be effective in preventing protein aggregation. Chen et al also disclose the fact that other negatively charged small ions such as phosphate (a multivalent ion) also have moderate stabilizing effects on preventing aggregation of recombinant keratinocyte growth factor (rhKGF).

Thus, given the disclosure in the cited prior art, a person of ordinary skill in the art would have modified the composition comprising purified AAV virus particles taught by Zolotukhin et al taken with Andersson et al such that said composition additionally comprise a surfactant (to help prevent aggregation during freezing-thawing cycles, for example) such as Pluronic® F68, as explicitly taught by Zhang et al, and a suitable amount of sodium citrate as a multivalent ion in Tris buffer solution (at a suitable pH; as specifically disclosed by Andersson et al) in order to stabilize the viral particles as suggested by Chen et al (for suppressing/reducing aggregation of fairly unstable proteins such as rhKGF; see discussion above, thus providing a conceptual basis for including multivalent ions in the buffer containing purified AAV particles), in addition to

other stabilizing components such as a surfactant. Since, the benefits of including a surfactant and high ionic strength multivalent ions have been disclosed in the cited prior art of Zhang et al and Chen et al, an artisan of ordinary skill in the art would have established suitable concentrations required to help stabilize viral preparations (i.e. by preventing aggregation, etc.) with a reasonable expectation of success.

The limitations of claims 7 and 8 (average particle radius and percent recovery following filtration, etc.) are also met by the prior art as these are taken to be intrinsic characteristic features of the purified viral compositions as disclosed by Zolotukhin et al when taken with the teachings of Andersson et al, Zhang et al and Chen et al. Since, all the components of the product, as recited in the claims are the same as disclosed and/or suggested in the cited prior art, these features will necessarily follow from the composition disclosed in the art, as they do not structurally change the composition as claimed.

Thus, the invention as a whole would have been *prima facie* obvious to a person of ordinary skill in the art at the time the claimed invention was made.

As per MPEP 2144.06, "*It is prima facie obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose.... [T]he idea of combining them flows logically from their having been individually taught in the prior art." In re Kerkhoven, 626 F.2d 846, 850, 205 USPQ 1069, 1072 (CCPA 1980).*

As per MPEP 2144.06, *In order to rely on equivalence as a rationale supporting an obviousness rejection, the equivalency must be recognized in the prior art, and cannot be based on applicant's disclosure or the mere fact that the components at issue are functional or mechanical equivalents. In re Ruff, 256 F.2d 590, 118 USPQ 340 (CCPA 1958).*

Response to Arguments over 103(a) Rejection

Applicant's arguments filed 05/09/2012 (as they pertain to the obviousness rejection of record) have been fully considered but they are not persuasive for the following reasons of record:

Applicants argument that "*(N)one of the cited art, either alone or in combination, teaches or suggests a purified rAAV particle preparation with an ionic strength of greater than 200 mM to prevent aggregation*" (see remarks, page 7), is duly noted and considered. However it is not found to be persuasive because Zolotukhin et al disclose such a product composition as recited in instant claim 1 (see 102b rejection of record, above). The argument that Zolotukhin et al "*expressly teaches away from the claimed invention because Zolotukhin purposely eliminates high salt concentrations from the remainder of the iodixanol gradient...*" (see remarks, page 7, 3rd paragraph), is not found to be persuasive because instant claims are directed to product composition that has been fully disclosed in the cited art of record, *albeit* as an intermediate composition during the process of preparation of said rAAV particles. Thus, applicants argument that "*...even though Zolotukhin recognizes AAV virion-lysate protein aggregation as a problem, it does not teach or suggest that self-aggregation of rAAV virions is a problem at all, let alone a problem in a purified, concentrated AAV preparation*", is noted and fully considered but is not found to be persuasive for the same reasons of record. The arguments regarding Lactate Ringer's composition and its ionic strength as made by the applicants (see remarks page 8) is also not found be persuasive because Zolotukhin et al disclose all the elements of the claimed composition. Moreover, it is noted that the term "**purified**" is not specifically defined in the instant disclosure, as being tied to any particular step and/or degree of purification of the

AAV particles in said composition, as claimed. The cited reference of Zolotukhin et al disclose (*albeit* during the process of purification as an intermediate) the composition comprising purified rAAV virus particles, a pH buffer such as phosphate buffered-saline with magnesium chloride and potassium chloride (PBS-MK used as elution buffer containing 1M NaCl), and excipients comprising multivalent ions such phosphate and magnesium, wherein the ionic strength of said composition is greater than 200mM, and therefore meet the limitations of the claimed invention as currently presented in claim 1. Regarding the cited references of Andersson et al, Zhang et al and Chen et al, applicants seem to argue that they are non-analogous art and do not relate to AAV composition at hand (see remarks, page 8), and that "*.. .there was no motivation to combine the teachings of Zolotukhin with any of Andersson, Zhang and/or Chen at the time of filing the instant application and, even if such motivation existed, there was no reasonable expectation of a composition containing purified rAAV virions with components that resulted in the prevention of aggregation*", which is fully considered, but is not found to be persuasive because (as also discussed in the rejection of record above) the problems of viral particle aggregation has been known and/or recognized in the art (see disclosure of Zolotukhin et al, above), specifically as suggested by the disclosure of Chen et al (when taken in combination with Andersson et al and Zhang et al) that salts of multivalent ions such as citrate were found to be effective in preventing protein aggregation. They also disclose the fact that other negatively charged small ions such as phosphate (also a multivalent ion) also have moderate stabilizing effect on preventing aggregation of recombinant keratinocyte growth factor. Since, AAV virions are encapsulated in capsid proteins, an artisan of ordinary skill in the art would have been motivated to use multivalent ions such as citrate, phosphate, etc. (with or without suitable surfactants such as

Pluronic® F68) in order to stabilize the purified preparation of AAV virions, with a reasonable expectation of success. In the absence of evidence/data to the contrary, the limitations presented in instant claims 7 and 8 are taken to be intrinsic to the composition disclosed in the cited art of record (see arguments on page 9, 2nd paragraph), as all the components recited in instant claim 1 have been disclosed and/or fully suggested by the combined disclosure of the cited art of record. Applicants are advised to amend claim 1 in order to reflect the novelty of the invention commensurate in the scope of the claims and showing in the disclosure of record.

Double Patenting- Maintained

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting

ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Applicants are apprised that The United States Patent and Trademark Office now provides for an **eTerminal Disclaimer in EFS-Web**. For more information please see http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp. The new eTerminal Disclaimer provides applicants with many advantages and promotes greater efficiency in the patent examination process. This web-based eTerminal Disclaimer can be filled out completely online through web-screens and no EFS-Web fillable forms are required. eTerminal Disclaimers are auto-processed and approved immediately upon submission if the request meets all of the requirements.

Fees must be paid immediately which will then provide users more financial flexibility. A paper filed Terminal Disclaimer requires a fee but does not guarantee a Terminal Disclaimer approval. Each eTerminal Disclaimer filed requires a single terminal disclaimer fee, but can include up to 50 "reference applications" and 50 "prior patents".

1. **Claims 1 and 3-8** (as presented) are/remain rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over at least **claim 1** of U.S. Patent No. **7,704,721 B2** (issued on April 27, 2010 to the same assignee and inventors). Although the conflicting claims are not identical, they are not patentably distinct from each other because issued claim 1 is directed to a method of preventing aggregation of rAAV virion preparations by adding "one or more salts of multivalent ions selected from the group consisting of citrate, phosphate, sulfate and magnesium to said purified virions to produce a preparation of virions with an ionic strength of at least 200 mM", which is suitable for long term storage without significant aggregation problems. Since, the composition disclosed in the issued '721

patent is essentially similar in scope to the composition as currently being claimed by applicants in the instant application, an ODP rejection is deemed proper.

Response to ODP Arguments

Applicant's arguments filed 5/9/2012 (regarding the ODP rejection of record) have been fully considered but they are not persuasive for the following reasons of record:

First, it is noted that applicant correction that it was patent **7,704,721** over which the ODP rejection was made, is acknowledged and appreciated. Regarding the ODP arguments that "*…composition claims corresponding to those pending herein were restricted out in the application that ultimately issued as the '721 patent. Accordingly, it is completely improper to require a Terminal Disclaimer in the present application over the '721 patent. See, MPEP 804.01, citing 35 U.S.C. 121*", it is noted that instant application has been filed as a CON of the 11/141,996 (allowed and issued as US 7,704,721; see page 1 of the instant disclosure filed on 3/19/2010). Moreover, the issued method claims deal with a composition prepared using a nuclease, whereas the composition as currently claimed only requires AAV particles in a buffer containing one or more multivalent ions having ionic strength as recited in claim 1. Thus, instant application has not been treated as a true DIV of earlier application 11/141,996, even though a restriction was made between methods and the product. Since, the composition as claimed is fully contemplated in the issued patent '721, and is co-extensive in scope, the ODP rejection is deemed proper.

Conclusion

NO claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SATYENDRA SINGH whose telephone number is (571)272-8790. The examiner can normally be reached on 9-5MF.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, JON P. WEBER can be reached on 571-272-0925. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Satyendra K. Singh/
Examiner, Art Unit 1657

/JON P WEBER/
Supervisory Patent Examiner, Art Unit 1657

PTO/SB/30 (09-04)

Request for Continued Examination (RCE) Transmittal

Address to:
Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Application Number	12/661,553
Filing Date	March 19, 2010
First Named Inventor	John Fraser Wright, et al.
Art Unit	1653
Examiner Name	Satyendra K. Singh
Attorney Docket Number	0800-0045.01

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. See Instruction Sheet for RCEs (not to be submitted to the USPTO) on page 2.

1. Submission required under 37 CFR 1.114 Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

   a. ☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.
      i. ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____
      ii. ☐ Other _____

   b. ☒ Enclosed
      i. ☒ Amendment/Reply
      ii. ☐ Affidavit(s)/ Declaration(s)
      iii ☐ Information Disclosure Statement (IDS)
      iv. ☐ Other _____

2. Miscellaneous

   a. ☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of ___months. (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

   b. ☐ Other _____

3. Fees The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.

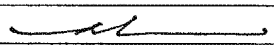
   a. ☒ The Director is hereby authorized to charge the following fees, or credit any overpayments, to Deposit Account No. 50-5826. **I have enclosed a duplicate copy of this sheet.**
      i. ☐ RCE fee required under 37 CFR 1.17(e)
      ii. ☐ Extension of time fee (37 CFR 1.136 and 1.17)
      iii. ☒ Other **any fees not already included**

   b. ☐ Check in the amount of $ _____ enclosed

   c. ☒ Payment by credit card

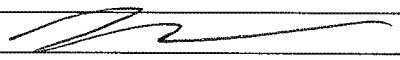
WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization of PTO-2038.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

Signature		Date	1/17/2013
Name (Print /Type)	Roberta L. Robins	Registration No.	33,208

CERTIFICATE OF MAILING OR TRANSMISSION

I hereby certify pursuant to 37 CFR §1.8 that this correspondence is being transmitted via EFS to the United States Patent and Trademark Office on the date shown below.

Signature			
Name (Print /Type)	Denise M. Vaillancourt	Date	1/17/13

PTO/SB/22 (12-04)

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) **FY 2012** *(Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).)*	Docket Number (Optional) 0800-0045.01
Application Number: 12/661,553	Filed: March 19, 2010
For COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION	
Art Unit: 1653	Examiner: Satyendra K. Singh

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	
☐ One month (37 CFR 1.17(a)(1))	$150	$75	$
☐ Two months (37 CFR 1.17(a)(2))	$570	$285	$
☒ Three months (37 CFR 1.17(a)(3))	$1290	$645	$ 1290
☐ Four months (37 CFR 1.17(a)(4))	$2010	$1005	$
☐ Five months (37 CFR 1.17(a)(5))	$2730	$1365	$

☐ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check in the amount of the fee is enclosed.

☒ Payment by credit card.

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 50-5826.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

☐ applicant/inventor.

☐ assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed (Form PTO/SB/96).

☐ attorney or agent of record. Registration Number _____

☒ attorney or agent under 37 CFR 1.34.
Registration number if acting under 37 CFR 1.34 33,208



Signature

1/17/2013
Date

Roberta L. Robins
Typed or printed name

(650) 493-3400
Telephone Number

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.

☒ Total of 1 forms are submitted.

Atty Dkt No: 0800-0045.01
PATENT

CERTIFICATE OF TRANSMISSION PURSUANT TO 37 CFR § 1.8

I hereby certify pursuant to 37 CFR §1.8 that this correspondence is being transmitted via EFS to the United States Patent and Trademark Office on the date shown below.

Date 1/17/13 Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:	Customer No.: 105379
JOHN FRASER WRIGHT et al.	Confirmation No.: 4726
Application No.: 12/661,553	Art Unit: 1653
Filing Date: March 19, 2010	Examiner: Satyendra K. Singh

Title: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

SUBMISSION UNDER 37 CFR 1.114

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

This Submission under 37 C.F.R. §1.114 is being filed in response to the Final Office Action mailed July 17, 2012. A request for an extension of time, as well as the requisite fee, accompany this Submission. Applicants request reconsideration of the above-referenced patent application in view of the following amendments and remarks.

Amendments to the **specification** begin on page 2 of this paper.

A **listing of claims** begins on page 3 of this paper.

Remarks begin on page 5 of this paper.

I. AMENDMENT

Amendments to the Specification

Please amend the first paragraph of the Specification at page 1 to read as follows:

This application is a continuation of U.S. application serial no. 11/141,996, now U.S. Patent No. 7,704,721, from which application priority is claimed pursuant to 35 U.S.C. §120; which application claims the benefit under 35 U.S.C. § 119(e) of provisional applications 60/575,997 filed June 1, 2004 and 60/639,222 filed December 22, 2004. The foregoing applications are hereby incorporated by reference in their entireties.

Amendments to the Claims:

The following listing reflects amendments to the claims and replaces all prior versions and listings of claims in this application.

1. (Currently amended) A composition for the storage of purified adeno-associated virus (AAV) particles, comprising:

purified (AAV) particles at a concentration exceeding $1x10^{13}$ vg/ml up to $6.4x10^{13}$ vg/ml.;

a pH buffer; and

excipients comprising one or more multivalent ions; wherein the ionic strength of the composition is greater than 200 mM, wherein aggregation of the purified AAV particles in the composition is prevented.

2. (Canceled)

3. (Original) The composition of claim 1, wherein one of the one or more multivalent ions is citrate.

4. (Previously presented) The composition of claim 1, further comprising Pluronic® F68 (ethylene oxide/propylene oxide block copolymer).

5. (Previously presented) The composition of claim 4, wherein the Pluronic® F68 is present at 0.001% (w/v).

6. (Original) The composition of claim 1, wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate.

7. (Previously presented) The composition of claim 1, wherein the purified AAV particles have an average particle radius (*Rh*) of less than about 20nm as measured by dynamic light scattering.

8. (Original) The composition of claim 1, wherein recovery of the purified virus particles is at least about 90% following filtration of the composition of virions through a 0.22μm filter.

9-11. (Canceled)

II. REMARKS

Introductory Comments

Claims 1 and 3-8 were examined in the Office Action under reply and stand variously rejected under (1) 35 U.S.C. §102 (claims 1, 7 and 8); (2) 35 U.S.C. §103(a) (claims 1 and 3-8); and (3) the judicially created doctrine of nonstatutory double patenting (claims 1 and 3-8). These grounds of rejection are believed to be overcome by this response and are otherwise traversed for reasons discussed in detail below.

Applicants note with appreciation the withdrawal of the previous rejections under 35 U.S.C. §112, second paragraph.

Overview of the Above Amendments

Claim 1 has been amended to recite that the concentration of purified rAAV virions in the composition exceeds $1x10^{13}$ vg/ml up to $6.4x10^{13}$ vg/ml. Support for this recitation can be found throughout the specification at, for example, page 6, lines 5-6 and in the examples.

The foregoing amendment is made without prejudice, without intent to abandon any originally claimed subject matter, and without intent to acquiesce in any rejection of record. Applicants expressly reserve the right to file one or more continuing applications containing the unamended claims.

Rejections Under 35 U.S.C. §102

Claims 1, 7 and 8 were rejected under 35 U.S.C. §102(b) over U.S. Patent No. 6,146,874 to Zolotukhin et al. ("Zolotukhin"). The Office argues Zolotukhin teaches a preparation of purified rAAV virions with a pH buffer and excipients comprising one or more multivalent ions, wherein the ionic strength of the composition is greater than 200 mM. Office Action, page 3. However, Applicants respectfully disagree that Zolotukhin anticipates the present claims.

Applicants reiterate the Office has applied Zolotukhin under 35 U.S.C. §102(b) against claims 1, 7 and 8, and then combined Zolotukhin with additional art under 35 U.S.C. §103(a) against the same claims. Implicitly, then, the Examiner appears to be acknowledging that

Zolotukhin does not disclose each and every element in claims 1, 7 and 8 without the benefit of additional art. For this reason alone, the rejection must fail.

Moreover, Applicants submit Zolotukhin does not teach a composition as currently claimed, including purified AAV particles exceeding more than $1x10^{13}$ up to $6.4x10^{13}$ vg/ml, wherein the ionic strength of the composition including such purified particles is greater than 200 mM. Rather, in the passage at column 11, lines 35-40, cited by the Examiner to evidence purified rAAV particles in excipients greater than 200 mM ionic strength, the rAAV is in the process of being purified. The Examiner appears to agree with this assessment in the rejection under 35 U.S.C. §103(a), acknowledging that the disclosure relied on in Zolotukhin relates to "an intermediate composition during the process of preparation of rAAV particles." Office Action, page 9. High salt concentrations as claimed are not used in the Zolotukhin's preparation. On the contrary, as previously explained, Zolotukhin's final, purified AAV product is formulated in Lactated Ringer's buffer (see, column 12, lines 49-54) which according to the formula for ionic strength in the present specification, provides an ionic strength of 138.5 mM, not greater than 200 mM as claimed.

Thus, Zolotukhin fails to disclose all of the claimed elements arranged as in the claim. Withdrawal of this basis for rejection is therefore respectfully requested.

Rejections Under 35 U.S.C. §103(a)

Claims 1 and 3-8 remain rejected under 35 U.S.C. §103(a) as being unpatentable over Zolotukhin, taken with U.S. Patent No. 4,138,287 to Andersson et al. ("Andersson"); U.S. Patent No. 6,194,191 to Zhang et al. ("Zhang"); and Chen et al., *J. Pharm. Sci.* (1994) 83:1657-1661 ("Chen").

The Office applies Zolotukhin as above. Andersson is said to disclose a composition of purified HBsAg particles, a pH buffer and excipients comprising a multivalent ion such as sodium citrate wherein the ionic strength of the composition is greater than about 200 mM. The Examiner correctly notes neither of Zolotukhin or Andersson describes a composition further comprising Pluronic (claims 4 and 5) or a composition wherein the pH buffer is 10 mM Tris, pH 8.0 and wherein the excipients comprise 100 mM sodium citrate (claim 6). Office Action, page 6. Zhang is said to disclose the use of Pluronic® F68 for producing adenoviral particles. Office

Action, page 7. Chen allegedly discloses strategies to suppress aggregation of keratinocyte growth factor (KGF) in liquid formulations by adding sulfated polysaccharides and citrate salts. Office Action, page 7. Finally, the limitations of claims 7 and 8 are said to be an intrinsic characteristic of the purified viral compositions. However, Applicants submit the Office has failed to establish a *prima facie* case of obviousness.

The Office disputes Applicants' previous arguments alleging "the instant claims are directed to product composition that has been fully disclosed in the cited art of record, ***albeit* as an intermediate composition during the process of preparation of rAAV particles**." Office Action, page 9, emphasis added. The Examiner argues the term "purified" is not specifically defined by Applicants as being tied to any particular step and/or degree of purification of the AAV particles in the composition. Office Action, pages 9-10, bridging paragraph. The Office concludes the combined teachings in the cited prior art references of record disclose and/or fully suggest all the claimed elements, including the recognition of the problem of self aggregation of rAAV virions associated with purified preparations of the virions. However, Applicants submit that all elements of the claimed invention are not taught or suggested by the cited combination.

To reiterate, all pending claims pertain to compositions for storing purified rAAV particles such that aggregation of rAAV particles in the composition is prevented. The concentration of purified rAAV particles in the preparation exceeds $1x10^{13}$ up to $6.4x10^{13}$ vg/ml. One or more excipients is also present in the composition such that an ionic strength of at least 200 mM is achieved. None of the cited art, either alone or in combination, teaches or suggests such a composition.

First, all claims now define the purity level of the rAAV particles in the composition. Thus, it is clear that highly purified rAAV particles are present in the claimed composition which has an ionic strength exceeding 200 mM. As the Examiner has expressly acknowledged, the passages in Zolotukhin relied upon to evidence the teaching of excipients greater than 200 mM ionic strength, relate to intermediate steps where the rAAV is in the process of being purified. See, for example, Figure 1 where all of the steps using such buffers are purification steps. High salt concentrations are not used in Zolotukhin's final preparation. Rather, the final AAV product is formulated in Lactated Ringer's buffer. See, column 12, lines 49-54, where virus is concentrated by centrifugation through a BIOMAX 100K filter and desalted into Lactated

Ringer's. Only after this step is the virus considered "purified" in Zolotukhin's disclosure and, as explained above, Lactated Ringer's has an ionic strength of 138.5 mM. Applicants' purified product, on the other hand, has an ionic strength of at least 200 mM. There is absolutely no suggestion in Zolotukhin or any of the other cited references that a final preparation with an ionic strength of at least 200 mM and more than $1x10^{13}$ up to $6.4x10^{13}$ vg/ml is desirable or necessary.

Moreover, contrary to the Office's assertions, Zolotukhin does not recognize that rAAV virions **self-aggregate** and therefore does not provide any suggestions regarding a composition as claimed. Rather, Zolotukhin recognizes AAV aggregates with **proteins** in the cell lysate and uses high salt in the **first step** of the iodixanol gradient to destabilize AAV-lysate protein interactions. High salt concentrations are purposefully eliminated from the remainder of the iodixanol gradient, including the gradient from which the rAAV is collected after centrifugation, because the elimination of high salt is important for subsequent purification steps. See, column 15, lines 22-35 of Zolotukhin. Accordingly, this passage expressly teaches away from Applicants' invention. Absent Applicants' teaching regarding this problem, there is no recognition that rAAV virion self-aggregation is a concern that may be addressed by manipulating ionic strength in any of the cited art.

The secondary references do not fill the gaps present in Zolotukhin. Applicants reiterate the present claims are directed to compositions of purified **rAAV** particles with an ionic strength of at least 200 mM and more than $1x10^{13}$ up to $6.4x10^{13}$ vg/ml. None of Andersson, Zhang or Chen even relates to AAV and hence would not suggest a composition with the particular purity and ionic strength as claimed. Andersson is directed to methods for isolating Hepatitis B virus (HBV), a virus completely unrelated to AAV. Zhang relates to methods for producing adenoviral vectors. Adenovirus, like HBV, is unrelated to AAV. A skilled artisan in the AAV field would not look to art pertaining to unrelated viruses in order to determine proper conditions to prevent aggregation at the recited purity levels.

As for Chen, this reference does not relate to viruses, but rather pertains to methods for preventing aggregation of keratinocyte growth factor (KGF). There is absolutely no reason to believe that art directed to growth factors is in any way pertinent to virion production. Moreover, Chen used sulfated polysaccharides in combination with citrate to prevent aggregation. There is no suggestion in Chen to use citrate alone, or to provide a composition as

claimed in order to prevent aggregation of rAAV virions, wherein the composition includes more than $1x10^{13}$ up to $6.4x10^{13}$ vg/ml of rAAV virions.

With respect to the Office's assertion regarding claims 7 and 8, namely, that the average particle radius and percent recovery are inherent characteristics, Applicants reiterate inherency is not a proper standard on which to base an obviousness rejection. It is axiomatic that a retrospective view of inherency is not a substitute for some teaching or suggestion to arrive at the claimed invention. That which may be inherent is not necessarily known, and obviousness **cannot** be predicated on the unknown. See, e.g., *In re Newell*, 13 USPQ2d 1248 (Fed. Cir. 1989).

Applicants' composition provides a commercially viable solution for providing high amounts of rAAV particles in stored compositions. As explained at page 20, second paragraph of the specification and in Table 3, rAAV particles prepared and stored in elevated ionic strength solutions remain soluble and can be stored for an extended period.

Applicants continue to submit the Examiner has chosen bits and pieces of the cited references to arrive at the allegation that this combination of references suggests the claimed invention. It is axiomatic that statements in the prior art must be considered in the context of the teaching of the entire reference, and that rejection of claims **cannot** be predicated on mere identification in a reference of individual components of claimed limitations. In this regard, the Federal Circuit has consistently reversed a finding of obviousness, even when all claimed elements are individually present in the references. *See, e.g., In re Kotzab*, 55 USPQ2d 1313, 1317 (CAFC 2000, emphasis added):

> While the test for establishing an implicit teaching, motivation or suggestion is what the combination of these two statements [in the reference] would have suggested to those of ordinary skill in the art, the two statements cannot be viewed in the abstract. Rather, they must be considered in the context of the teaching of the entire reference. Further, a rejection **cannot** be predicated on the mere identification [in the reference] of individual components of claimed limitations. Rather, particular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed.

Virtually all inventions are combinations of elements that can be individually identified in multiple references. See, e.g., *In re Rouffet*, 47 USPQ2d 1453 (Fed. Cir. 1998), noting that the

Office cannot rely on a high level of skill in the art to overcome the differences between the selected elements in the references, it cannot rely on a high level of skill in the art to provide the necessary motivation.

As explained in Section 2143.01 of the MPEP, the mere fact that references can be combined or modified does not render the resultant combination obvious, unless the prior art also suggests the desirability of the combination. Since the suggestion or motivation to combine the references to arrive at the claimed invention is not in the references, the Examiner is required to cite to some knowledge generally available to one of ordinary skill in the art for the motivation to combine the references. It is respectfully submitted that the Examiner has not provided such knowledge. Instead, the Examiner has merely asserted that it would have been obvious to combine the various methods of the cited art to arrive at Applicants' specifically claimed composition that prevents aggregation of rAAV virions in a purified preparation.

For at least these reasons, withdrawal of the rejections under 35 U.S.C. § 103(a) is respectfully requested.

The Double Patenting Rejection

Claims 1 and 3-8 remain rejected under the judicially created doctrine of nonstatutory obviousness-type double patenting over claim 1 of U.S. Patent No. 7,704,721. Applicants respectfully traverse.

Obviousness-type double patenting requires that the claimed subject matter is **not patentably distinct** from the subject matter in the cited patent. As previously explained, the Examiner considered the method and composition claims patentably distinct in a Restriction Requirement in USSN 11/941,996 which ultimately matured into the '721 patent. In particular, the Office required Applicants to elect, *inter alia*, between methods of preventing aggregation of virions (Group I) and compositions for the storage of purified virus particles (Group II). The composition claims included in Group II were identical to claims 1-8 as filed in the present application. Additionally, the Patent Office required restriction in the present application between the currently elected composition claims and methods of preventing aggregation of virions.

The restriction requirements quoted above clearly set forth the subject matter and the claims that the PTO considered patentably distinct. Accordingly, the present double patenting rejection is in direct contradiction to the Examiner's previous position regarding the claims.

Moreover, Applicants dispute the Examiner's argument that the composition as claimed is co-extensive in scope with the methods issued in the '721 patent. The '721 patent claims are directed to methods of preventing aggregation of rAAV virions which include specific steps in addition to the use of multivalent ions, such as (1) providing a lysate comprising rAAV virions; and (2) purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography. The present composition claims require no such steps.

Based on the foregoing, Applicants submit the claims in the '721 patent and those in the present application are patentably distinct. Thus, the double patenting rejection is believed to be in error and withdrawal thereof is respectfully requested.

III. CONCLUSION

Applicants respectfully submit that the claims are now in condition for allowance and request early notification to that effect. The Examiner is encouraged to contact the undersigned if the Examiner notes any further matters which might be resolved by a telephone interview.

Respectfully submitted,

Date: 1/17/2013

By: _____
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Electronic Patent Application Fee Transmittal

Application Number:	12661553
Filing Date:	19-Mar-2010
Title of Invention:	Compositions and methods to prevent AAV vector aggregation
First Named Inventor/Applicant Name:	John Fraser Wright
Filer:	Roberta L. Robins/Denise Vaillancourt
Attorney Docket Number:	0800-0045.01

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Extension - 3 months with $0 paid	1253	1	1290	1290

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
			Total in USD ($)	**1290**

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/661,553	03/19/2010	John Fraser Wright	0800-0045.01	4726

20855 7590 08/15/2013

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EXAMINER	
SINGH, SATYENDRA K	
ART UNIT	PAPER NUMBER
1657	

MAIL DATE	DELIVERY MODE
08/15/2013	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 12/661,553	Applicant(s) WRIGHT ET AL.	
	Examiner SATYENDRA SINGH	Art Unit 1657	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *17 January 2013*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *1 and 3-8* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1 and 3-8* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some * c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on **01/17/2013** has been entered.

Claims 2, and 9-11 (invention of group II) were previously canceled by applicants.

Claims 1 and 3-8 (elected invention of group I), as currently amended, have been examined on their merits in this office action.

Objection to Specification-Withdrawn

In view of current amendments to page 1 first paragraph of the specification, the objection as previously made by the examiner has been withdrawn.

Claim Rejections - 35 USC § 102-Withdrawn

In view of current amendments to claim 1, the rejection of **Claims 1, 7 and 8** under 35 U.S.C. 102(b) as being anticipated by **Zolotukhin et al** (2000; US 6,146,874), as previously made by the examiner has been withdrawn.

Claim Rejections - 35 USC § 103

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under pre-AIA 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names **joint inventors**. In considering patentability of the claims under pre-AIA 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of pre-AIA 35 U.S.C. 103(c) and potential pre-AIA 35 U.S.C. 102(e), (f) or (g) prior art under pre-AIA 35 U.S.C. 103(a).

1. **Claims 1 and 3-8** (as currently amended) **are/remain** rejected under 35 U.S.C. 103(a) as being unpatentable over Zolotukhin et al (2000; US 6,146,874) taken with Andersson et al (1979; US 4,138,287), Zhang et al (2001; US 6,194,191) and Chen et al (1994).

Claims (as amended) are directed to "a composition (for the storage) of purified adeno-associated virus (AAV) particles, comprising: purified (AAV) particles at a concentration exceeding 1 x 10^{13} vg/ml up to 6.4x 10^{13} vg/ml; a pH buffer; and excipients comprising one or

more multivalent ions; wherein the ionic strength of the composition is greater than 200 mM, wherein aggregation of the purified AAV particles in the composition is prevented (instant claim 1); wherein one of the one or more multivalent ions is citrate (claim 3); further comprising Pluronic® F68 (ethylene oxide/propylene oxide block copolymer) at 0.001% (w/v) (claims 4-5); wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate (claim 6); wherein the purified AAV particles have an average particle radius (*Rh*) of less than about 20 nm as measured by dynamic light scattering (instant claim 7); and wherein recovery of the purified virus particles is at least about 90% following filtration of the composition of virions through a 0.22 μm filter." (claim 8).

Zolotukhin et al (2000) disclose the composition comprising: purified recombinant AAV virus particles; a pH buffer such as **phosphate buffered-saline with magnesium chloride and potassium chloride** (PBS-MK); and excipients comprising one or more multivalent ions such as phosphate and magnesium; wherein the ionic strength of the composition is greater than 200 mM (see Zolotukhin et al, column 11, 4th paragraph, lines 35-40, in particular), wherein they elute the purified rAAV particles in **PBS-MK buffer having 1M NaCl**, i.e. elution buffer; and wherein they disclose the fact that highly purified stocks having titers up to **about 10^{13} particles/ml** are obtained using their purification steps within 24 hours or less (see abstract, and column 16, 1st paragraph, in particular), and in fact has the potential to produce **10^{14} virus particles**. In addition Zolotukhin et al also disclose the problems facing the purification of high titer AAV particles specifically related to the **problem of aggregation**, which was alleviated and/or reduced significantly by the use of high concentrations of salts (i.e. high ionic strength buffer

such as 1M NaCl in PBS-MK; see column 3, last paragraph; column 15, 3rd paragraph, for examples) during purification, wherein the excess salt can be later removed, if required for downstream applications.

However, the composition having AAV particles at a concentration exceeding 1 x 10^{13} vg/ml up to 6.4x 10^{13} vg/ml (see instant claim 1), further comprising **Pluronic® F68** at 0.001% w/v (claims 4-5); and wherein the pH buffer is **10 mM Tris, pH 8.0** and the excipients comprise **100 mM sodium citrate** (claim 6), is not explicitly exemplified (although the number of virus particles potentially purified have been disclosed to be in the vicinity of **about 10^{13} and about 10^{14}**; see Zolotukhin et al above) and/or disclosed by the inventions of Zolotukhin et al.

Andersson et al (1979) disclose the composition comprising purified hepatitis virus (HB_sAg) particles (see abstract, in particular), a pH buffer such as Tris-sodium citrate buffer, pH 7.5, and excipients comprising a multivalent ion such as **sodium citrate**, wherein the ionic strength of the composition is greater than about 200 mM (see elution of purified virus from column using 0.5 M NaCl in Tris-citrate buffer; see Andersson et al, example 2 and claim 5, in particular).

Zhang et al (2001) disclose the use of a surfactant such as **Pluronic® F68** (0.1% in growth medium for adenovirus infection and viral production, etc.; see column 4, 2nd paragraph and columns 53-54, in particular) for production of adenoviral particles in serum-free suspension cultures using spinner flasks, and also use in the cryopreservation media (see column 53, last paragraph).

Chen et al (1994) disclose strategies to suppress aggregation of proteins (such as recombinant keratinocyte growth factor) during liquid formulation development (see Chen et al,

abstract, table 1-2, in particular) by adding sulfated polysaccharides (such as heparin) and **citrate salts** (such as **0.1 to 0.5 M sodium citrate**; see Chen et al, page 1661, left column, in particular) that were found to be effective in preventing protein aggregation. Chen et al also disclose the fact that other negatively charged small ions such as phosphate (a multivalent ion) also have moderate stabilizing effects **on preventing aggregation** of recombinant keratinocyte growth factor (rhKGF).

Thus, given the disclosure in the cited prior art, a person of ordinary skill in the art would have modified the composition comprising purified AAV virus particles taught by Zolotukhin et al taken such that said composition additionally comprise a surfactant (to help prevent aggregation during freezing-thawing cycles, for example) such as Pluronic® F68, as explicitly taught by Zhang et al, and a suitable amount of sodium citrate as a multivalent ion in Tris buffer solution (at a suitable pH; as specifically disclosed by Andersson et al) in order to stabilize the viral particles as suggested by Chen et al (for suppressing/reducing aggregation of fairly unstable proteins such as rhKGF; see discussion above, thus providing a conceptual basis for including multivalent ions in the buffer containing purified AAV particles), in addition to other stabilizing components such as a surfactant. Since, the benefits of including a surfactant and high ionic strength multivalent ions have been disclosed in the cited prior art of Zhang et al and Chen et al, an artisan of ordinary skill in the art would have established suitable concentrations required to help stabilize viral preparations (i.e. by preventing aggregation, etc.) with a reasonable expectation of success, especially given the disclosure from Zolotukhin et al, for example, for the use of high salt concentrations for reducing the potential aggregation of virus particles (see discussion of Zolotukhin et al, above). Such modification in the use of suitable concentrations of

the multivalent ions (such as sodium citrate) for reducing aggregation in place of high salt concentrations (i.e. 1 M NaCl) used by Zolotukhin et al would have been therefore obvious and fully contemplate by an artisan of ordinary skill in the art, given the combined disclosure provided by the cited references of Andersson et al, Zhang et al and Chen et al, as discussed above.

The limitations of claims 7 and 8 (average particle radius and percent recovery following filtration, etc.) are also met by the prior art as these are taken to be intrinsic characteristic features of the purified viral compositions as disclosed by Zolotukhin et al when taken with the teachings of Andersson et al, Zhang et al and Chen et al. Since, all the components of the product, as recited in the claims are the same as disclosed and/or suggested in the cited prior art, these features will necessarily follow from the composition disclosed in the art, as they do not structurally change the composition as claimed.

Thus, the invention as a whole would have been *prima facie* obvious to a person of ordinary skill in the art at the time the claimed invention was made.

As per MPEP 2144.06, "*It is prima facie obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose.... [T]he idea of combining them flows logically from their having been individually taught in the prior art." In re Kerkhoven, 626 F.2d 846, 850, 205 USPQ 1069, 1072 (CCPA 1980).*

As per MPEP 2144.06, *In order to rely on equivalence as a rationale supporting an obviousness rejection, the equivalency must be recognized in the prior art, and cannot be based on applicant's disclosure or the mere fact that the components at issue are functional or mechanical equivalents. In re Ruff, 256 F.2d 590, 118 USPQ 340 (CCPA 1958).*

Response to Applicant's Arguments

Applicant's arguments filed **01/17/2013** (as they pertain to the prior art rejection of record) have been fully considered but they are not persuasive for the following reasons of record:

Regarding the 103a rejection of record, applicants argue that "*…all pending claims pertain to compositions for storing purified rAAV particles such that aggregation of rAAV particles in the composition is prevented. The concentration of purified rAAV particles in the preparation exceeds 1 x 10^{13} up to 6.4x 10^{13} vg/ml. One or more excipients is also present in the composition such that an ionic strength of at least 200 mM is achieved. None of the cited art, either alone or in combination, teaches or suggests such a composition*" (see remarks, page 7), which is noted and fully considered. However, as discussed in the obviousness rejection above, the cited prior art of Zolotukhin et al disclose the range of viral particles that can be purified up to or greater than 10^{14} vp/ml (see column 6, 1st paragraph,; column 16, 1st paragraph, in particular), and therefore, when taken with the disclosure from Andersson et al, Zhang et al and Chen et al for the benefits of using suitable concentrations of a multivalent ions such as sodium citrate, a person of ordinary skill in the art would have fully contemplated the modification in the buffer composition disclosed by Zolotukhin et al such that it uses multivalent ions as stabilizing buffer excipient in order to prevent viral aggregation, as already intended by the disclosure of Zolotukhin et al and supported by Zhang et al and Chen et al, as discussed above.

The argument that "*…Zhang relates to methods for producing adenoviral vectors. Adenovirus, like HBV, is unrelated to AAV. A skilled artisan in the AAV field would not look to art pertaining to unrelated viruses in order to determine proper conditions to prevent*

aggregation at the recited purity levels", is noted and considered, but is not found to be persuasive because the problems of viral particle aggregation has been well known and/or recognized in the art (see disclosure of Zolotukhin et al, as discussed above), specifically as suggested by the disclosure of Chen et al (when taken in combination with Andersson et al and Zhang et al) that salts of multivalent ions such as citrate were found to be effective in preventing protein aggregation. They also disclose the fact that other negatively charged small ions such as phosphate (also a multivalent ion) also have moderate stabilizing effect on preventing aggregation of recombinant keratinocyte growth factor. Since, AAV virions are also encapsulated in capsid proteins, an artisan of ordinary skill in the art, at the time this invention was made, would have been motivated to use multivalent ions such as citrate, phosphate, etc. (in place of very high salt concentrations as used by Zolotukhin et al that are generally removed before clinical applications; with or without suitable surfactants such as Pluronic® F68) in order to stabilize the purified preparation of AAV virions, with a reasonable expectation of success. In addition, applicants have not provided evidence/data on the record that demonstrates that such modification would not have been feasible or applicable to the viral formulation and/or buffer system used by Zolotukhin et al when taken with the disclosure of Andersson et al, Zhang et al and Chen et al, as discussed in the 103a rejection, above. Therefore, the argument that ".. *As for Chen, this reference does not relate to viruses, but rather pertains to methods for preventing aggregation of keratinocyte growth factor (KGF). There is absolutely no reason to believe that art directed to growth factors is in any way pertinent to virion production…*" (see remarks, page 8), is fully considered, but is not found to be persuasive for the above discussed reasons of record. The argument regarding the intrinsic features as recited in claims 7 and 8 (i.e. the

average particle radius and recovery following filtration) are also not found to be persuasive because such features are taken to be intrinsic in the composition disclosed by the cited art of record, as the use of suitable concentration of multivalent ions in the buffer reduce and/or prevent viral aggregation in order to provide said features as currently recited in the claims of record.

Thus, the 103(a) rejection of record is properly made and maintained.

Double Patenting- Withdrawn

In view of applicant's remarks (see remarks, dated 01/17/2013, pages 10-11) regarding the ODP rejection over US 7,704,721 B2 (issued to Wright et al on April 27, 2010) as previously made by the examiner, the rejection of record has been fully withdrawn.

Conclusion

NO claims are currently allowed.

Pertinent art:

1. **Evans et al.** (published on 08/26/2004; **US 2004/0166122 A1**; cited as ref. [A] on PTO 892 form) - Adenovirus formulations (disclose stable viral formulations for gene therapy and other clinical applications generally comprising up to about $1x10^{13}$ viral particles/ml in a suitable buffer, a sugar, a salt, a divalent cation, a non-ionic detergent as well as sodium citrate, wherein the typical formulations comprise 5 mM Tris buffer, pH 7.5, 250 mM NaCl, sucrose to provide suitable osmolarity in the range of 200-800 mOsm/L, $MgCl_2$ in the range of 0.1mM to about 10 mM, 0.001% to about 2% of polysorbate-80 as surfactant, and sodium citrate at about 10 mM; see page 8, example 1, in particular; paragraphs [0051], [0056], [0060], [0079], entire disclosure at pages 5-6, in particular and claims).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SATYENDRA SINGH whose telephone number is (571)272-8790. The examiner can normally be reached on 9-5MF.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, JON P. WEBER can be reached on 571-272-0925. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Satyendra K. Singh/
Examiner, Art Unit 1657

Notice of References Cited	Application/Control No. 12/661,553	Applicant(s)/Patent Under Reexamination WRIGHT ET AL.	
	Examiner SATYENDRA SINGH	Art Unit 1657	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-2004/0166122	08-2004	Evans et al.	424/204.1
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

PTO/SB/22 (12-04)

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) **FY 2014** *(Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).)*	Docket Number (Optional) 0800-0045.01
Application Number: 12/661,553	Filed: March 19, 2010
For COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION	
Art Unit: 1653	Examiner: Satyendra K. Singh

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	
☐ One month (37 CFR 1.17(a)(1))	$200	$100	$
☐ Two months (37 CFR 1.17(a)(2))	$600	$300	$
☒ Three months (37 CFR 1.17(a)(3))	$1400	$700	$ 1400
☐ Four months (37 CFR 1.17(a)(4))	$2200	$1100	$
☐ Five months (37 CFR 1.17(a)(5))	$3000	$1500	$

☐ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check in the amount of the fee is enclosed.

☒ Payment by credit card.

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 50-5826.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

☐ applicant/inventor.

☐ assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed (Form PTO/SB/96).

☐ attorney or agent of record. Registration Number _____

☒ attorney or agent under 37 CFR 1.34.
Registration number if acting under 37 CFR 1.34 33,208

Signature	Date
	FEB. 18, 2014
Roberta L. Robins	(650) 493-3400
Typed or printed name	Telephone Number

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.

☒ Total of 1 forms are submitted.

Atty Dkt No: 0800-0045.01
PATENT

CERTIFICATE OF TRANSMISSION PURSUANT TO 37 CFR § 1.8

I hereby certify pursuant to 37 CFR §1.8 that this correspondence is being transmitted via EFS to the United States Patent and Trademark Office on the date shown below.

Date 2/18/14 Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:	Customer No.: 105379
JOHN FRASER WRIGHT et al.	Confirmation No.: 4726
Application No.: 12/661,553	Art Unit: 1653
Filing Date: March 19, 2010	Examiner: Satyendra K. Singh

Title: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

AMENDMENT UNDER 37 CFR 1.111

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

This paper is responsive to the Office Action mailed August 15, 2013, with a shortened statutory period of three months for response. Accordingly, a request for an extension of time, as well as the requisite fee, accompany this response. Applicants request reconsideration of the above-referenced patent application in view of the following amendments and remarks.

A **listing of claims** begins on page 2 of this paper.

Remarks begin on page 4 of this paper.

I. AMENDMENT

Amendments to the Claims:

The following listing reflects amendments to the claims and replaces all prior versions and listings of claims in this application.

1. (Currently amended) A composition for the storage of purified adeno-associated virus (AAV) particles, comprising:

purified (AAV) particles at a concentration exceeding $1x10^{13}$ vg/ml up to $6.4x10^{13}$ vg/ml[[,]];

a pH buffer, wherein the pH of the composition is between 7.5 and 8.0; and

excipients comprising one or more multivalent ions; wherein the ionic strength of the composition is greater than 200 mM, wherein aggregation of the purified AAV particles in the composition is prevented.

2. (Canceled)

3. (Original) The composition of claim 1, wherein one of the one or more multivalent ions is citrate.

4. (Previously presented) The composition of claim 1, further comprising Pluronic® F68 (ethylene oxide/propylene oxide block copolymer).

5. (Previously presented) The composition of claim 4, wherein the Pluronic® F68 is present at 0.001% (w/v).

6. (Original) The composition of claim 1, wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate.

7. (Previously presented) The composition of claim 1, wherein the purified AAV particles have an average particle radius (*Rh*) of less than about 20nm as measured by dynamic light scattering.

8. (Original) The composition of claim 1, wherein recovery of the purified virus particles is at least about 90% following filtration of the composition of virions through a 0.22μm filter.

9-11. (Canceled)

II. REMARKS

Introductory Comments

Claims 1 and 3-8 were examined in the Office Action under reply and stand rejected solely under 35 U.S.C. §103(a). This ground of rejection is believed to be overcome by this response and is otherwise traversed for reasons discussed in detail below.

Applicants note with appreciation the withdrawal of the previous rejections under 35 U.S.C. §102 and under the judicially created doctrine of nonstatutory double patenting.

Overview of the Above Amendments

Claim 1 has been amended to eliminate a typographical error and to recite that the pH of the composition is between 7.5 and 8.0. Support for this recitation can be found throughout the specification at, e.g., in the examples.

The foregoing amendment is made without prejudice, without intent to abandon any originally claimed subject matter, and without intent to acquiesce in any rejection of record. Applicants expressly reserve the right to file one or more continuing applications containing the unamended claims.

Rejection Under 35 U.S.C. §103(a)

Claims 1 and 3-8 remain rejected under 35 U.S.C. §103(a) as being unpatentable over U.S. Patent No. 6,146,874 to Zolotukhin et al. ("Zolotukhin"), taken with U.S. Patent No. 4,138,287 to Andersson et al. ("Andersson"); U.S. Patent No. 6,194,191 to Zhang et al. ("Zhang"); and Chen et al., *J. Pharm. Sci.* (1994) 83:1657-1661 ("Chen").

The Office reiterates the previous rejection and disputes Applicants' arguments, asserting the primary reference, Zolotukhin, discloses that viral particles can be purified up to or greater than 10^{14} vp/ml and Andersson et al., Zhang et al. and Chen et al. disclose the benefits of using suitable concentrations of multivalent ions as stabilizing buffer excipients in order to prevent viral aggregation. Office Action, page 6. The Examiner also dismisses Applicants' arguments regarding the additional references as directed to non-relevant art, alleging:

> Since AAV virions are also encapsulated in capsid proteins, an artisan of ordinary skill in the art, at the time this invention was made, would have been motivated to use multivalent ions such as citrate, phosphate, etc. (in place of very high salt concentrations as used by Zolotukhin et al that are generally removed before clinical applications; with or without suitable surfactants such as Pluronic® F68) in order to stabilize the purified preparation of AAV virions, with a reasonable expectation of success.

Office Action, page 9. However, Applicants continue to submit the Office has failed to establish a *prima facie* case of obviousness.

First, Applicants do not agree that self-aggregation of AAV virions would necessarily be prevented using the same mechanisms as used for other non-related viruses and proteins. Applicants direct the Examiner's attention to Qu et al., *Molec. Ther.* (2003) 5:S348, Abstract 901 ("Qu") of record herein and which was successfully overcome in the parent application, USSN 11/141,996. Qu proposed that ion bridges between charged amino acids on the surface of vector particles contribute to inter-particle interactions. However, as explained at page 9, lines 18-20 of the instant specification, contrary to the above, it has been found that amino acids with charged side chains are not effective in preventing AAV2 vector aggregation beyond their contribution to ionic strength. Additionally, Qu specifically states that other types of interactions may play a role in aggregation and concludes: "In conjunction with further elucidation of the mechanism(s) of AAV vector aggregation, these observations will facilitate formulation development for optimal large-scale vector purification and clinical use." Thus, Qu explicitly acknowledges that the causes of self-aggregation of rAAV virions had not yet been determined and further work was needed in order to provide a viable purification procedure. Thus, the Examiner's implication that rAAV virion self-aggregation is based on a simple, universal mechanism, is misplaced.

Additionally, Zolotukhin does not recognize that rAAV virions self-aggregate and therefore does not provide any suggestions regarding a composition as claimed. Rather, Zolotukhin recognizes AAV aggregates with cellular debris, especially proteins present during an **intermediate stage** of purification. Further, Zolotukhin actually teaches away from the use of high ionic strength because it describes the use of I M NaCl as "unnecessary or unwarranted" in the other steps of the purification process, and it teaches that it is desirable to "remove or reduce the concentration of salt ... prior to use of, or further purification of, the rAAV." See, column 3,

line 63 to column 4, line 5 of Zolotukhin. Thus, Zolotukhin uses high salt in the **first step** of the iodixanol gradient to destabilize AAV-lysate protein interactions. High salt concentrations are purposefully eliminated from the remainder of the iodixanol gradient, including the gradient from which the rAAV is collected after centrifugation, because the elimination of high salt is important for subsequent purification steps. See, column 15, lines 22-35 of Zolotukhin. Absent Applicants' teaching regarding the problem of rAAV self-aggregation, there is no recognition that this is a concern that may be addressed by manipulating ionic strength in any of the cited art.

The secondary references do not cure the defects present in Zolotukhin. The present claims are directed to compositions of purified **rAAV** particles with an ionic strength of at least 200 mM and more than $1x10^{13}$ up to $6.4x10^{13}$ vg/ml. Additionally, the pH of the composition is between 7.5 and 8.0. None of Andersson, Zhang or Chen even relates to AAV and hence would not suggest a composition with the particular purity, ionic strength and pH as claimed. As explained above, the mechanisms of rAAV virion self-aggregation was not understood at the time of the invention. Hence, relying on the teachings of the secondary references to provide the motivation to make a composition as claimed, in order to prevent self-aggregation of rAAV virions, is misplaced.

With respect to the Office's continued assertion regarding claims 7 and 8, namely, that the average particle radius and percent recovery are intrinsic characteristics, Applicants again assert that inherency is not a proper standard on which to base an obviousness rejection. It is axiomatic that a retrospective view of inherency is not a substitute for some teaching or suggestion to arrive at the claimed invention. That which may be inherent is not necessarily known, and obviousness **cannot** be predicated on the unknown. See, e.g., *In re Newell*, 13 USPQ2d 1248 (Fed. Cir. 1989).

Applicants' composition provides a commercially viable solution for providing high amounts of rAAV particles in stored compositions. As explained at page 20, second paragraph of the specification and in Table 3, rAAV particles prepared and stored in elevated ionic strength solutions remain soluble and can be stored for an extended period.

Finally, Applicants' claims now also recite that the pH of the composition is between 7.5 and 8.0. As acknowledged by the Examiner in the parent application, such a composition was considered nonobvious over the art.

For at least the above reasons, withdrawal of the rejections under 35 U.S.C. § 103(a) is respectfully requested.

III. CONCLUSION

Applicants respectfully submit that the claims are now in condition for allowance and request early notification to that effect. The Examiner is encouraged to contact the undersigned if the Examiner notes any further matters which might be resolved by a telephone interview.

Respectfully submitted,

Date: FEB. 18, 2014

By: _____
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/661,553	03/19/2010	John Fraser Wright	0800-0045.01	4726

20855 7590 05/21/2014

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EXAMINER	
SINGH, SATYENDRA K	
ART UNIT	PAPER NUMBER
1657	

MAIL DATE	DELIVERY MODE
05/21/2014	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 12/661,553	Applicant(s) WRIGHT ET AL.	
	Examiner SATYENDRA SINGH	Art Unit 1657	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *2/8/2014*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1 and 3-8* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1 and 3-8* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

The present application is being examined under the pre-AIA first to invent provisions.

DETAILED ACTION

Applicant's submission filed on **02/18/2014** is duly acknowledged.

Claims 2, and 9-11 (invention of group II) were previously canceled by applicants.

Claims 1 and 3-8 (elected invention of group I), as currently amended, have been examined on their merits in this office action.

The following is a **NON-FINAL action** on the pending claims as currently amended by applicants:

Claim Rejections - 35 USC § 101- NEW

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

The claimed invention is **not directed to patent eligible subject matter**. Based upon an analysis with respect to the claim as a whole, claim(s) 1 and 3-8 do not recite something significantly different than a judicial exception. The rationale for this determination is explained below:

Claim 1 is directed to the following product composition:

"**A composition** for the storage of purified adeno-associated virus (AAV) particles, comprising:

purified (AAV) particles at a concentration exceeding 1 x 10^{13} vg/ml up to 6.4x 10^{13} vg/ml;

a pH buffer, wherein the pH of the composition is between 7.5 and 8.0; and

excipients comprising one or more multivalent ions; wherein the ionic strength of the composition is greater than 200 mM, wherein aggregation of the purified AAV particles in the composition is prevented."

It is noted that instant claims are directed to a product composition comprising purified AAV particles (i.e. virions or viral particles) in combination with excipients such as buffer and salt(s) (see also instant claim 3 and 6, in particular) that appears to be a composition comprising a natural AAV product that does not seem to be "**markedly different in structure**" from the naturally occurring AAV particles. The elements/components (i.e. excipients such as pH buffer, salts, detergent, etc.; see claims 3-6, in particular) recited in the claims do not seem to effectuate significant structural change in the purified viral particles *per se*, as claimed (see also claims 7-8 directed to intrinsic features of the purified AAV preparation), and are known in the art for use in buffering and/or stabilization of viral preparations in the prior art (see the art rejection based on the cited prior art references, as discussed below), therefore instant invention reads on a natural product, i.e. a judicial exception.

Thus, based upon an analysis with respect to the claim as a whole, claims 1 and 3-8 (as presented) do not recite something significantly different than a judicial exception, and are therefore deemed to be patent ineligible (see MPEP 2106).

Appropriate correction is required.

Claim Rejections - 35 USC § 103- Made/Maintained

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the

> invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under pre-AIA 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names **joint inventors**. In considering patentability of the claims under pre-AIA 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of pre-AIA 35 U.S.C. 103(c) and potential pre-AIA 35 U.S.C. 102(e), (f) or (g) prior art under pre-AIA 35 U.S.C. 103(a).

1. **Claims 1 and 3-8** (as currently amended) **are/remain** rejected under 35 U.S.C. 103(a) as being unpatentable over Zolotukhin et al (2000; US 6,146,874) taken with Andersson et al (1979; US 4,138,287), Zhang et al (2001; US 6,194,191) and Chen et al (1994).

Claims (as currently amended) are directed to "a composition (for the storage) of purified adeno-associated virus (AAV) particles, comprising: purified (AAV) particles at a

concentration exceeding 1 x 10^{13} vg/ml up to 6.4x 10^{13} vg/ml; a pH buffer, wherein the pH of the composition is between 7.5 and 8.0; and excipients comprising one or more multivalent ions; wherein the ionic strength of the composition is greater than 200 mM, wherein aggregation of the purified AAV particles in the composition is prevented (instant claim 1); wherein one of the one or more multivalent ions is citrate (claim 3); further comprising Pluronic® F68 (ethylene oxide/propylene oxide block copolymer) at 0.001% (w/v) (claims 4-5); wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate (claim 6); wherein the purified AAV particles have an *average particle radius* (*Rh*) of less than about 20 nm as measured by dynamic light scattering (instant claim 7); and wherein *recovery of the purified virus particles* is at least about 90% following filtration of the composition of virions through a 0.22 μm filter." (claim 8).

Zolotukhin et al (2000) disclose the composition comprising: purified **recombinant** AAV virus particles; a pH buffer such as **phosphate buffered-saline with magnesium chloride and potassium chloride** (PBS-MK); and excipients comprising one or more multivalent ions such as phosphate and magnesium; wherein the ionic strength of the composition is greater than 200 mM (see Zolotukhin et al, column 11, 4th paragraph, lines 35-40, in particular), wherein they elute the purified rAAV particles in **PBS-MK buffer having 1M NaCl**, i.e. elution buffer; and wherein they disclose the fact that highly purified stocks having titers up to **about 10^{13} particles/ml** are obtained using their purification steps within 24 hours or less (see abstract, and column 16, 1st paragraph, in particular), and in fact has the potential to produce **10^{14} virus particles**. In addition Zolotukhin et al also disclose the problems facing the purification of high titer AAV particles specifically related to the **problem of aggregation**, which was alleviated

and/or reduced significantly by the use of high concentrations of salts (i.e. high ionic strength buffer such as 1M NaCl in PBS-MK; see column 3, last paragraph; column 15, 3rd paragraph, for examples) during purification, wherein the excess salt can be later removed, if required for downstream applications.

However, the composition having AAV particles at a concentration exceeding 1 x 10^{13} vg/ml up to 6.4x 10^{13} vg/ml (see instant claim 1), further comprising **Pluronic® F68** at 0.001% w/v (claims 4-5); and wherein the pH buffer is **10 mM Tris, pH 8.0** and the excipients comprise **100 mM sodium citrate** (claims 1 and 6), is not explicitly exemplified and/or disclosed by the inventions of Zolotukhin et al (although the number of virus particles potentially purified have been disclosed to be in the vicinity of **about 10^{13} and about 10^{14}**; see Zolotukhin et al above).

Andersson et al (1979) disclose the composition comprising purified hepatitis virus (HB_sAg) particles (see abstract, in particular), a pH buffer such as Tris-sodium citrate buffer, pH 7.5, and excipients comprising a multivalent ion such as **sodium citrate**, wherein the ionic strength of the composition is greater than about 200 mM (see elution of purified virus from column using 0.5 M NaCl in Tris-citrate buffer; see Andersson et al, example 2 and claim 5, in particular).

Zhang et al (2001) disclose the use of a surfactant such as **Pluronic® F68** (0.1% in growth medium for adenovirus infection and viral production, etc.; see column 4, 2nd paragraph and columns 53-54, in particular) for production of adenoviral particles in serum-free suspension cultures using spinner flasks, and also use in the cryopreservation media (see column 53, last paragraph).

Chen et al (1994) disclose strategies to suppress aggregation of proteins (such as recombinant keratinocyte growth factor) during liquid formulation development (see Chen et al, abstract, table 1-2, in particular) by adding sulfated polysaccharides (such as heparin) and **citrate salts** (such as **0.1 to 0.5 M sodium citrate**; see Chen et al, page 1661, left column, in particular) that were found to be effective in preventing protein aggregation. Chen et al also disclose the fact that other negatively charged small ions such as phosphate (a multivalent ion) also have moderate stabilizing effects **on preventing aggregation** of recombinant keratinocyte growth factor (rhKGF).

Thus, given the disclosure in the cited prior art, a person of ordinary skill in the art would have modified the composition comprising purified recombinant AAV virus particles taught by Zolotukhin et al such that said composition additionally comprise a surfactant (to help prevent aggregation during freezing-thawing cycles, for example) such as Pluronic® F68, as explicitly taught by Zhang et al, and a suitable amount of sodium citrate as a multivalent ion in Tris buffer solution (at a suitable pH; as specifically disclosed by Andersson et al) in order to stabilize the viral particles as suggested by Chen et al (*albeit* for suppressing/reducing aggregation of fairly unstable proteins such as rhKGF; see discussion above, thus providing a conceptual basis for including multivalent ions in the buffer containing purified recombinant AAV particles having capsid proteins), in addition to other stabilizing components such as a surfactant. Since, the benefits of including a surfactant and high ionic strength multivalent ions have been disclosed in the cited prior art of Zhang et al and Chen et al, an artisan of ordinary skill in the art would have established suitable concentrations required to help stabilize purified recombinant AAV viral preparations (i.e. for preventing aggregation, etc.) with a reasonable expectation of success,

especially given the disclosure from Zolotukhin et al, for example, for the use of high salt concentrations for reducing the potential aggregation of virus particles (see discussion of Zolotukhin et al, above). Such modification in the use of suitable concentrations of the multivalent ions (such as sodium citrate) for reducing aggregation in place of high salt concentrations (i.e. 1 M NaCl) used by Zolotukhin et al would have been therefore obvious and fully contemplate by an artisan of ordinary skill in the art, given the combined disclosure provided by the cited references of Andersson et al, Zhang et al and Chen et al, as discussed above.

The limitations of claims 7 and 8 (average particle radius and percent recovery following filtration, etc.) are also met by the prior art as these are taken to be intrinsic features of the purified AAV viral compositions as disclosed by Zolotukhin et al when taken with the teachings of Andersson et al, Zhang et al and Chen et al. Since, all the components of the product, as recited in the claims are the same as disclosed and/or suggested in the cited prior art, these features will necessarily follow from the composition disclosed in the art, as they do not structurally change the composition as claimed.

Thus, the invention as a whole would have been *prima facie* obvious to a person of ordinary skill in the art at the time the claimed invention was made.

As per MPEP 2144.06, "*It is prima facie obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose.... [T]he idea of combining them flows logically from their having been individually taught in the prior art." In re Kerkhoven, 626 F.2d 846, 850, 205 USPQ 1069, 1072 (CCPA 1980).*

As per MPEP 2144.06, *In order to rely on equivalence as a rationale supporting an obviousness rejection, the equivalency must be recognized in the prior art, and cannot be based on applicant's disclosure or the mere fact that the components at issue are functional or mechanical equivalents. In re Ruff, 256 F.2d 590, 118 USPQ 340 (CCPA 1958).*

Response to Applicant's 103(a) Rejection Arguments

Applicant's arguments filed on **02/18/2014** (as they pertain to the prior art rejection over pending claims) have been fully considered but they are not persuasive for the following reasons of record:

First, it is noted that instant claims are drawn to a judicial exception (i.e. preparation comprising a naturally occurring AAV particles) as discussed above in the 101 rejection above.

Regarding the 103a rejection of record, applicant's argument that "…*Qu explicitly acknowledges that the causes of self-aggregation of rAAV virions had not yet been determined and further work was needed in order to provide a viable purification procedure. Thus, the Examiner's implication that rAAV virion self-aggregation is based on a simple, universal mechanism, is misplaced*" (see remarks on page 5 regarding the abstract by Au et al, 2003; not relied upon in the instant rejection of record), is duly noted and considered. However first, it is noted that instant claims are directed to any AAV particles (not necessarily limited to recombinant AAV discussed by Qu et al. Secondly, applicant's allegation that " *Examiner's implication that rAAV virion self-aggregation is based on a simple, universal mechanism"* does not represent required evidence and/or data providing sufficient reasons in order to obviate the rejection of record. As discussed in the 103a rejection of record, the cited prior art of Zolotukhin et al disclose the range of viral particles that can be purified up to or greater than 10^{14} vp/ml (see column 6, 1st paragraph,; column 16, 1st paragraph, in particular), and therefore, when taken with the disclosure from Andersson et al, Zhang et al and Chen et al for the benefits of using suitable concentrations of a multivalent ions such as sodium citrate under suitable pH conditions, a person of ordinary skill in the art would have fully contemplated the modification in the buffer

composition disclosed by Zolotukhin et al such that it uses multivalent ions as stabilizing buffer excipient in order to reduce/prevent viral aggregation, as already intended by the disclosure of Zolotukhin et al and supported by Zhang et al and Chen et al, as discussed above.

Applicant's argument that "…*Zolotukhin does not recognize that rAAV virions self-aggregate and therefore does not provide any suggestions regarding a composition as claimed. Rather, Zolotukhin recognizes AAV aggregates with cellular debris, especially proteins present during an intermediate stage of purification*" (see remarks, page 5, last paragraph) is also not found to be persuasive because instant claim is drawn to a composition, not a process of "*preventing self-aggregation of AAV particles*", and since the components required to provide such desired effects (i.e. in terms of stabilization of the viral preparation) have already been disclosed and/or suggested by the combined teachings in the cited prior art for the same purposes of reducing aggregation (irrespective of the mode of viral aggregation), a person of ordinary skill in the viral vector purification art would have fully contemplated such adjustments in the concentrations of excipients/surfactants and/or pH as already suggested in the art of record. Moreover, viral capsid proteins would be reasonably assumed to be interacting with each other, as well as other proteins and/or cellular debris contained in the preparation of Zolotukin et al, and therefore, a person of ordinary skill in the art, at the time this invention was made, would have reasonably contemplated the teachings related to protein-based aggregation of particles (see teachings of Chen et al, above), and would have employed multivalent ions in suitable concentration under appropriate pH condition, as disclosed by the combined teachings and/or suggestions of the cited art of record.

Applicant's arguments regarding the limitations of claims 7 and 8, that "…*Applicants again assert that inherency is not a proper standard on which to base an obviousness rejection. It is axiomatic that a retrospective view of inherency is not a substitute for some teaching or suggestion to arrive at the claimed invention*…" (see remarks on page 6), is duly noted and considered. However, applicants have not provided any data or evidence on the record to demonstrate that under the conditions as claimed, such average particle radius would not be an intrinsic feature of the composition comprising purified AAV particles (i.e. would be structurally different), to which the percent filtration recovery would be considered an added feature, as already contemplated in the cited art of record.

Applicants seem to argue benefit of the composition as disclosed in the instant specification of record on page 20 and Table 3 (see remarks, page 6), which is duly noted and considered. However, it is noted that the scope of the showing must be commensurate with the scope of claims to consider evidence probative of unexpected results, for example. *In re Dill, 202 USPQ 805 (CCPA, 1979), In re Lindner 173 USPQ 356 (CCPA 1972), In re Hyson, 172 USPQ 399 (CCPA 1972), In re Boesch, 205 USPQ 215, (CCPA 1980), In re Grasselli, 218 USPQ 769 (Fed. Cir. 1983), In re Clemens, 206 USPQ 289 (CCPA 1980).* It should be clear that the probative value of the data is not commensurate in scope with the degree of protection sought by the instant claims. Thus, the argument that "…*Applicants' claims now also recite that the pH of the composition is between 7.5 and 8.0. As acknowledged by the Examiner in the parent application, such a composition was considered nonobvious over the art*", is also not found to be persuasive because the claims in the parent case were directed to the "*method of preventing*

aggregation of recombinant AAV virions", the scope of which is very different from the product claims under consideration.

Thus, the 103(a) rejection of record is properly made and/or maintained.

Applicants are advised to amend claims appropriately in order to limit the scope of the claims commensurate with the unexpected results in order to further the prosecution of this case.

Conclusion

NO claims are allowed.

Pertinent art:

1. **Evans et al.** (published on 08/26/2004; **US 2004/0166122 A1**; previously cited by the examiner) - Adenovirus formulations (disclose stable **viral vector formulations** for gene therapy and other clinical applications generally comprising up to about $1x10^{13}$ viral particles/ml in a suitable buffer, a sugar, a salt, a divalent cation, a non-ionic detergent as well as sodium citrate, wherein the typical formulations comprise 5 mM Tris buffer, pH 7.5, 250 mM NaCl, sucrose to provide suitable osmolarity in the range of 200-800 mOsm/L, $MgCl_2$ in the range of 0.1mM to about 10 mM, 0.001% to about 2% of polysorbate-80 as surfactant, and sodium citrate at about 10 mM; see page 8, example 1, in particular; paragraphs [0051], [0056], [0060], [0079], entire disclosure at pages 5-6, in particular and claims).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SATYENDRA SINGH whose telephone number is (571)272-8790. The examiner can normally be reached on 9-5MF.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, JON P. WEBER can be reached on 571-272-0925. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/SATYENDRA SINGH/
Primary Examiner,
Art Unit 1657

Atty Dkt No: 0800-0045.01
PATENT

CERTIFICATE OF TRANSMISSION PURSUANT TO 37 CFR § 1.8

I hereby certify pursuant to 37 CFR §1.8 that this correspondence is being transmitted via EFS to the United States Patent and Trademark Office on the date shown below.

Date 11/18/14 Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:	Customer No.: 105379
JOHN FRASER WRIGHT et al.	Confirmation No.: 4726
Application No.: 12/661,553	Art Unit: 1653
Filing Date: March 19, 2010	Examiner: Satyendra K. Singh

Title: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

AMENDMENT UNDER 37 CFR 1.111

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

This paper is responsive to the Office Action mailed May 21, 2014, with a shortened statutory period of three months for response. Accordingly, an extension of time in which to respond is requested and the requisite fee accompanies this response. Applicants request reconsideration of the above-referenced patent application in view of the following amendments and remarks.

A **listing of claims** begins on page 2 of this paper.

Remarks begin on page 4 of this paper.

I. AMENDMENT

Amendments to the Claims:

The following listing reflects amendments to the claims and replaces all prior versions and listings of claims in this application.

1. (Currently amended) A composition for the storage of purified, recombinant adeno-associated virus (AAV) particles, comprising:

purified, recombinant ~~(AAV)~~ AAV particles at a concentration exceeding $1x10^{13}$ vg/ml up to $6.4x10^{13}$ vg/ml;

a pH buffer, wherein the pH of the composition is between 7.5 and 8.0; and

excipients comprising one or more multivalent ions; wherein the ionic strength of the composition is greater than 200 mM, wherein aggregation of the purified AAV particles in the composition is prevented.

2. (Canceled)

3. (Original) The composition of claim 1, wherein one of the one or more multivalent ions is citrate.

4. (Previously presented) The composition of claim 1, further comprising Pluronic® F68 (ethylene oxide/propylene oxide block copolymer).

5. (Previously presented) The composition of claim 4, wherein the Pluronic® F68 is present at 0.001% (w/v).

6. (Original) The composition of claim 1, wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate.

7. (Currently amended) The composition of claim 1, wherein the purified, recombinant AAV particles have an average particle radius (*Rh*) of less than about 20nm as measured by dynamic light scattering.

8. (Currently amended) The composition of claim 1, wherein recovery of the purified, recombinant virus particles is at least about 90% following filtration of the composition of virions through a 0.22μm filter.

9-11. (Canceled)

II. REMARKS

Introductory Comments

Claims 1 and 3-8 were examined in the Office Action under reply and stand rejected under (1) 35 U.S.C. §101; and (2) 35 U.S.C. §103(a). These rejections are believed to be overcome by this response and are otherwise traversed for reasons discussed in detail below.

Overview of the Above Amendments

Claims 1, 7 and 8 have been amended to clarify that the purified AAV particles are "recombinant" AAV particles. Support for this recitation can be found throughout the specification at, e.g., page 8, lines 6-7. Claim 1 has also been amended to correct a minor typographical error.

The foregoing amendments are made without prejudice, without intent to abandon any originally claimed subject matter, and without intent to acquiesce in any rejection of record. Applicants expressly reserve the right to file one or more continuing applications containing the unamended claims.

Rejection Under 35 U.S.C. §101

Claims 1 and 3-8 were rejected under 35 U.S.C. §101 as being directed to nonpatentable subject matter. The Office alleges the claims "do not recite something significantly different than a judicial exception" because they appear to cover "a composition comprising a natural AAV product that does not seem to be '**markedly different in structure**' from the naturally occurring AAV particles." Office Action, page 3, emphasis in original. Applicants submit the present claims indeed recite patentable subject matter.

As explained above, the claims have been amended to recite that the AAV particles are "recombinant" particles. It is well known that such particles represent an artificial AAV which does not contain any AAV rep and cap genes that encode viral replication and structural proteins, respectively. Rather, these genes are replaced with a gene or construct of interest which is flanked by the ITRs which include cis-acting elements necessary for replication and packaging.

Accordingly, a "recombinant AAV particle" is not an AAV product as found in nature. Based on the foregoing, withdrawal of the rejection under 35 U.S.C. §101 is respectfully requested.

Rejection Under 35 U.S.C. §103(a)

Claims 1 and 3-8 remain rejected under 35 U.S.C. §103(a) as being unpatentable over U.S. Patent No. 6,146,874 to Zolotukhin et al. ("Zolotukhin"), taken with U.S. Patent No. 4,138,287 to Andersson et al. ("Andersson"); U.S. Patent No. 6,194,191 to Zhang et al. ("Zhang"); and Chen et al., *J. Pharm. Sci.* (1994) 83:1657-1661 ("Chen").

The Office reiterates the previous rejection and disputes Applicants' arguments, asserting the primary reference, Zolotukhin, discloses that viral particles can be purified up to or greater than 10^{14} vp/ml and Andersson et al., Zhang et al. and Chen et al. allegedly disclose the benefits of using suitable concentrations of multivalent ions under suitable pH conditions. Office Action, page 9. With respect to these secondary references, the Examiner dismisses Applicants' citation of Qu et al., *Molec. Ther.* (2003) 5:S348, Abstract 901 ("Qu") which Applicants pointed out evidenced that the causes of self-aggregation of AAV virions had not yet been determined. The Examiner alleges:

> [F]irst, it is noted that instant claims are directed to any AAV particles not necessarily limited to recombinant AAV discussed by Qu et al. Secondly, applicant's allegation that 'Examiner's implication that rAAV virion self-aggregation is based on a simple, universal mechanism' does not represent required evidence and or data providing sufficient reasons in order to obviate the rejection of record.

Office Action, page 9. However, Applicants continue to submit the Office has failed to establish a *prima facie* case of obviousness.

As explained above, Applicants' claims are directed to compositions comprising "recombinant" AAV particles. Qu's abstract is also directed to recombinant AAV particles. Contrary to the Office's assertion above, this article, when read in combination with the present application, does evidence that it would **not** be obvious to provide a composition as claimed. To reiterate, Qu proposed that ion bridges between charged amino acids on the surface of recombinant AAV particles contribute to inter-particle interactions. However, page 9, lines 18-

20 of the instant application explain that, contrary to the above, it has been found that amino acids with charged side chains are not effective in preventing AAV vector aggregation beyond their contribution to ionic strength. Additionally, Qu specifically states other types of interactions may play a role in aggregation and concludes: "In conjunction with further elucidation of the mechanism(s) of AAV vector aggregation, these observations will facilitate formulation development for optimal large-scale vector purification and clinical use." Thus, Qu **explicitly** acknowledges that the causes of self-aggregation of **rAAV virions** had not yet been determined and further work was needed in order to provide a viable composition as claimed "wherein aggregation of the purified AAV particles in the composition is prevented." Accordingly, contrary to the Examiner's allegation, Applicants have indeed provided evidence in order to obviate the assertion that the references provide a motivation to formulate recombinant AAV particles in a composition as claimed.

To reiterate, the secondary references which are **not** even related to AAV would not suggest a composition with the particular purity, ionic strength and pH as alleged by the Office as causes of aggregation of recombinant AAV particles **were not known** prior to Applicants' invention. Hence, relying on the teachings of the secondary references which do not pertain to recombinant AAV particles, to provide the motivation to make a composition as claimed, in order to prevent self-aggregation of rAAV virions, is misplaced.

The Examiner also disputes Applicants' argument that Zolotukhin does not recognize that rAAV virions self-aggregate and therefore does not provide any suggestions regarding a composition as claimed, asserting this argument is unpersuasive because the claims are drawn to compositions and not to a process of preventing self-aggregation. The Examiner further asserts:

> [T]he components required to provide such desired effects i.e. in terms of stabilization of the viral preparation have already been disclosed and/or suggested by the combined teachings in the cited prior art for the same purposes of reducing aggregation (irrespective of the mode of viral aggregation), a person of ordinary skill in the viral vector purification art would have fully contemplated such adjustments in the concentrations of excipients/surfactants and/or pH as already suggested in the art of record.

Office Action, page 10. Applicants disagree with this assertion.

First, although the claims relate to compositions, not methods, the claims explicitly require that "aggregation of the purified AAV particles in the composition is prevented."

Accordingly, Applicants' unique mixture of components in the purified composition as claimed must be factored into the assessment of obviousness. To reiterate, Zolotukhin teaches away from the use of high ionic strength because it teaches that it is desirable to "remove or reduce the concentration of salt ... prior to use of, or further purification of, the rAAV." See, column 3, line 63 to column 4, line 5 of Zolotukhin. Thus, high salt concentrations are purposefully eliminated from later purification steps, and hence from the final product. Moreover, Applicants have provided evidence that the causes of aggregation of recombinant AAV particles **were not known** prior to Applicants' invention and hence a composition as claimed would not be obvious in view of Zolotokhin in combination with the secondary references which are not in any way directed to recombinant AAV preparations. Absent Applicants' teaching regarding the problem of rAAV self-aggregation, there is no recognition that this is a concern that may be addressed by manipulating ionic strength in any of the cited art.

With respect to the Office's continued assertion regarding claims 7 and 8, namely, that the average particle radius and percent recovery are intrinsic characteristics, the Office argues "applicants have not provided any data or evidence on the record to demonstrate that under conditions as claimed, such average particle radius would not be an intrinsic feature of the composition comprising purified particles." Office Action, page 11. However, this argument assumes Zolotukhin's final composition is the same as Applicants' composition since none of the secondary references pertain to recombinant AAV particles. In fact, the Examiner recognizes Zolotukhin's final composition, as well as the compositions of the additional cited art, are not the same as Applicants' since the previous rejections under 35 U.S.C. §102 over Zolotukhin and Andersson were withdrawn.

Even if appropriate, the fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic. *In re Rijckaert,* 28 USPQ2d 1955, 1957 (Fed. Cir. 1993) (reversed rejection because inherency was based on what would result due to optimization of conditions, not what was necessarily present in the prior art); *In re Oelrich,* 212 USPQ 323, 326 (CCPA 1981). "To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact

that a certain thing may result from a given set of circumstances is not sufficient.' " *In re Robertson,* 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999) (citations omitted).

The Examiner further disputes Applicants' showing that the claimed composition provides a commercially viable solution for providing high amounts of recombinant AAV particles in stored compositions. The Examiner states "the scope of the showing must be commensurate with the scope of claims to consider evidence probative of unexpected results." This is indeed the case. As explained at page 20, second paragraph of the specification and in Table 3, recombinant AAV particles prepared and stored in elevated ionic strength solutions (greater than 200 mM as claimed) remain soluble with no aggregation and can be stored for an extended period.

Finally, the Examiner dismisses the fact that Applicants' current claims also recite that the pH of the composition is between 7.5 and 8.0 as in the issued parent case. Indeed, the recitation "wherein the pH of the purified preparation of rAAV virions is between 7.5 and 8.0" was added to the parent claims in an Examiner's amendment. Applicants are aware the present claims relate to compositions and not methods, however, as explained above, the composition is one where "aggregation of the purified AAV particles in the composition is prevented" and the causes of aggregation, and hence the proper formulation for preventing aggregation, were unknown prior to Applicants' discovery.

Accordingly, the combination cited by the Office does not provide evidence that the claimed invention is a "predictable use of prior art elements according to their established functions." *KSR Int'l Co. v. Teleflex, Inc.*, 82 USPQ2d 1385, 1396 (U.S. 2007). Rather, as explained above, the evidence is to the contrary. Applicants submit the Examiner has chosen bits and pieces of the cited references to arrive at the allegation that this combination of references suggests the claimed invention. This is improper. As stated in *KSR*, "a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." *KSR*, page 1396; see also, *In re Kotzab*, 55 USPQ2d 1313, 1317 (Fed. Cir. 2000). A rejection cannot be predicated on the mere identification of individual components of claimed limitations. Rather, particular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner.

Additionally, as set forth in MPEP 2142, impermissible hindsight must be avoided and the conclusion of obviousness must be reached on the basis of the facts gleaned from the prior art. Should the Examiner continue to maintain the rejection over the cited combination, the only conclusion that can be drawn is that the rejection is premised on an impermissible hindsight reconstruction of the invention based on Applicants' disclosure. As stated by the Court of Appeals for the Federal Circuit, "[i]t is impermissible to use the claimed invention as an instruction manual or 'template' to piece together the teachings of the prior art so that the claimed invention is rendered obvious." *In re Fritch*, 23 USPQ2d 1780, 1784 (Fed. Cir. 1992). See, also, *In re Fine*, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988): "One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention." Thus, it is insufficient merely to show that some or all of the elements of the invention are present in the prior art and possess characteristics of the elements of the instant invention.

For at least the above reasons, withdrawal of the rejections under 35 U.S.C. § 103(a) is respectfully requested.

III. CONCLUSION

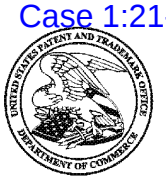
Applicants respectfully submit that the claims are now in condition for allowance and request early notification to that effect. The Examiner is encouraged to contact the undersigned if the Examiner notes any further matters which might be resolved by a telephone interview.

Respectfully submitted,

Date: Nov. 18, 2014

By: _____
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/661,553	03/19/2010	John Fraser Wright	0800-0045.01	4726

20855 7590 01/27/2015

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EXAMINER	
SINGH, SATYENDRA K	

ART UNIT	PAPER NUMBER
1657	

MAIL DATE	DELIVERY MODE
01/27/2015	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Examiner-Initiated Interview Summary	Application No. 12/661,553	Applicant(s) WRIGHT ET AL.	
	Examiner SATYENDRA SINGH	Art Unit 1657	

All participants (applicant, applicant's representative, PTO personnel):

(1) *SATYENDRA SINGH*. (3)_____.

(2) *ROBERTA ROBINS (ATTORNEY)*. (4)_____.

Date of Interview: *22 January 2015*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☒ Yes ☐ No.
If Yes, brief description: *see a copy of the proposed EXAM AMEND faxed to attorney Robins on 12/23/14*.

Issues Discussed ☒101 ☐112 ☐102 ☒103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *1, in particular*.

Identification of prior art discussed: *of the record*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

after cosiderations of applicant's arguments (and discussion with SPE, Jon Weber regarding 101 rejection of record; dated 12/18/14), examiner called applicant's attorney of record Miss Roberta Robins (ph=650-493-3400 x303) and provided with (via fascimile) a proposed Examiner's Amendment to pending claims (the scope of which was found to be tentatively allowable; see attached copy of the EXAM AMEND faxed to attorney Robins on 12/23/2014) for applicant's quick considerations. Attorney Robins informed the examiner that applicants are on leave and once they respond, she will contact the examiner. Upon multiple calling by the examiner (from 1/7/15 to 1/21/15), attorney Robins told the examiner that for some reasons, she was not able to contact the applicants, and that they are not responding to her emails.
On 01/22/15, in the absence of any response from the applicants, examiner called the attorney Robins again, and was informed that applicants have not yet responded, and she suggested the examiner to go ahead and issue an appropriate office action, as she is not able to get in touch with the applicants. Examiner told attorney Robins that in this situation, a FINAL rejection will be mailed by the office soon.

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☒ Attachment

/SATYENDRA SINGH/ Primary Examiner, Art Unit 1657	

Office Action Summary	Application No. 12/661,553	Applicant(s) WRIGHT ET AL.	
	Examiner SATYENDRA SINGH	Art Unit 1657	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *11/18/14*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1 and 3-8* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1 and 3-8* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date *11/18/14*.

3) ☒ Interview Summary (PTO-413) Paper No(s)/Mail Date. *1/22/15*.

4) ☐ Other: _____.

The present application is being examined under the pre-AIA first to invent provisions.

DETAILED ACTION

Applicant's submission filed on **11/18/2014** has been entered.

Claims 2, and 9-11 (invention of group II) were previously canceled by applicants.

Claims 1 and 3-8 (elected invention of group I), as currently amended, have been examined on their merits in this office action.

Examiner-Initiated Interview

In the spirit of compact prosecution, an attempt by the examiner was made to offer applicants the allowable scope of the claimed invention (see attached interview summary, and the attached copy of the proposed examiner's amendment faxed to the Attorney of record **Miss Roberta Robins** on 12/23/2014). However, no response from the applicants was received by attorney Robins, and therefore, finally no agreement could be reached.

Claim Rejections - 35 USC § 101- Withdrawn

In view of applicant's arguments (see remarks, page 4) and amendment to claim 1, the 101 rejection of record, as previously made by the examiner, has been withdrawn. The "recombinant AAV particles" have been taken as "recombinant AAV **vector** particles" for the purposes of this office action hereinafter.

Claim Rejections - 35 USC § 103- Maintained

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under pre-AIA 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names **joint inventors**. In considering patentability of the claims under pre-AIA 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of pre-AIA 35 U.S.C. 103(c) and potential pre-AIA 35 U.S.C. 102(e), (f) or (g) prior art under pre-AIA 35 U.S.C. 103(a).

1. **Claims 1 and 3-8** (as currently amended) **remain** rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Zolotukhin et al (2000; US 6,146,874) taken with Andersson et al (1979; US 4,138,287), Zhang et al (2001; US 6,194,191) and Chen et al (1994).

Claims (as currently amended) are directed to "a composition (for the storage) of purified, recombinant adeno-associated virus (AAV) particles, comprising: purified , recombinant (AAV) particles at a concentration exceeding 1 x 10^{13} vg/ml up to 6.4x 10^{13} vg/ml;

a pH buffer, wherein the pH of the composition is between 7.5 and 8.0; and excipients comprising one or more *multivalent ions*; wherein the ionic strength of the composition is greater than 200 mM, wherein aggregation of the purified AAV particles in the composition is prevented (instant claim 1); wherein one of the one or more multivalent ions is citrate (claim 3); further comprising Pluronic® F68 (ethylene oxide/propylene oxide block copolymer) at 0.001% (w/v) (claims 4-5); wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate (claim 6); wherein the purified AAV particles have an *average particle radius* (*Rh*) of less than about 20 nm as measured by dynamic light scattering (instant claim 7); and wherein *recovery of the purified, recombinant virus particles* is at least about 90% following filtration of the composition of virions through a 0.22 μm filter." (claim 8).

Zolotukhin et al (2000) disclose the composition comprising: purified **recombinant** AAV (rAAV) virus particles; a pH buffer such as **phosphate buffered-saline with magnesium chloride and potassium chloride** (PBS-MK); and excipients comprising one or more multivalent ions such as phosphate and magnesium; wherein the ionic strength of the composition is greater than 200 mM (see Zolotukhin et al, column 11, 4th paragraph, lines 35-40, in particular), wherein they elute the purified rAAV particles in **PBS-MK buffer having 1M NaCl**, i.e. elution buffer; and wherein they disclose the fact that highly purified stocks having titers up to **about 10^{13} particles/ml** are obtained using their purification steps within 24 hours or less (see abstract, and column 16, 1st paragraph, in particular), and in fact has the potential to produce **10^{14} virus particles**. In addition Zolotukhin et al also disclose the problems facing the purification of high titer AAV particles specifically related to the **problem of aggregation**, which was alleviated and/or reduced significantly by the use of high concentrations of salts (i.e.

high ionic strength buffer such as 1M NaCl in PBS-MK; see column 3, last paragraph; column 15, 3rd paragraph, for examples) during purification, wherein the excess salt can be later removed, if required for downstream applications.

However, the composition having AAV particles at a concentration exceeding 1 x 10^{13} vg/ml up to 6.4x 10^{13} vg/ml (see instant claim 1), further comprising **Pluronic® F68** at 0.001% w/v (claims 4-5); and wherein the pH buffer is **10 mM Tris, pH 8.0** and the excipients comprise **100 mM sodium citrate** (claims 1 and 6), is not explicitly exemplified and/or disclosed by the inventions of Zolotukhin et al (although the number of virus particles potentially purified have been disclosed to be in the vicinity of **about 10^{13} and about 10^{14}**; see Zolotukhin et al above).

Andersson et al (1979) disclose the composition comprising purified hepatitis virus (HB_sAg) particles (see abstract, in particular), a pH buffer such as Tris-sodium citrate buffer, pH 7.5, and excipients comprising a multivalent ion such as **sodium citrate**, wherein the ionic strength of the composition is greater than about 200 mM (see elution of purified virus from column using 0.5 M NaCl in Tris-citrate buffer; see Andersson et al, example 2 and claim 5, in particular).

Zhang et al (2001) disclose the use of a surfactant such as **Pluronic® F68** (0.1% in growth medium for adenovirus infection and viral production, etc.; see column 4, 2nd paragraph and columns 53-54, in particular) for production of adenoviral particles in serum-free suspension cultures using spinner flasks, and also use in the cryopreservation media (see column 53, last paragraph).

Chen et al (1994) disclose strategies to suppress aggregation of proteins (such as recombinant keratinocyte growth factor) during liquid formulation development (see Chen et al,

abstract, table 1-2, in particular) by adding sulfated polysaccharides (such as heparin) and **citrate salts** (such as **0.1 to 0.5 M sodium citrate**; see Chen et al, page 1661, left column, in particular) that were found to be effective in preventing protein aggregation. Chen et al also disclose the fact that other negatively charged small ions such as phosphate (a multivalent ion) also have moderate stabilizing effects **on preventing aggregation** of recombinant keratinocyte growth factor (rhKGF).

Thus, given the disclosure in the cited prior art, a person of ordinary skill in the art would have modified the composition comprising purified recombinant AAV virus particles taught by Zolotukhin et al such that said composition additionally comprise a surfactant (to help prevent aggregation during freezing-thawing cycles, for example) such as Pluronic® F68, as explicitly taught by Zhang et al, and a suitable amount of sodium citrate as a multivalent ion in Tris buffer solution (at a suitable pH; as specifically disclosed by Andersson et al) in order to stabilize the viral particles as suggested by Chen et al (*albeit* for suppressing/reducing aggregation of fairly unstable proteins such as rhKGF; see discussion above, thus providing a conceptual basis for including multivalent ions in the buffer containing purified recombinant AAV particles having capsid proteins), in addition to other stabilizing components such as a surfactant. Since, the benefits of including a surfactant and high ionic strength multivalent ions have been disclosed in the cited prior art of Zhang et al and Chen et al, an artisan of ordinary skill in the art would have established suitable concentrations required to help stabilize purified recombinant AAV viral preparations (i.e. for preventing aggregation, etc.) with a reasonable expectation of success, especially given the disclosure from Zolotukhin et al, for example, for the use of high salt concentrations for reducing the potential aggregation of virus particles (see discussion of

Zolotukhin et al, above). Such modification in the use of suitable concentrations of the multivalent ions (such as sodium citrate) for reducing aggregation in place of high salt concentrations (i.e. 1 M NaCl) used by Zolotukhin et al would have been therefore obvious and fully contemplated by an artisan of ordinary skill in the art, given the combined disclosure provided by the cited references of Andersson et al, Zhang et al and Chen et al, as discussed above.

The limitations of claims 7 and 8 (average particle radius and percent recovery following filtration, etc.) are also met by the prior art as these are taken to be intrinsic features of the purified AAV viral compositions as disclosed by Zolotukhin et al when taken with the teachings of Andersson et al, Zhang et al and Chen et al. Since, all the components of the product, as recited in the claims are the same as disclosed and/or suggested in the cited prior art, these features will necessarily follow from the composition disclosed in the art, as they do not structurally change the composition as claimed.

Thus, the invention as a whole would have been *prima facie* obvious to a person of ordinary skill in the art at the time the claimed invention was made.

As per MPEP 2144.06, "*It is prima facie obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose.... [T]he idea of combining them flows logically from their having been individually taught in the prior art." In re Kerkhoven, 626 F.2d 846, 850, 205 USPQ 1069, 1072 (CCPA 1980).*

As per MPEP 2144.06, *In order to rely on equivalence as a rationale supporting an obviousness rejection, the equivalency must be recognized in the prior art, and cannot be based on applicant's disclosure or the mere fact that the components at issue are functional or mechanical equivalents. In re Ruff, 256 F.2d 590, 118 USPQ 340 (CCPA 1958).*

Response to Applicant's Arguments

Applicant's arguments filed on **11/18/2014** (as they pertain to the prior art rejection of record) have been fully considered but they are not persuasive for the following reasons of record:

Regarding the 103a rejection of record, applicants essentially argue that there is no motivation and/or suggestion to combine the cited prior art of record, and earlier cited abstract of Qu et al shows that there is no clear understanding for the causes of self-aggregation of purified rAAV particles (see remarks, pages 5-7), which is duly note d and considered. However, it is noted that Qu et al 2003 abstract has not been currently relied upon in the instant rejection of record. In response to applicant's argument that there is no teaching, suggestion, or motivation to combine the references, the examiner recognizes that obviousness may be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art. See *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988), *In re Jones*, 958 F.2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992), and *KSR International Co. v. Teleflex, Inc.*, 550 U.S. 398, 82 USPQ2d 1385 (2007). In this case, as discussed in the 103a rejection of record, the cited prior art of Zolotukhin et al disclose the range of viral particles that can be purified up to or greater than 10^{14} vp/ml (see column 6, 1st paragraph; column 16, 1st paragraph, in particular), and therefore, when taken with the disclosure from Andersson et al, Zhang et al and Chen et al for the benefits of using suitable concentrations of a multivalent ions such as sodium citrate under suitable pH conditions, a person of ordinary skill in the art would have fully contemplated the modification

in the buffer composition disclosed by Zolotukhin et al such that it uses multivalent ions as stabilizing buffer excipient in order to reduce/prevent viral aggregation, as already recognized and/or intended by the disclosure of Zolotukhin et al (see column 3, last paragraph,; column 14,,last paragraphs, for instances) and supported by the teachings of Zhang et al and Chen et al, as discussed above.

Regarding the limitations of claims 7 and 8, applicants appear to argue that the average particle radius and the percent recovery are not intrinsic properties of the composition (see remarks, page 7), which is duly considered. However, if the components of the composition have been fully disclosed and/or suggested in the art, one would reasonably presume that the resulting characteristics of the composition comprising rAAV particles (such as average particle radius, and recovery, etc.) stabilized with suitable concentrations of multivalent ions such as citrate under similar conditions of number of particles, buffer, pH and temperature, would provide the same characteristics, as currently being claimed by the applicants. Moreover, instant claim 1 is not limited to such characteristics, as currently argued by applicants.

Applicant's arguments regarding the beneficial aspects and scope of the claimed composition and unexpected results (see remarks, page 8), is duly noted and fully considered. However, the scope of the showing must be commensurate with the scope of claims to consider evidence probative of unexpected results, for example. *In re Dill, 202 USPQ 805 (CCPA, 1979), In re Lindner 173 USPQ 356 (CCPA 1972), In re Hyson, 172 USPQ 399 (CCPA 1972), In re Boesch, 205 USPQ 215, (CCPA 1980), In re Grasselli, 218 USPQ 769 (Fed. Cir. 1983), In re Clemens, 206 USPQ 289 (CCPA 1980).* It should be clear that the probative value of the data is not commensurate in scope with the degree of protection sought by the instant claims.

Applicants are advised to amend scope of the claims (as already suggested by the examiner during the examiner-initiated interview, attached herewith) in order to commensurate with the showings, for favorable considerations in future prosecution.

Conclusion

NO claims are allowed.

Pertinent art:

1. **Evans et al.** (published on 08/26/2004; **US 2004/0166122 A1**; previously cited by the examiner) - Adenovirus formulations (disclose stable **viral vector formulations** for gene therapy and other clinical applications generally comprising up to about $1x10^{13}$ viral particles/ml in a suitable buffer, a sugar, a salt, a divalent cation, a non-ionic detergent as well as sodium citrate, wherein the typical formulations comprise 5 mM Tris buffer, pH 7.5, 250 mM NaCl, sucrose to provide suitable osmolarity in the range of 200-800 mOsm/L, $MgCl_2$ in the range of 0.1mM to about 10 mM, 0.001% to about 2% of polysorbate-80 as surfactant, and sodium citrate at about 10 mM; see page 8, example 1, in particular; paragraphs [0051], [0056], [0060], [0079], entire disclosure at pages 5-6, in particular and claims).

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period

will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SATYENDRA SINGH whose telephone number is (571)272-8790. The examiner can normally be reached on 9-5MF.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, JON P. WEBER can be reached on 571-272-0925. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/SATYENDRA SINGH/
Primary Examiner,
Art Unit 1657

Examiner-Initiated Interview Summary	Application No. 12/661,553	Applicant(s) WRIGHT ET AL.	
	Examiner SATYENDRA SINGH	Art Unit 1657	

All participants (applicant, applicant's representative, PTO personnel):

(1) *SATYENDRA SINGH*. (3)_____.

(2) *ROBERTA ROBINS (ATTORNEY)*. (4)_____.

Date of Interview: *22 January 2015*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☒ Yes ☐ No.
If Yes, brief description: *see a copy of the proposed EXAM AMEND faxed to attorney Robins on 12/23/14*.

Issues Discussed ☒101 ☐112 ☐102 ☒103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *1, in particular*.

Identification of prior art discussed: *of the record*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

after cosiderations of applicant's arguments (and discussion with SPE, Jon Weber regarding 101 rejection of record; dated 12/18/14), examiner called applicant's attorney of record Miss Roberta Robins (ph=650-493-3400 x303) and provided with (via fascimile) a proposed Examiner's Amendment to pending claims (the scope of which was found to be tentatively allowable; see attached copy of the EXAM AMEND faxed to attorney Robins on 12/23/2014) for applicant's quick considerations. Attorney Robins informed the examiner that applicants are on leave and once they respond, she will contact the examiner. Upon multiple calling by the examiner (from 1/7/15 to 1/21/15), attorney Robins told the examiner that for some reasons, she was not able to contact the applicants, and that they are not responding to her emails.
On 01/22/15, in the absence of any response from the applicants, examiner called the attorney Robins again, and was informed that applicants have not yet responded, and she suggested the examiner to go ahead and issue an appropriate office action, as she is not able to get in touch with the applicants. Examiner told attorney Robins that in this situation, a FINAL rejection will be mailed by the office soon.

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☒ Attachment

/SATYENDRA SINGH/ Primary Examiner, Art Unit 1657	

UNITED STATES PATENT AND TRADEMARK OFFICE

Facsimile Transmission

To:	**Name:**	ROBERTA L. ROBINS
	Company:	
	Fax Number:	6504933440
	Voice Phone:	
From:	**Name:**	
	Voice Phone:	

37 C.F.R. 1.6 sets forth the types of correspondence that can be communicated to the Patent and Trademark Office via facsimile transmissions. Applicants are advised to use the certificate of facsimile transmission procedures when submitting a reply to a non-final or final Office action by facsimile (37 CFR 1.8(a)).

Fax Notes:

Hi Attorney Robins,
after considerations of applicant's claim amendments and arguments prsented on 11/18/14, I am sending you by fax, a proposed Examiner's Amendments to pending claims (the scope of which has been tentatively found to be allowable) for applicant's quick considerations. Pl. let me know at your earlieast, if there are any issues with the claims currently being amended and considered for allowance (pref. by COB 12/26/2014).
Sincerely,
Satyendra Singh
Examiner, AU 1657

Date and time of transmission: Tuesday, December 23, 2014 3:39:24 PM
Number of pages including this cover sheet: 04

The present application is being examined under the pre-AIA first to invent provisions.

DRAFT

DETAILED ACTION

Applicant's submission filed on **11/18/2014** has been entered.

Claims 2, and 9-11 (invention of group II) were previously canceled by applicants.

Claims 1 and 3-8 (elected invention of group I), as currently amended, have been examined on their merits in this office action.

PROPOSED EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with **Roberta L. Robins** (attorney of record) on December xxxx 2014.

The application has been amended as follows:

In The Claims

Claim 3 has been **canceled** by this Examiner's Amendment.

Claims 1 and 4-8 have been allowed by this Examiner's amendment as follows:

Claims 1, 4, 5, 7 and 8 have been amended, and the entire set of allowed claims have been recited below:

1. (Currently amended) A composition for the storage of purified, recombinant adeno-associated virus (AAV) vector particles, comprising:

purified, recombinant AAV vector particles at a concentration exceeding $1x10^{13}$ vg/ml up to $6.4x10^{13}$ vg/ml;

a pH buffer, wherein the pH of the composition is between 7.5 and 8.0; and

excipients comprising one or more multivalent ions selected from the group consisting of citrate, sulfate, magnesium, and phosphate; wherein the ionic strength of the composition is greater than 200 mM, and wherein ~~aggregation of~~ the purified AAV vector particles are stored in the composition ~~is prevented~~ without significant aggregation.

4. (Currently amended) The composition of claim 1, further comprising ethylene oxide/propylene oxide block copolymer Pluronic® F68 ~~(ethylene oxide/propylene oxide block copolymer)~~.

5. (Currently amended) The composition of claim 4, wherein the Pluronic® F68 is present at a concentration of 0.001% (w/v).

6. (Original) The composition of claim 1, wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate.

7. (Currently amended) The composition of claim 1, wherein the purified, recombinant AAV vector particles have an average particle radius (Rh) of less than about 20nm as measured by dynamic light scattering.

8. (Currently amended) The composition of claim 1, wherein recovery of the purified, recombinant virus particles is at least about 90% following filtration of the composition of ~~virions~~ said AAV vector particles through a 0.22μm filter.

Conclusion

Claims 1 and 4-8 are being allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SATYENDRA SINGH whose telephone number is (571)272-8790. The examiner can normally be reached on 9-5MF.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, JON P. WEBER can be reached on 571-272-0925. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

SATYENDRA SINGH
Primary Examiner,
Art Unit 1657

Applicant-Initiated Interview Summary	Application No.	Applicant(s)	
	12/661,553	WRIGHT ET AL.	
	Examiner	Art Unit	
	SATYENDRA SINGH	1657	

All participants (applicant, applicant's representative, PTO personnel):

(1) *SATYENDRA SINGH*. (3)_____.

(2) *ROBERTA ROBINS (ATTORNEY)*. (4)_____.

Date of Interview: *28 January 2015*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☒ Yes ☐ No.
If Yes, brief description: *previously discussed EXAM AMEND that was faxed on 12/23/14 to attorney Robins (see IMIS dated 1/27/15, already on record)*.

Issues Discussed ☐101 ☐112 ☐102 ☒103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *of the record*.

Identification of prior art discussed: *of the record*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

After the FINAL rejection was mailed by the office on 1/27/2015, applicant's attorney Miss Roberta Robins called the examiner on 01/28/2015, and informed that she has finally received the response from applicants, and they have agreed to the proposed EXAM. AMEND. as previously offered by the examiner on 12/23/14 (see paper IMIS dated 1/27/15 already on the record), and even if the FINAL rejection has been mailed, Examiner is authorized amend the claims per proposed examiner's amendments that were discussed before.

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/SATYENDRA SINGH/
Primary Examiner, Art Unit 1657

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews
Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.
All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:
- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:
1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

Notice of Allowability	Application No. 12/661,553	Applicant(s) WRIGHT ET AL.	
	Examiner SATYENDRA SINGH	Art Unit 1657	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *11/18/14; and interview on 1/28/15*.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on_____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *1 and 4-8*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov .
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   Certified copies:
   a) ☐ All b) ☐ Some *c) ☐ None of the:
   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☒ Interview Summary (PTO-413), Paper No./Mail Date *1/28/15* .
5. ☒ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/SATYENDRA SINGH/
Primary Examiner, Art Unit 1657

The present application is being examined under the pre-AIA first to invent provisions.

DETAILED ACTION

Applicant's submission filed on **11/18/2014** is duly acknowledged.

Claims 2, and 9-11 (invention of group II) were previously canceled by applicants.

Claims 1 and 3-8 (elected invention of group I), as currently amended, have been examined on their merits in this office action.

WITHDRAWAL OF FINALITY

In view of applicant's acceptance of the previously proposed Examiner's Amendment (see IMIS and Examiner-initiated interview summary, dated 01/27/15 of record) to the pending claims, the FINALITY of the previous rejection is withdrawn.

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with **Miss Roberta L. Robins** (attorney of record) on **January 28th 2015** (see also attached interview summary regarding the previously proposed Examiner's Amendment to claims that was finally agreed upon on 01/28/15 by the applicants, after the FINAL rejection was sent by the office on 01/27/15).

The application has been amended as follows:

In The Claims

Claim 3 has been **canceled** by this Examiner's Amendment.

Claims 1 and 4-8 have been allowed by this Examiner's amendment as follows:

Claims 1, 4, 5, 7 and 8 have been amended, and the allowed claims have been recited below:

1. (Currently amended) A composition for the storage of purified, recombinant adeno-associated virus (AAV) vector particles, comprising:

purified, recombinant AAV vector particles at a concentration exceeding $1x10^{13}$ vg/ml up to $6.4x10^{13}$ vg/ml;

a pH buffer, wherein the pH of the composition is between 7.5 and 8.0; and

excipients comprising one or more multivalent ions selected from the group consisting of citrate, sulfate, magnesium, and phosphate; wherein the ionic strength of the composition is greater than 200 mM, and wherein ~~aggregation of~~ the purified AAV vector particles are stored in the composition ~~is prevented~~ without significant aggregation.

4. (Currently amended) The composition of claim 1, further comprising ethylene oxide/propylene oxide block copolymer Pluronic® F68 ~~(ethylene oxide/propylene oxide block copolymer)~~.

5. (Currently amended) The composition of claim 4, wherein the Pluronic® F68 is present at a concentration of 0.001% (w/v).

6. (Original) The composition of claim 1, wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate.

7. (Currently amended) The composition of claim 1, wherein the purified, recombinant AAV vector particles have an average particle radius (Rh) of less than about 20nm as measured by dynamic light scattering.

8. (Currently amended) The composition of claim 1, wherein recovery of the purified, recombinant virus particles is at least about 90% following filtration of the composition of ~~virions~~ said AAV vector particles through a 0.22μm filter.

Conclusion

Claims 1 and 4-8 have been allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SATYENDRA SINGH whose telephone number is (571)272-8790. The examiner can normally be reached on 9-5MF.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, JON P. WEBER can be reached on 571-272-0925. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/SATYENDRA SINGH/
Primary Examiner,
Art Unit 1657

Applicant-Initiated Interview Summary	Application No.	Applicant(s)	
	12/661,553	WRIGHT ET AL.	
	Examiner	Art Unit	
	SATYENDRA SINGH	1657	

All participants (applicant, applicant's representative, PTO personnel):

(1) *SATYENDRA SINGH*. (3)_____.

(2) *ROBERTA ROBINS (ATTORNEY)*. (4)_____.

Date of Interview: *28 January 2015*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☒ Yes ☐ No.
If Yes, brief description: *previously discussed EXAM AMEND that was faxed on 12/23/14 to attorney Robins (see IMIS dated 1/27/15, already on record)*.

Issues Discussed ☐101 ☐112 ☐102 ☒103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *of the record*.

Identification of prior art discussed: *of the record*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

After the FINAL rejection was mailed by the office on 1/27/2015, applicant's attorney Miss Roberta Robins called the examiner on 01/28/2015, and informed that she has finally received the response from applicants, and they have agreed to the proposed EXAM. AMEND. as previously offered by the examiner on 12/23/14 (see paper IMIS dated 1/27/15 already on the record), and even if the FINAL rejection has been mailed, Examiner is authorized amend the claims per proposed examiner's amendments that were discussed before.

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/SATYENDRA SINGH/ Primary Examiner, Art Unit 1657	

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews
Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.
All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:
- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:
1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

Exhibit 4

18379 U.S.PTO 060104

PTO/SB/16 (04-04)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

13441 U.S.PTO 60/575997 060104

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV 130294782 US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
John Fraser	Wright	Mill Valley, California

Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Characterization of AAV2 Vector Aggregation, and Recommended Excipients/Formulations to Achieve Vector Concentrations of 2E13 vg/mL (or Greater)

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☑ Customer Number: 26154

OR

☐ Firm or Individual Name					
Address					
Address					
City		State		Zip	
Country		Telephone		Fax	

ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 16 ☐ CD(s), Number _____

☐ Drawing(s) *Number of Sheets* _____ ☐ Other (specify) _____

☐ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees.

☑ The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 502082

☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE Amount ($): $80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☑ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

[Page 1 of 2]

Respectfully submitted,

SIGNATURE M Christina Thomson

TYPED or PRINTED NAME Mary Christina Thomson

TELEPHONE 510-748-7150

Date 01 June 2004

REGISTRATION NO. 43,190 *(if appropriate)*

Docket Number: 1039.PROV

EXHIBIT 17 Planet Depos, LLC

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

CERTIFICATE OF MAILING

"Express Mail" Mailing Label No. EV 130294782 US
Date of Deposit: 06/01/2004

I hereby certify that this Provisional Application for Patent is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 on the date indicated above and is addressed to MS Provisional Patent Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Danna R. Kramer
Typed or Printed Name of Person Mailing Paper or Fee

Danna R Kramer
Signature of Person Mailing Paper or Fee

Attorney Docket No. 1039.PROV
USPTO Customer No. 26154
Avigen, Inc.
1301 Harbor Bay Parkway
Alameda, CA 94502-6541

Applicant: John Fraser Wright
Title: Characterization of AAV2 Vector Aggregation, and Recommended Excipients/Formulations to Achieve Vector Concentrations of 2E13 vg/mL (or Greater)

Characterization of AAV2 vector aggregation, and recommended excipients / formulations to achieve vector concentrations of 2E13 vg/mL (or greater)

Summary

Studies were undertaken to elucidate the mechanism(s) of AAV vector aggregation, and to identify formulations excipients and purification methods to enable concentration of vectors to ≥2E13 vg/mL. The results of these studies are summarized in three major findings and associated recommendations:

1) Ionic strength ($\mu = \frac{1}{2} \sum c_i z_i^2$) of solution is the major factor that influences AAV vector aggregation. The ionic strength of common buffered-saline solutions ($\mu \sim$ 150mM) is insufficient to reliably prevent vector aggregation at high vector concentrations. Purified vector formulation μ values preferably >300mM are required to substantially prevent aggregation. The use of appropriate concentrations of parenterally acceptable salt species with charge valency >1 (e.g. phosphates, sulfates, citrates) enables the preparation of isotonic solutions with sufficient ionic strength to prevent vector aggregation. A formulation composed of 10mM Tris, 100mM sodium citrate, pH8 (osmolarity ~350 mOsm; μ ~500mM) was demonstrated to enable concentration of AAV2 AADC vector to a concentration of ~6.5E13 vg/mL without aggregation. A series of other useful formulations supported by these findings are listed in the 'Claims' section of this document.

2) Vector surface 'impurity' or 'residual' DNA was implicated in the low ionic strength -induced aggregation of AAV2 vectors. Extensive DNAse treatment of AAV2 vectors (e.g. treatment of AAV2 vectors immobilized on a cation exchange resin using 100 U/mL Benzonase and 10 U/mL DNAseI (G Qu *et al*, Avigen)) was observed to result in vector that demonstrated a markedly reduced tendency to aggregate at reduced ionic strength. While DNAse treatment of unpurified vector has been frequently used during vector purification, more efficient methods to remove 'impurity' DNA from the surface of AAV vectors, i.e. treatment of purified vector with DNAses, are useful to further reduce vector aggregation.

3) Productivity and/or purification yield of vector (e.g. following production of AAV vectors by triple transfection of HEK293 cells) correlates with the tendency of purified vector to aggregate. A retrospective analysis indicates that vector purified from low producing cell cultures (i.e. < 50,000 vg / cell (< 5E12 vg/RB pre purification)) is more likely to aggregate than vector purified from high producing cell cultures (i.e. > 200,000 vg/ cell (>2E13 vg / RB)). Therefore maintaining high cell culture productivity (recommend > 100,000 vg / cell) will reduce vector aggregation.

Background and Objective

A major technical problem that has been encountered frequently during the manufacturing of AAV vectors to support pre-clinical and clinical studies is the tendency of these particles to aggregate. At Avigen, AAV2 vector aggregation is frequently observed when isotonic solutions of vector are prepared at a concentration ≥1E13 vg/mL, and especially at vector concentrations ≥2E13 vg/mL, and most especially at vector concentrations ≥5E13 vg/mL. Other literature describing the problem of AAV vector aggregation has been described (Wright *et al* (2003) Recombinant adeno-associated virus: Formulation challenges and strategies for a gene therapy vector. Current Opinion in Drug Discovery a& Devleopment, Vol 6, No 2, pg174-178). Aggregation of vector particles leads to unacceptable product quality (heterogeneity, instability, inconsistency), reduced yield (loss of vg following filtration steps), and limitations in final product concentration. The studies reported herein aimed to elucidate the mechanisms of AAV vector aggregation, and to achieve AAV vector concentrations of ≥ 2E13 vg/mL using excipients and conditions compatible with pre-clinical and clinical use of the vector. The studies were performed with AAV2 vectors (hFIX and AADC), deemed to represent the greatest challenge to achieve this target concentration (AAV2 vectors have shown the greatest tendency to aggregate in various studies at Avigen).

Excipient screening by dilution stress

Initial screening experiments were performed to elucidate the mechanism of AAV vector aggregation, and to identify classes of excipients that could reduce / prevent aggregation. A 'dilution stress' model was used in these screening studies, and aggregation was assessed by dynamic light scattering (DLS). A broad range of excipients, including amino acids, inorganic salts, simple and compound carbohydrates, surfactants, and human serum albumin (see **Appendix A**), were assessed for their ability to affect aggregation, and it was determined that the primary mechanism of aggregation was ionic (interaction of charged moieties on vector particles) in nature. Concurrent studies (G Qu *et al*, Avigen) support the hypothesis that residual DNA on the surface of vector particles is (at least in part) responsible for ionic strength-dependent vector aggregation. Vector aggregation was assessed as a function of osmolarity and ionic strength (μ) of solution (see **Appendices B, C** and **D**).Vector aggregation was found to be inhibited by charged molecular species to a degree proportional to the ionic strength (μ) of the solution. In these experiments, AAV2 vectors prepared using column- or CsCl- based methods were found to require solution ionic strengths of at least 250-300 mM to consistently prevent aggregation. The ionic strength of frequently used formulations such as phosphate buffered saline ('PBS') or tris buffered saline ('TBS') is in the range 150-175mM, and one of Avigen's standard formulations, 'PBS-sorbitol' (10mM sodium phosphate, 140mM sodium chloride, 5% sorbitol, 0.01% Tween 80 (or 0.001% F68) has an approx. μ = 160mM.

Studies were performed to assess aggregation as a function of ionic strength for AAV2 AADC vectors prepared using various purification techniques and with an additional DNAse treatment. As shown in **Appendix D**, vectors prepared using column chromatography only (HS, containing empty capsids, 'Process 2'), by combined column and cesium chloride gradient ultracentrifugation ('hybrid', essentially empty capsid free, 'Process 3'), and by double cesium chloride gradient ultracentrifugation without column chromatography (CsCl, essentially empty capsid free, 'Process 1') each demonstrated a similar aggregation in

response to decreasing ionic strength in the dilution stress model. In contrast, purified vector that was immobilized on a Poros HS column and treated with 100 U/mL Benzonase and 10U/mL DNAse I (prepared by Guang Qu *et al*, Avigen) demonstrated a significantly lower degree of aggregation when subsequently subjected to decreasing ionic strengths using the dilution model. These data implicate vector surface 'residual' DNA as a contributing factor in vector aggregation, and support efficient DNAse treatment of vector particles to reduce aggregation.

Excipient screening by freeze / thaw stress

A second series of screening experiments was performed to test selected high ionic strength formulations using parenterally acceptable excipients at isotonic concentrations using a second stress, namely freeze thaw (F/T) cycling. These high ionic strength formulations were tested alone or with one of five selected simple carbohydrates (approx. 110mM: sorbitol, mannitol, sucrose, trehalose, or glycerol) during storage at 2-8 °C or following 10 F/T cycles at -20 °C and -80 °C, and aggregation was assessed by DLS analysis. These studies indicated that the high ionic strength formulations, in particular those using sodium citrate, effectively prevented aggregation during multiple F/T cycles. The studies also demonstrated that the addition of sugars (to approx 110mM) had only a marginal effect in reducing aggregation during F/T cycling. Of the simple carbohydrates examined, glycerol was most effective.

Test Formulation (FTF1, 2, and 3) assessment by hollow fiber UF/DF

A final series of experiments was performed to test three selected 'finalist' formulations in ultrafiltration / diafiltrations studies using AAV-AADC2 vector (AAV2), concurrent with the control formulation (CF: PBS / 5% sorbitol). Details / recipes of the three test formulations, FTF1, FTF2, and FTF3, are given in **Appendix E**. A single starting pool of vector prepared as part of Process 1b process development (approx 1.7E15 vg total in cesium chloride) (prepared by J Bahr Davidson *et al*, Avigen). Aliquots of the common pool were used in individual UF/DF runs. AG Technologies hollow fiber units (8" Midgees) were chosen for these experiments because they enable preparation of small final volumes (as little as 3mL) and hence the ability to achieve high concentrations of vector using relatively small volumes of starting material often required for AAV vector purification. The results obtained using this 'smallest' configuration of the AG Technologies hollow fiber systems were assumed to be transferable to larger iterations of the same system (e.g. extended (12") Midgees, Examplers, etc.) that are available for larger target volumes, using reasonable scale-up considerations. In a first study (Experiment 25: E25), diafiltration was carried out at a target vector concentration of 1E13 vg/mL, and after diafiltration the vector solution was concentrated to a target final concentration of 2.5E13 vg/mL (assumes 100% recovery). The recovered material was then subjected to 0.22µm filtration (varying times post UF/DF to model larger scale manufacturing: 0,1,2,5,7 days), and pre- and post- filtration samples assessed for aggregation by DLS and vg concentration by Q-PCR (P Smith). The filtered materials were further challenged by a short stability study (1wk at 2-8 °C, 5F/T cycles at -20 °C and -80 °C). The control formulation (PBS / 5% sorbitol) gave 63-77% recovery post-filtration (concentrations of 1.5E13 and 1.9E13 vg/mL were obtained post-filtration). Use of the control formulation also resulted in obvious visible precipitates in the concentrated product observed prior to filtration. In contrast, all three test formulations (FTF1,2,3) gave **>93% recovery** post filtrations (concentrations ranging from **2.33E13 to 2.39E13 vg/mL**

were obtained post filtration). For all three test formulations, there were no visible precipitates in the concentrated product prior to filtration. Of the three test formulations, **FTF3 (10mMTris, 100mM NaCitrate, 0.001% F68, pH8.0)** best prevented signs of aggregation during the stability study. In a second study (Experiment 26: E26), test formulation **FTF3** was further assessed. Compared to Experiment 25 (E25), an approx. 2.3-fold higher concentration was targeted. Diafiltration was carried out at a vector concentration of 2E13 vg/mL, and after diafiltration the vector solution was concentrated to a target final concentration of 6.7E13 vg/mL. In this 'hyperconcentration' study, use of TF3 resulted in **96% recovery** post filtrations (concentration of **6.45E13 vg/mL** was obtained post-filtration). The solutions of the concentrated vector (AAV AADC2) had no visible signs of precipitated material before or after filtration

In a subsequent corroborating study, a further assessment of the usefulness of FTF3 (10mM Tris, 100mM sodium citrate, pH8) to reduce / prevent aggregation was performed. FTF3 was prepared and provided to Avigen's Research Vector Core (RVC) (Shangzhen Zhou *et al*, Avigen). AAV2 'null vector' prepared by Avigen's Research Vector Core was concentrated and diafiltered into FTF3 using RVC's standard methodology (Midgee hollow fiber units). In this study, no signs of aggregation were observed, and a final post 0.22µm filtration concentration of **3.5E13** vg/mL was achieved using the FTF3 sodium citrate formulation, representing **93%** recovery (personal communication Shangzhen Zhou and Dave Donoho, Avigen).

Correlation of vector aggregation and cell culture productivity

The data obtained in experiments with Avigen's standard formulation (**CF**: 10mM sodium phosphate, 140mM sodium chloride, 5% sorbitol, and 0.001% pluronic F68 (surfactant added after UF/DF)), in conjunction with past experience using our implicates cell culture productivity as a significant factor in vector to aggregate. This correlation is observed by examining experience with three large lots of AAV2 AADC vectors produced and concentrated using similar purification protocols. Table 1 below indicates the estimated upstream productivity (vector genomes produced per cell transfected) and the observed recovery following vector concentration and subsequent 0.22µm filtration.

Table 1. Yield / recovery of AAV2 AADC vector following UF/DF (Midgee) concentration and 0.22µm filtration using Avigen's 'PBS-sorbitol' formulation – Correlation with cell culture productivity in three large lots

AAV2 AADC vector lot	Process	Cell culture productivity: Est'd vg / cell*	UF/DF target vg/mL (100%)	Actual vg/mL, post 0.22µm	Yield (approx.)
AADC GLP campaign (Apr03)	1	~1E5 vg / cell (~1E13 vg/RBeq)	1E13	~5E12	50%
AADC GMP campaign (Jul03)	1	< 5E4 vg / cell (<5E12 vg/RBeq)	5E12	~1.4E12	28%
AADC Process 1b development (Apr04)	1b	~2.5E5 vg / cell (~2.5E13 vg/RB)	2.5E13 6.7E13	~1.6E13 ~4.0E13	63% 59%

*estimated: assumes 5% vg recovery from harvest to post CsCl stage for Process 1, 10% vg for Process1b

These data demonstrate the deficiency in Avigen's current 'PBS-sorbitol' formulation, the use of which resulted in yields ranging from 28-63% in three large scale purification campaigns. The data also support the hypothesis that AAVs vector purified from high yielding cell cultures have less tendancy to aggregation compared to low yielding cultures. This may be related to the relative amounts of impurities to vector product (one explanation is that the presence, on average, of higher levels of vector surface residual DNA on vectors purified from relative low yielding transfected cell cultures increase aggregation of the purified product). These data support the implementation of a 'productivity' specification to ensure consistency and stability of purified vector lots.

Issues / future perspectives

Although FTF3 uses approved parenteral excipients, concentrations of these excipients, and their suitability for all possible routes of administration of vector (e.g. CNS) needs to be critically reviewed. In **Appendix F** is shown a search performed of the FDA Center for Drug Evaluation and Research database of Inactive Ingredients for Approved Drug Products. A number of products have been approved for various routes of administration using sodium citrate at concentrations approximating the 100mM used in FTF3. Therefore FTF3 or variations of FTF3 that retain the positive effects characterized in this study report is proposed as a useful formulation to prevent aggregation of AAV vectors during their preparation and concentration for **Process 1b** (CsCl based purification of AAV vectors (various capsid variants) to support pre-clinical and early stage clinical studies). FTF3 is not explicitly proposed for **Process 4** (empty capsid-free column process for AAV2 vectors for clinical development and commercialization). The highly efficient DNAse step performed on purified AAV2 vector particles planned for Process 4 may obviate the need for the high ionic strength of FTF3, and alternate formulations such as saline or weakly buffered saline may be preferable, especially for some CNS applications. Elucidation of key elements of the mechanism of AAV2 aggregation reported herein, supported by the results obtained with FTF3 suggest that yet higher concentrations of AAV vectors are achievable. Avigen should consider further formulations development to achieve high concentration stocks (e.g. 1E14 vg/mL (approx 1 mg/mL) to 1E15 vg/mL (approx 10 mg/mL) of AAV vectors, for use with commercial diluents at investigational sites.

AAV vector aggregation, especially AAV2 vector aggregation, often occurs when the purified vector is concentrated to concentrations >5E12 vg/mL, especially when concentrated to concentrated to concentrations >1E13 vg/mL, more especially when concentrated to concentrations >2E13 vg/mL, most especially when concentrated to concentrations >5E13 vg/mL. A useful method to prevent aggregation of AAV vectors, especially AAV2 vectors, to be used for research, pre-clinical and clinical studies, is to use an isotonic formulation that has an ionic strength preferably in the range 200-1000mM, more preferably in the range 250-750mM, and most preferably in the range 300-500mM. Such formulations can be prepared with approximately isotonic concentrations of salts containing one or more species having a charge valency >1. Examples of such salts included sodium citrate (preferably in the range 20-150mM, more preferably in the range 50-100mM); magnesium sulfate (preferably in the range 20-250mM, more preferably in the range 50-150mM); calcium sulfate (preferably in the range 20-250mM, more preferably in the range 50-150mM); sodium sulfate (preferably in the range 20-250mM, more preferably in the range 50-150mM); sodium phosphate (preferably in the range 20-250mM, more preferably in the range 50-150mM).

The use of human serum albumin, preferably recombinant human serum albumin, at a concentration in the range 0.1%-10%, preferably in the range 0.5%-2% is also claimed to be useful in preventing AAV vector aggregation.

Fragments of deoxyribonucleic acid (DNA) on the surface of AAV vectors particles ('residual' or 'impurity' vector surface DNA) has been implicated, in these and associated studies, to contribute to AAV vector aggregation. The low ionic strength -induced aggregation observed using AAV2 vectors purified by a variety of purification methods is likely mediated in whole or in part by the vector surface DNA. Efficient removal of vector surface DNA is useful, alone or in conjunction with the use of high ionic strength formulations as described above and / or in conjunction with the use of human serum albumin, to reduce or prevent aggregation of purified AAV vectors, especially AAV2 vectors. In particular, treatment of already purified AAV vectors in liquid suspension or bound to a cation exchange resin (e.g. Poros HS resins) or an anion exchange resin (e.g. Poros HQ resins) with Benzonase at a concentration in the range 1U/mL to 1000U/mL, more preferably with Benzonase at a concentration in the range 10U/mL to 200U/mL, or most preferably with Benzonase at a concentration of 100U/mL; and / or with DNAseI at a concentration in the range 1-100 U/mL, more preferably with DNAseI at a concentration in the range 5-20 U/mL, and most preferably with DNAseI at a concentration of 10U/mL. Other nucleases that can be used alone at effective concentrations, or used in conjunction with Benzone and/or DNAseI, to efficiently remove DNA from the surface of AAV vectors and thereby prevent vector aggregation are also included as a claim herein.

CLAIM:

1. A method for preventing AAV vector aggregation, comprising:
   a. treating a preparation of AAV with DNAses, and
   b. providing the preparation in a formulation having a μ value of >300 mM.

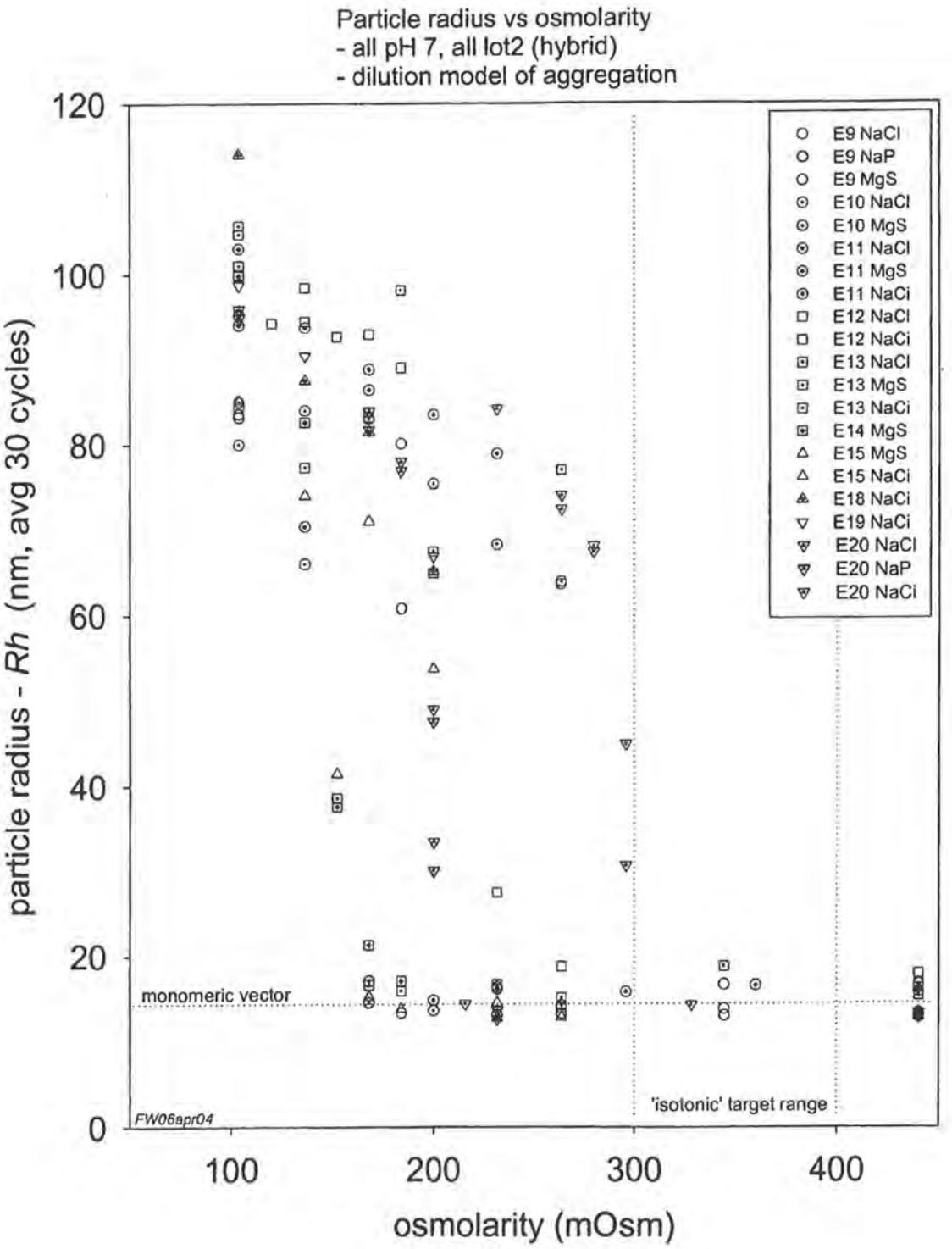
Appendix A

List of candidate excipients examined in initial screening by dilution stress model

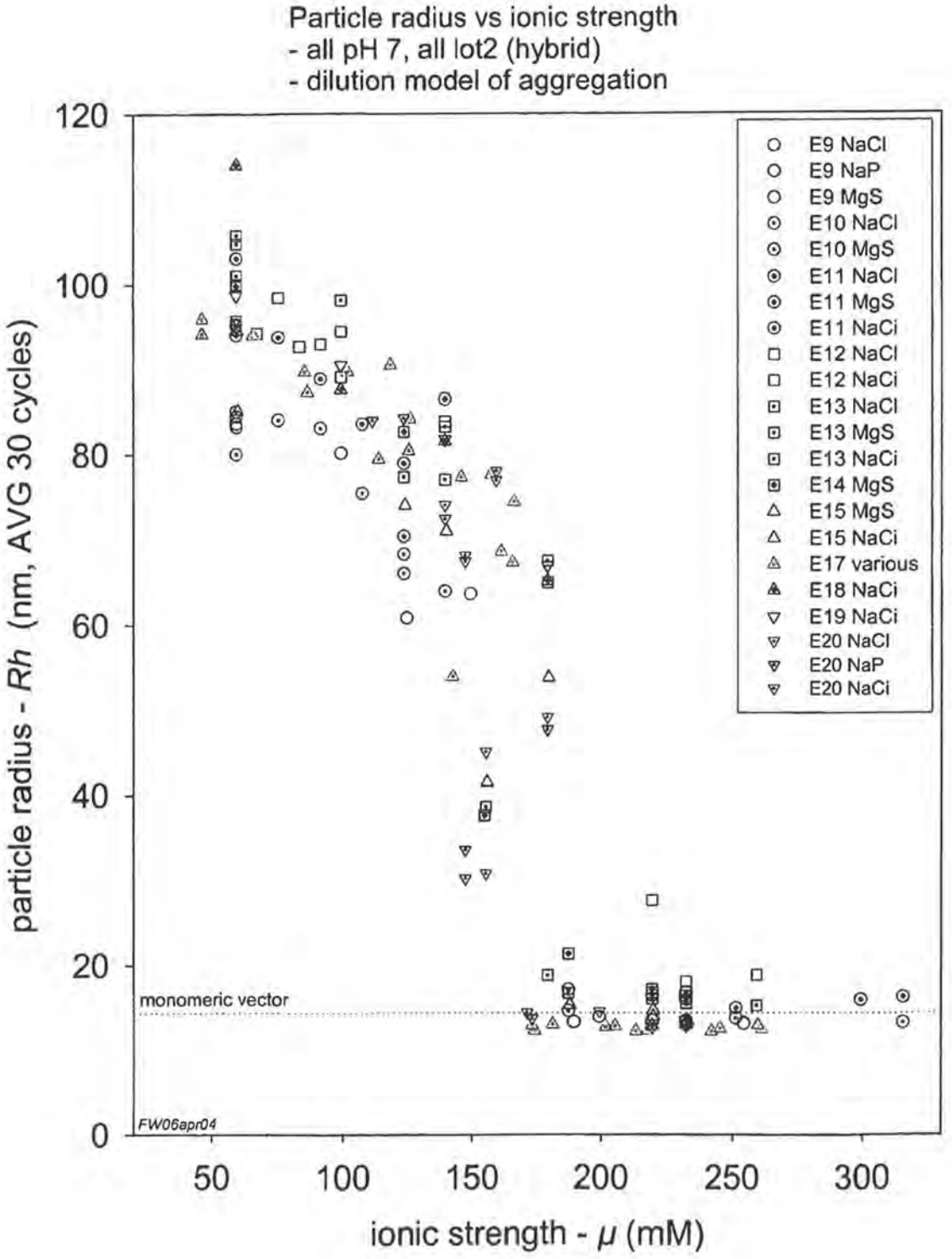
Excipient species tested	*Range examined*	*Effect on aggreg'n*
Magnesium chloride	0 – 100mM	++
Magnesium sulfate	0 – 100mM	+++
Potassium sulfate	0 – 100mM	++
Sodium citrate	0 – 100mM	+++
Sodium chloride	0 – 300mM	+
Sodium phosphate	0 – 200mM	++
Tris	0 – 100mM	+
Dextran sulfate	0 – 2%	variable
Arginine	0 – 100mM	+
Aspartic acid	0 – 100mM	+
Histidine	0 – 100mM	+
Glutaminc acid	0 – 100mM	+
Glycine	0 – 100mM	+
Histidine	0 – 100mM	+
Lysine	0 – 100mM	+
Pluronic F68	0 – 10%	-
Polysorbate 20/80	0 – 1%	-
Glycerol	0 – 5%	-
Iodixanol	0 – 5%	-
Mannitol	0 – 5%	-
Polyethylene glycol	0 – 5%	-
Sorbitol	0 – 5%	-
Sucrose	0 – 5%	-
Trehalose	0 – 5%	-
Human serum albumin	0 – 2%	++
pH	3.0 – 10.0	++, pH >9

Appendix B

Effect of osmolarity on AAV vector aggregation using various charged species

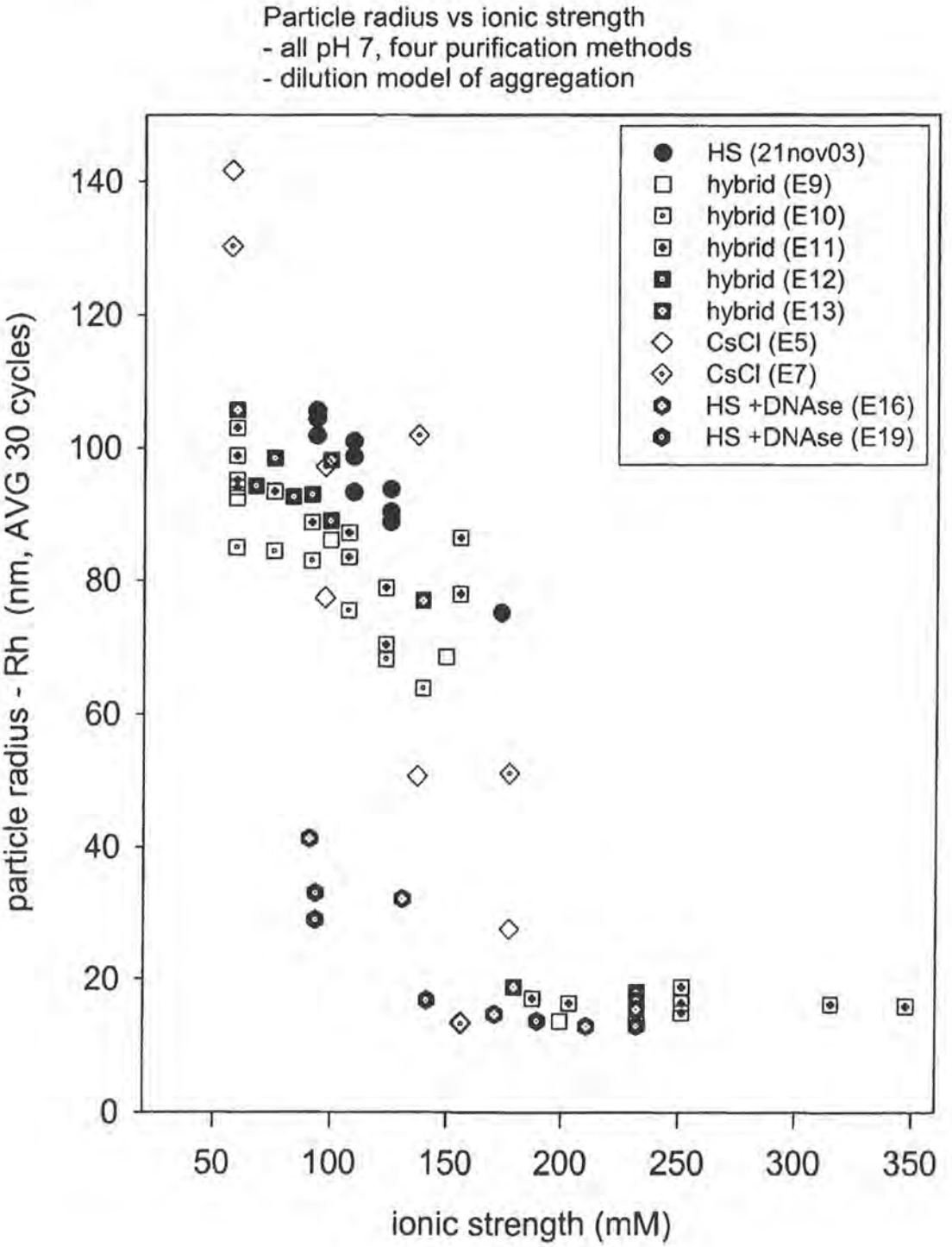


Appendix C
Effect of ionic strength on AAV vector aggregation using various charged species



Appendix D

Effect of ionic strength on AAV vector aggregation using vector purified using four methods, including DNAse treatment of purified vector



Appendix E:
Recipes for control and test formulations for UF/DF studies, and results:

			Calculated: mOsm	μ
Control formulation		10mM Na Phosphate, pH 7.3 140mM NaCl 5% Sorbitol (275mM) 0.001% F68*	585	160
Test formulations:	FTF1	150mM Na Phosphate, pH 7.5 0.001% F68*	390	310
	FTF2	100mM Na Phosphate, pH 7.5 1% glycerol (109 mM) 0.001% F68*	369	210
	FTF3	10mM Tris, pH 8.0 100mM Na Citrate 0.001% F68*	366	510

*Note: in all cases, UF/DF was performed using formulation without added F68. Following concentration and recovery of material, F68 was added to a final concentration of 0.001%.

Results:
E25 (08May04, notebook 670)

Formulation	Vg / mL* (SD)	100% target	Recovery	DLS (Rh, nm) (post filtration)
CF post filtr (2d)	1.93E13 (0.30E13)	2.5e13	77%	14.46
CF post filtr (7d)	1.58E13 (0.15E13)	2.5E13	63%	
TF1 post filtr (2d)	2.38E13 (0.18E13)	2.5E13	95%	13.76
TF1 post filtr (7d)	2.46E13 (0.19E13)	2.5E13	98%	14.66
TF2 post filtr (2d)	2.39E13 (0.20E13)	2.5E13	96%	13.85
TF2 post filtr (7d)	2.35E13 (0.19E13)	2.5E13	94%	13.95
TF3 post filtr (2d)	2.33E13 (0.18E13)	2.5E13	93%	13.82
TF3 post filtr (7d)	2.31E13 (0.17E13)	2.5E13	92%	13.73

E26 (11May04, notebook 670) / **E27** (14May04, notebook 670)

Formulation	Vg / mL* (SD)	100% target	Recovery	DLS (Rh,nm) (post filtration)
CF post filtr (2d)	Pending	6.7E13		13.62
CF post filtr (5d)	3.98E13 (0.24E13)	6.7E13	59%	13.54
TF3 post filtr (2d)	6.42E13 (0.28E13)	6.7E13	96%	13.05
TF3 post filtr (7d)	6.83E13 (0.69E13)	6.7E13	(102%)	13.09

* by Q-PCR, P Smith
**theoretical, based on vg input

Appendix F:
CDER Inactive Ingredient for Approved Drug Products – Search for Sodium Citrate (edited to exclude oral and topical formulations):

U.S. Food and Drug Administration - Center for Drug Evaluation and Research

Inactive Ingredient Search for Approved Drug Products

CDER Home Page | Site Info | Contact Us | What's New

About this Database Back to Search Page

Search Results for: "citrate"

INACTIVE INGREDIENT	ROUTE;DOSAGE FORM	CAS NUMBER	MAXIMUM POTENCY
SODIUM CITRATE	AN,INFILTRATION; INJECTION	006132043	0.0395%
SODIUM CITRATE	**EPIDURAL; INJECTION**	**006132043**	**2.2%**
SODIUM CITRATE	IM - IV - SC; INJECTION	006132043	0.94%
SODIUM CITRATE	**IM - IV; INJECTION**	**006132043**	**40%**
SODIUM CITRATE	IM - SC; INJECTION	006132043	0.228%
SODIUM CITRATE	INHALATION; INHALANT	006132043	
SODIUM CITRATE	INHALATION; SOLUTION	006132043	0.6%
SODIUM CITRATE	INTRA-ARTERIAL; INJECTION	006132043	
SODIUM CITRATE	INTRA-ARTICULAR; INJECTION	006132043	2.2%
SODIUM CITRATE	INTRACARDIAC; INJECTION	006132043	0.8%
SODIUM CITRATE	INTRACAVITARY; INJECTION	006132043	0.15%
SODIUM CITRATE	INTRACAVITARY; POWDER, FOR INJECTION SOLUTION, LYOPHILIZED	006132043	0.0053%

SODIUM CITRATE	INTRALESIONAL; INJECTION	006132043	1%
SODIUM CITRATE	INTRAMUSCULAR; INJECTABLE	006132043	0.304%
SODIUM CITRATE	**INTRAMUSCULAR; INJECTION**	**006132043**	**6.6%**
SODIUM CITRATE	INTRAMUSCULAR; POWDER, FOR INJECTION SOLUTION	006132043	4.62%
SODIUM CITRATE	INTRAMUSCULAR; SOLUTION, INJECTION	006132043	
SODIUM CITRATE	INTRAOCULAR; SOLUTION	006132043	0.17%
SODIUM CITRATE	**INTRAPERITONEAL; INJECTION**	**006132043**	**6.6%**
SODIUM CITRATE	INTRAPLEURAL; INJECTION	006132043	
SODIUM CITRATE	INTRAPLEURAL; POWDER, FOR INJECTION SOLUTION	006132043	4.62%
SODIUM CITRATE	INTRASYNOVIAL; INJECTION	006132043	1%
SODIUM CITRATE	**INTRATHECAL; INJECTION**	**006132043**	
SODIUM CITRATE	**INTRATHECAL; POWDER, FOR INJECTION SOLUTION**	**006132043**	**4.62%**
SODIUM CITRATE	**INTRATRACHEAL; SUSPENSION**	**006132043**	
SODIUM CITRATE	INTRAUTERINE; SOLUTION	006132043	
SODIUM CITRATE	INTRAVASCULAR; INJECTION	006132043	0.32%
SODIUM CITRATE	INTRAVENOUS; INJECTABLE	006132043	
SODIUM CITRATE	**INTRAVENOUS; INJECTION**	**006132043**	**30%**
SODIUM CITRATE	**INTRAVENOUS; POWDER, FOR INJECTION SOLUTION**	**006132043**	**16.4%**
SODIUM CITRATE	INTRAVENOUS; POWDER, FOR	006132043	16.35%

	INJECTION SOLUTION, LYOPHILIZED		
SODIUM CITRATE	INTRAVENOUS; SOLUTION	006132043	0.8295%
SODIUM CITRATE	INTRAVENOUS; SOLUTION, INJECTION	006132043	0.065%
SODIUM CITRATE	**IRRIGATION; INJECTION**	**006132043**	**2.2%**
SODIUM CITRATE	**IV(INFUSION); INJECTION**	**006132043**	**40%**
SODIUM CITRATE	IV(INFUSION); POWDER, FOR INJECTION SOLUTION	006132043	8%
SODIUM CITRATE	IV(INFUSION); POWDER, FOR INJECTION SOLUTION, LYOPHILIZED	006132043	14%
SODIUM CITRATE	IV(INFUSION); SOLUTION, INJECTION	006132043	0.6%
SODIUM CITRATE	NASAL; GEL	006132043	
SODIUM CITRATE	NASAL; SOLUTION	006132043	70%
SODIUM CITRATE	NASAL; SPRAY	006132043	0.44%
SODIUM CITRATE	NASAL; SPRAY, METERED	006132043	0.028%
SODIUM CITRATE	**NERVE BLOCK; INJECTION**	**006132043**	**0.0395%**
SODIUM CITRATE	OPHTHALMIC; POWDER, FOR SOLUTION	006132043	0.17%
SODIUM CITRATE	OPHTHALMIC; SOLUTION	006132043	2%
SODIUM CITRATE	OPHTHALMIC; SOLUTION, DROPS	006132043	2.2%
SODIUM CITRATE	OPHTHALMIC; SUSPENSION, DROPS	006132043	0.45%
SODIUM CITRATE	**SOFT TISSUE; INJECTION**	**006132043**	**1%**
SODIUM CITRATE	SUBCUTANEOUS; POWDER, FOR INJECTION SOLUTION, LYOPHILIZED	006132043	0.645%
SODIUM CITRATE	SUBCUTANEOUS; SOLUTION,	006132043	

	INJECTION		
SODIUM CITRATE	SUBLINGUAL; TABLET	006132043	2.68MG
SODIUM CITRATE	URETERAL; SOLUTION	006132043	
SODIUM CITRATE DIHYDRATE	IM - IV; INJECTION		1.67%
SODIUM CITRATE, ANHYDROUS	IM - IV; INJECTION	000068042	16%
SODIUM CITRATE, ANHYDROUS	INTRA-ARTERIAL; INJECTION	000068042	
SODIUM CITRATE, ANHYDROUS	INTRA-ARTICULAR; INJECTION	000068042	1%
SODIUM CITRATE, ANHYDROUS	INTRABURSAL; INJECTION	000068042	1%
SODIUM CITRATE, ANHYDROUS	INTRAVENOUS; POWDER, FOR INJECTION SOLUTION	000068042	6.99%
SODIUM CITRATE, ANHYDROUS	IV(INFUSION); INJECTION	000068042	16%
SODIUM CITRATE, ANHYDROUS	OPHTHALMIC; SOLUTION	000068042	
SODIUM CITRATE, ANHYDROUS	SOFT TISSUE; INJECTION	000068042	

Database Last Updated: April 5, 2004

Appendix

PATENT APPLICATION SERIAL NO. _____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

06/04/2004 MBERHE 00000040 502082 60575997
01 FC:2005 80.00 DA

PTO-1556
(5/87)

*U.S. Government Printing Office: 2002 — 489-267/69033

Exhibit 5

20427 U.S. PTO 122204

PTO/SB/16 (12-04)
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112960 U.S. PTO 60/639222 122204

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV 130294840 US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
John Fraser	Wright	Philadelphia, PA
Guang	Qu	Alameda, CA

Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max):

Compositions and Methods to Prevent AAV Vector Aggregation

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☑ The address corresponding to Customer Number: 26154

OR

☐ Firm or Individual Name

Address

City	State	Zip
Country	Telephone	Fax

ENCLOSED APPLICATION PARTS *(check all that apply)*

☐ Application Data Sheet. See 37 CFR 1.76
☐ CD(s), Number of CDs _____
☑ Specification *Number of Pages* 29
☐ Other (specify) _____
☑ Drawing(s) *Number of Sheets* 3

Application Size Fee: If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

METHOD OF PAYMENT OF FILING FEES AND APPLICATION SIZE FEE FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.
☐ A check or money order is enclosed to cover the filing fee and application size fee (if applicable).
☐ Payment by credit card. Form PTO-2038 is attached
☑ The Director is hereby authorized to charge the filing fee and application size fee (if applicable) or credit any overpayment to Deposit Account Number: 502082. A duplicative copy of this form is enclosed for fee processing.

TOTAL FEE AMOUNT ($): 100.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.
☑ No.
☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

SIGNATURE Gregory R. Bellomy

Date 22 December 2004

TYPED or PRINTED NAME Gregory R. Bellomy

REGISTRATION NO. 48,451 *(if appropriate)*

TELEPHONE 510.748.7280

Docket Number: 1039.PROV2

EXHIBIT 18 Planet Depos, LLC

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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CERTIFICATE OF MAILING

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I hereby certify that this Provisional Application for Patent is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 on the date indicated above and is addressed to MS Provisional Patent Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Regina J. Pugh
Typed or Printed Name of Person Mailing Paper or Fee

Regina J. Pugh
Signature of Person Mailing Paper or Fee

Attorney Docket No. 1039.PROV2
USPTO Customer No. 26154
Avigen, Inc.
1301 Harbor Bay Parkway
Alameda, CA 94502-6541

Applicant: John Fraser Wright *et al.*
Title: Compositions and Methods to Prevent AAV Vector Aggregation

COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

FIELD OF THE INVENTION

The present invention relates to compositions and methods of preparing and storing AAV virions that prevent aggregation.

BACKGROUND

Recombinant adeno-associated virus (rAAV) is a promising vector for human gene transfer [1-3]. AAV is a member of the Dependovirus genus of the parvoviruses. AAV serotype 2 (AAV2) is composed of a single-strand DNA molecule of 4680 nucleotides encoding replication (*rep*) and encapsidation (*cap*) genes flanked by inverted terminal repeat (ITR) sequences [4]. The genome is packaged by three capsid proteins (VP1, VP2 and VP3), which are amino-terminal variants of the *cap* gene product. The resulting icosahedral virus particle has a diameter of ~26 nm. A high resolution crystal structure of AAV2 has been reported [5].

The solubility of purified AAV2 virus particles is limited, and aggregation of AAV2 particles has been described as a problem [6-9]. In commonly used buffered-saline solutions, significant aggregation occurs at concentrations of 10^{13} particles/mL, and aggregation increases at higher concentrations. Huang and co-workers reported that AAV vectors undergo concentration-dependent aggregation [7]. Xie and coworkers [9] similarly reported that at concentrations exceeding 0.1mg/mL, AAV2 vectors require elevated concentrations of salt to prevent aggregation. Aggregation of AAV2 vectors occurs at particle concentrations exceeding 10^{13} particles/mL in commonly used neutral-buffered solutions such as phosphate- and Tris-buffered saline. This corresponds to a protein concentration of ~0.06 mg/mL, and emphasizes

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the low solubility of AAV2 under these conditions. The effective vector concentration limit may be even lower for vectors purified using column chromatography techniques because excess empty capsids are co-purified and contribute to particle concentration.

Particle aggregation is a significant and not fully resolved issue for adenovirus vectors as well. Stability of a recently established adenovirus reference material (ARM) was recently reported [21]. Aggregation of the reference material, formulated in 20mM Tris, 25 mM NaCl, and 2.5% glycerol at pH 8.0, was assessed by dynamic light scattering, photon correlation spectroscopy and visual appearance. A variable level of vector aggregation following either freeze-thaw cycling or non-frozen storage was observed, resulting in restrictive protocols for the use of the ARM.

Aggregation can lead to losses during purification and inconsistencies in testing of purified vector preparations. The *in vivo* administration of AAV2 vectors to certain sites, such as the central nervous system, may require small volumes of highly concentrated vector, and the maximum achievable dose may be limited by low vector solubility.

Vector aggregation is also likely to influence biodistribution following *in vivo* administration, and cause adverse immune responses to vectors following their administration. As has been reported for proteins [10], aggregation of vector may increase immunogenicity by targeting the vector to antigen presenting cells, and inducing enhanced immune responses to the capsid proteins and transgene product. The reports of immune responses to AAV vectors in pre-clinical [11-13] and clinical [14] studies illustrate the need to address all factors that may contribute to vector immunogenicity.

Testing protocols to characterize purified vectors are also likely to be affected by vector aggregation. Determination of the infectivity titer of vector was reported to be highly sensitive

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to vector aggregation [15]. An important concern is that vector aggregates may have deleterious consequences following their *in vivo* administration because their transduction efficiency, biodistribution and immunogenicity may differ from monomeric particles. For example, intravascular delivery of AAV vectors to hepatocytes requires that the vectors pass through the fenestrated endothelial cell lining of hepatic sinusoids. These fenestrations have a radius ranging from 50 to 150 nm [20] that is predicted to allow the passage of monomeric AAV vectors (diameter ~26 nm), but prevent the passage of larger vector aggregates. In biodistribution studies in mice, aggregated AAV2 vectors labeled with the fluorescent molecule Cy3 were sequestered in liver macrophages following vascular delivery [7].

Formulation development for virus-based gene transfer vectors is a relatively recent area of investigation, and only a few studies have been reported describing systematic efforts to optimize AAV vector formulation and stability [6,8,9]. Defining formulations compatible with pre-clinical and clinical applications that minimize changes in vector preparations is an important requirement to achieve consistently high vector safety and functional characteristics. As is well established for protein therapeutics [16-19], an important aspect of vector stability is solubility during preparation and storage, and vector aggregation is a problem that needs to be fully addressed. Vector aggregation leads to losses during vector purification, and while aggregates can be removed by filtration, the loss in yield results in higher costs and capacity limitations when producing vector for pre-clinical and clinical studies. Even after filtration to remove aggregates, new aggregates can form in concentrated preparations of AAV2 vector in buffered-saline solutions.

The need exists for improved formulations and methods for purification and storage of AAV vectors, such as rAAV2, that prevent aggregation of virus particles.

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SUMMARY OF THE INVENTION

These and other needs in the art are met by the present invention, which provides high ionic strength solutions for use in preparing and storing AAV vectors that maintain high infectivity titer and transduction efficiency, even after freeze-thaw cycles.

The effect of ionic strength (μ) on virus particle interactions is determined to elucidate the mechanism of vector aggregation. The ionic strength of neutral-buffered isotonic saline (μ = 150mM) is insufficient to prevent aggregation of AAV2 vectors purified by gradient ultracentrifugation or by cation exchange chromatography at concentrations exceeding ~10^{13} particles/mL. Inclusion of sugars (sorbitol, sucrose, mannitol, trehalose, glycerol) at concentrations up to 5% (w/v) or of surfactants Tween80 (1%) or Pluronic® F68 (10%) does not prevent aggregation of vector particles.

In contrast, vector particles remain soluble when elevated ionic strength solutions (μ > 200mM) are used during purification and for final vector formulation. Elevated ionic strength solutions using isotonic excipient concentrations for *in vivo* administration are prepared with salts of multivalent ions, including sodium citrate, sodium phosphate, and magnesium sulfate. An isotonic formulation containing 10mM Tris, 100mM sodium citrate, 0.001% Pluronic® F68, pH 8.0 (μ ~500mM) enables concentration of AAV2-AADC vectors to 6.4x10^{13} vg/mL with no aggregation observed during preparation and following ten freeze-thaw cycles at -20 °C. *See* Table 3, below, and accompanying discussion. AAV2-AADC vectors prepared and stored for an extended period in elevated ionic strength formulation retain high infectivity titer (13 IU/vg) and transduction efficiency.

Nuclease treatment of purified AAV2 vectors reduces the degree of vector aggregation, implicating vector surface nucleic acid impurities in inter-particle interactions. Hence,

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purification methods to efficiently remove vector surface residual nucleic acids, coupled with the use of elevated ionic strength isotonic formulations, are useful methods to prevent AAV2 vector aggregation.

In one embodiment the invention comprises AAV storage solutions with ionic strength (μ) greater than 200 mM. In other embodiments the ionic strength is 300, 400 or 500 mM or higher.

In some embodiments of the present invention, the surfactant Pluronic® F68 is added to a solution for preparing or storing AAV vectors, for example to 0.001%. In one embodiment, the solution comprises 10 mM Tris, 100mM sodium citrate and 0.001% Pluronic® F68, pH 8.0.

In one embodiment, AAV vectors can be stored in solutions of the present invention at concentrations up to $6.4x10^{13}$ vg/mL with no aggregation even after ten freeze-thaw cycles at -20°C.

In another aspect, the invention relates to methods of preparing and storing preparations of AAV vectors using high ionic strength solutions. In some embodiments, 0.001% Pluronic® F68 is included in the high ionic strength solutions.

In some embodiments of the present invention, purified AAV vectors are treated with one or more nucleases to reduce aggregation. In one embodiment such nuclease treatment is combined with storage in a high ionic-strength solution of the invention.

In yet another aspect, the invention relates to kits comprising the high ionic strength formulations of the invention. In one embodiment the kits comprise pre-mixed solution. In another embodiment the kit comprises two or more separate components of a high ionic strength solution of the present invention to be mixed by a user. In some embodiments the kit comprises one or more of the following: sodium citrate, sodium phosphate, magnesium sulfate, Tris and

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Pluronic® F68. In other embodiments the kit further comprises a recipe for mixing a solution of the present invention.

DESCRIPTION OF THE DRAWINGS

FIGS. 1A and **1B** present data showing aggregation of AAV2-FIX particles as a function of osmolarity (FIG. 1A) or ionic strength (FIG. 1B) for various buffer compositions. AAV2-FIX vectors are prepared by Method 2 of Example 1. Average particle radius is measured by dynamic light scattering (DLS) following vector dilution in varying concentrations of excipients buffered with 10 mM sodium phosphate at pH 7.5. Excipients include sodium chloride (●), sodium citrate (○), sodium phosphate (■), sodium sulfate (□), magnesium sulfate (▲), and glycerol (Δ).

FIG. 2 presents data on AAV2-FIX aggregation as a function of the method of purification. The average particle radius is measured by DLS following vector dilution in varying concentrations of sodium chloride buffered with10mM sodium phosphate at pH 7.5. Vectors are purified by Method 1 (double CsCl gradient) (○); Method 2 (cation exchange chromatography) (□); Method 2 plus nuclease digestion (■); or Method 3 (chromatography plus one CsCl gradient) (Δ). Purification Methods 1-3 are described in Example 1.

FIG. 3 presents data on transgene expression from D7/4 cells transduced with rAAV2-AADC virions prepared and stored in high ionic strength formulation (□) or in a control formulation (●). The concentration of AADC was measured by ELISA (in triplicate for each data point) 72 hours post-transduction. Error bars represent standard deviations.

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DETAILED DESCRIPTION OF THE INVENTION

AAV2 vector aggregation is frequently observed in concentrated preparations of vectors and can affect purification recovery, and *in vivo* potency and safety. Hence, an important objective for the development AAV2 vectors is to identify methods and formulations that prevent aggregation of vectors when concentrated stocks are prepared. Unless otherwise indicated, the term "vector" as used herein refers to a recombinant AAV virion, or viral particle.

The present invention is based in part on the observation that solution ionic strength is an important parameter in AAV vector aggregation, implicating the involvement of ionic interactions between virus particles in the aggregation process. The observation that elevated ionic strength increases AAV2 vector solubility regardless of the identity of the charged excipient supports the hypothesis that ionic strength of solution *per se*, rather than interactions involving a specific ionic species, is the relevant physico-chemical parameter. A threshold ionic strength of at least 200mM is required to prevent aggregation at vector particle concentrations examined herein.

Of practical concern, commonly used buffered saline solutions have insufficient ionic strength to prevent AAV2 vector aggregation at concentrations exceeding 10^{13} particles/mL. It is known that high salt concentrations increase AAV2 vector solubility (e.g. highly concentrated AAV2 vectors recovered from gradients generally remain soluble in concentrated CsCl). However, optimal formulations for pre-clinical and clinical studies should be close to isotonic (280-400 mOsm), especially for *in vivo* administration of vector to sites where dilution of hypertonic solutions may be slow. In embodiments of the present invention the exponential relationship of ionic strength with charge valency is used to develop isotonic formulations with high ionic strengths. Salt species with multiple charge valencies (e.g. salts of sulfate, citrate, and

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phosphate) that are commonly used as excipients in human parenteral formulations [22] can provide the level of ionic strength needed to prevent AAV2 vector aggregation when used at isotonic concentrations. While isotonic (150mM) sodium chloride has an ionic strength of 150mM, a value insufficient to maintain AAV2 solubility at high vector concentrations, isotonic sodium citrate, with an ionic strength of ~500mM, can support AAV2 vector concentrations of at least 6.4 x 10^{13} vg/mL without aggregation.

Without intending to be limited by theory, the low solubility of AAV2 particles may be caused by their highly symmetrical nature in conjunction with the stabilizing effect of complementary charged regions between neighbouring particles in aggregates. The surface charge density based on the crystal structure of AAV2 [5] reveals a pattern of positive and negative charges on the virus surface. Previous reports showed that AAV2 vector aggregation is pH dependent, and hypothesized that amino acids with charged side groups are involved in inter-particle binding [23]. These reports hypothesized that if charged amino acid side chains are involved in vector aggregation, high concentrations of free amino acids could block vector particle interactions. However, we have found that amino acids with charged side chains are not effective in preventing AAV2 vector aggregation beyond their contribution to ionic strength.

Vector aggregation at low ionic strength was also found to be reduced but not prevented by efficient nuclease treatment of purified vector particles. Digestion at an earlier stage of the purification process (clarified HEK cell lysate) did not reduce aggregation following vector purification. It is likely that digestion of already purified virions is more efficient because of a higher enzyme to nucleic acid substrate ratio. One mechanism to explain these results is that residual nucleic acid impurities (e.g. host cell and plasmid DNA) bound to the vector surface can bridge to binding sites on neighbouring virus particles and thus cause aggregation. Purified

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AAV2 vectors (empty capsid free) have been reported to contain approximately 1% non-vector DNA [24]. While >50% of this non-vector DNA was reported to be nuclease resistant and was packaged within capsid particles, some impurity DNA was nuclease resistant and appeared to be associated with the surface of purified vector particles. The observation that efficient nuclease treatment can reduce vector aggregation suggests that nucleic acids associated with the vector surface at an average level not greater than ~25 nucleotides per vector particle can contribute to AAV vector aggregation.

In summary, the use of high ionic strength solutions during AAV2 vector purification and final formulation, and efficient removal of residual vector surface DNA are two effective strategies to achieve highly concentrated solutions of AAV2 vectors for use in pre-clinical and clinical studies. High ionic strength solutions and nuclease treatment can be used in combination or separately. Although data were obtained using AAV2 vectors, the composition and methods of the present invention may also be useful with other AAV serotypes / variants, or other viral vectors such as adenoviruses, lentiviruses and retroviruses.

AAV Aggregation as a Function of Excipient Concentration

Initial screening experiments are performed to elucidate the mechanism of AAV vector aggregation and to identify classes of excipients that can reduce / prevent aggregation. Vector aggregation can be caused by dilution (5-fold) of vector in neutral-buffered saline with low concentration buffer (20mM sodium phosphate, pH 7.2). Excipients are screened using this "dilution-stress" method to identify excipients that are able to prevent vector aggregation when included in the diluent. For screening, aggregation is measured by dynamic light scattering

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(DLS). Classes of excipients examined included selected inorganic salts, amino acids, uncharged carbohydrates, and surfactants. Results are presented in Table 1.

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TABLE 1

SCREENING FOR EXCIPIENTS THAT PREVENT AAV2 VECTOR AGGREGATION USING DILUTION-STRESS METHOD

Excipient	Osm required to prevent aggregation (max tested)
Magnesium sulfate	180 mOsm
Sodium citrate	220 mOsm
Sodium chloride	320 mOsm
Sodium phosphate	220 mOsm
Sodium sulfate	220 mOsm
Arginine	NIA (200 mOsm)
Aspartic acid	320 mOsm
Glutamic acid	320 mOsm
Glycine	NIA (200 mOsm)
Histidine	NIA (200 mOsm)
Lysine	300 mOsm
Glycerol	NIA (5% w/v, 543 mOsm)
Iodixanol	NIA (5% w/v, 32 mOsm)
Mannitol	NIA (5% w/v, 275 mOsm)
Sorbitol	NIA (5% w/v, 275 mOsm)
Sucrose	NIA (5% w/v, 146 mOsm)
Trehalose	NIA (5% w/v, 146 mOsm)
Pluronic® F68	NIA (10% w/v, 12 mOsm)
Polysorbate 80	NIA (1% w/v)

NIA: No inhibition of aggregation

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As illustrated in Table 1, charged excipients (inorganic salts and amino acids) prevent aggregation when present at sufficient concentrations. However, salt concentrations required to prevent vector aggregation vary, ranging from 180 mOsm for magnesium sulfate, to 320 mOsm for sodium chloride. The amino acids arginine, aspartic acid, glutamic acid, glycine, histidine, and lysine do not prevent aggregation at 200 mOsm, but lysine, aspartic acid, and glutamic acid prevent aggregation at 300-320 mOsm. Arginine, glycine and histidine were not tested at concentrations other than 200 mOsm. Selected carbohydrates have no effect on vector particle aggregation when present at concentrations up to 5% w/v. For example, 5% w/v glycerol (543 mOsm) does not prevent aggregation. The surfactants Polysorbate80 (1% w/v) and Pluronic® F68 (10% w/v) similarly have no effect on aggregation using the "dilution-stress" method.

AAV Aggregation as a Function of Osmolarity and Ionic Strength

FIGS. 1A and 1B show the results of a more detailed analysis of vector aggregation as a function of the concentration of various salts. FIG. 1A shows vector aggregation as a function of the osmolarity of selected excipients. For charged species a concentration-dependent inhibition of AAV2 vector aggregation is observed. Salts with multivalent ions achieve a similar degree of inhibition of aggregation at lower concentrations than monovalent sodium chloride. For example, magnesium sulfate prevents aggregation at ≥200 mOsm whereas sodium chloride requires ≥350 mOsm to achieve a similar effect. Sodium citrate, sodium sulfate, and sodium phosphate are intermediate in their potency to prevent vector aggregation.

Although the results in FIG. 1A and Table 1 show no effect of glycerol and certain sugars at concentrations up to 5% on AAV2 vector aggregation induced by low ionic strength, the data

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cannot rule out improvement of AAV2 solubility at glycerol concentrations above 5%. For example, Xie and co-workers reported that 25% (w/v) glycerol enabled concentration of AAV2 to very high concentrations (4.4 to 18 x 10^{14} particles/ml) in low ionic strength solutions [9].

FIG. 1B shows the data of FIG. 1A plotted as a function of the calculated ionic strength, rather than osmolarity, for each excipient. FIG. 1B demonstrates that vector aggregation is prevented when ionic strength is ~200 mM or greater regardless of which salt is used. These data suggested that the ionic strength (μ) of a solution, a parameter that depends on both solute concentration and charge valency, is the primary factor affecting aggregation.

Other ionic strengths may also be used in embodiments of the present invention to prevent aggregation, such as 250 mM, 300 mM, 350 mM, 400 mM, 450 mM, 500 mM, 600 mM, 700 mM or higher ionic strengths. Multivalent ions are preferred to achieve these ionic strengths in methods and formulations of the present invention, such as divalent, trivalent, tetravalent, pentavalent ions and ions of even higher valency. The pH buffer in solutions and formulations of the present invention may be phosphate, Tris, or HEPES (or other Good's buffers), but any other suitable pH buffer may be used. In preferred embodiments, the multivalent ions and buffer are selected to be compatible with the target tissue for the vector being prepared.

AAV Aggregation as a Function of the Method of AAV Purification

Recombinant AAV2 purified using different methods (e.g. density gradient purification versus ion-exchange chromatography) would be expected to have different impurity profiles. FIG. 2 shows vector aggregation as a function of ionic strength for several preparations of AAV differing in the purification method. Purification methods are described in Example 1. Sodium chloride is used to vary the ionic strength. AAV2-FIX vectors purified by double cesium

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chloride gradient ultracentrifugation (Method 1), by cation exchange column chromatography (Method 2), or by combined column and cesium chloride gradient ultracentrifugation (Method 3) each demonstrate similar aggregation responses as ionic strength is decreased. In contrast, AAV2-FIX purified by the column method and then subjected to a nuclease digestion step (Method 2 + nuclease) shows reduced aggregation at low ionic strength.

AAV Aggregation at Preparative Scale

The data in Table 1 and FIGS. 1A, 1B and 2 involve vector aggregation *at an analytical scale*, employing a method to measure aggregation (DLS) that is difficult to quantify reliably. Table 2, in contrast, shows the effects of elevated ionic strength and nuclease treatment on AAV2 vector aggregation at a larger scale, using methods to induce and quantify vector aggregation that are relevant to *preparative scale* vector purification. Experimental details are provided in Example 2. Purified AAV vectors are diafiltered into solutions of various ionic strengths, the volume is reduced to achieve high vector concentrations, and aggregation is then assessed by measuring vector recovery after filtration through a 0.22μm filter. Aliquots from a single pool of AAV2-AADC vector purified by Method 1 through the second CsCl gradient centrifugation step ($1.8x10^{15}$ vg in 91mL, $1.8x10^{13}$ vg/mL, in ~3M CsCl) are used as starting material in the diafiltration experiments. Tangential flow filtration using hollow fibers is used for diafiltration because it is scalable and yet it still enables preparation of volumes (min. 1.4mL), and thus AAV concentrations, at which aggregation would be expected in neutral buffered saline.

In Experiment 1, three hollow fiber units are used to diafilter AAV2-AADC vector in formulations CF, TF1, or TF2, and the volume is reduced to a target of $2.5x10^{13}$ vg/mL. *See*

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Example 2. The samples are then filtered through a 0.22μm filter. Results are shown in Table 2. Vector recovery ("Yield %") for both elevated ionic strength formulations TF1 (95 ± 7.4%) and TF2 (93 ± 7.4%) are significantly higher than the recovery using the control formulation CF (77 ± 6.6%).

TABLE 2
AAV VECTOR RECOVERY AT PROCESS SCALE

Experiment	Formulation	μ (mM)	Target (vg/mL)	Actual (vg/mL)	Yield % (RSD)
1	CF	160	2.5E13	1.93E13	77 (6.6)
1	TF1	310	2.5E13	2.38E13	95 (7.4)
1	TF2	510	2.5E13	2.33E13	93 (7.4)
2	CF	160	6.7E13	3.98E13	59 (6.0)
2	TF2	510	6.7E13	6.42E13	96 (4.4)
3	CF (-Bz)	160	3.6E13	2.46E13	68 (11)
3	CF (+Bz)	160	3.6E13	3.29E13	91 (12)

In Experiment 2, AAV2-AADC is concentrated to a higher target value ($6.7x10^{13}$ vg/mL) in CF or TF2. Vector recovery using TF2 (96 ± 4.4%) is again significantly higher than recovery using CF (59 ± 6.0%). Within the variability of the assays used, vector was recovered fully at both target concentrations using TF2, indicating that aggregation was prevented. In contrast, significant aggregation was observed at both target concentrations using CF, and the extent of aggregation (i.e. loss following 0.22μm filtration) was higher at the higher target vector concentration. In an additional experiment (not shown), 50 μL samples of AAV2 vector are taken following concentration but prior to the 0.22μm filtration step of Experiment 2, and examined by light microscopy. Vector concentrated in CF contains obvious amounts of visible material (not shown), while no such material is seen in vector concentrated in TF2.

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Experiment 3 examines the effect of prior nuclease digestion of purified vector on aggregation. In the absence of nuclease digestion recovery of AAV2-AADC in CF is 68 ± 11%, similar to the recoveries in Experiments 1 and 2. In contrast, purified vector treated with nuclease and then concentrated in CF gives higher recovery (91 ± 12%). These prep scale results reflect the same effect of nuclease digestion shown in FIG. 2 using the "dilution-stress" (analytical scale) method.

AAV Stability and Activity Following Storage or Freeze-Thaw Cycling

Croyle and coworkers reported a significant loss of titer of AAV and adenovirus following multiple freeze-thaw cycling in sodium phosphate buffer, and demonstrated that the better pH buffering provided by potassium phosphate during freeze-thaw cycling prevented titer loss [6]. Results of our freeze-thaw stability study using sodium phosphate support these findings. We find that while 150mM sodium phosphate provides sufficient ionic strength to prevent aggregation during preparation and non-frozen storage of concentrated AAV2-AADC vector, even a single freeze-thaw cycle at -20 or -80 °C results in aggregation.

AAV stability after storage or freeze-thaw (F/T) cycling is assessed in buffers of the present invention as follows. The concentrated vectors prepared in CF, TF1, and TF2 (Table 2, Experiment 1) are subjected to a short stability study to investigate whether aggregation will occur during refrigerated storage, or following multiple freeze-thaw (F/T) cycles. Aggregation is assessed by DLS using undiluted samples, and *Rh* values >20nm are deemed to indicate the occurrence of some level of aggregation.

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TABLE 3

STABILITY OF AAV2 VECTORS

	Particle radius – Rh (nm)							
Formulation	Pre	4 °C	- 20 °C			- 80 °C		
		5d	1 F/T	5 F/T	10 F/T	1 F/T	5 F/T	10 F/T
CF	14.5	27.0	22.4	56.1	94.5	20.6	57.5	141
TF1	13.8	16.3	TH	TH	TH	TH	TH	TH
TF2	13.8	14.4	14.2	14.0	14.1	13.8	21.3	50.9

Pre: DLS radius measured immediately following 0.2μm filtration.

Vector concentrations (vg/mL): CF: 1.93E13, TF1: 2.38E13, TF2: 2.33E13.

TH: signal intensity is too high to measure because of extensive aggregation.

As shown in Table 3, AAV2-AADC vector prepared in CF shows some aggregation after 5 days of storage at 4 °C, as well as following one or more F/T cycles at -20 or -80°C. For vector prepared in TF1, no aggregation occurs after 5 days at 4°C, but aggregation occurs following a single F/T cycle at -20 or -80 °C as indicated by a DLS signal intensity that is too high to measure. Visual inspection of these samples reveals slight cloudiness, which is consistent with aggregation. For vector prepared in TF2, no aggregation is observed at 4 °C, or following up to 10 F/T cycles at -20 °C. Some aggregation is observed following 5 and 10 F/T cycles at -80 °C.

AAV activity after storage or F/T cycling in TF2 is assessed as follows. As described above, the high ionic strength, isotonic formulation TF2 effectively prevents vector aggregation during concentration and storage, and therefore represents a promising candidate for further study. An important question is whether preparation and storage of the vector in high ionic strength TF2 would adversely affect its functional activity. To assess this, assays are performed

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to measure the infectious titer and the transduction efficiency of vectors prepared and stored for an extended period of time in TF2.

For infectivity, a highly sensitive infectivity assay capable of detecting single infectious events is used [15]. AAV2-AADC is prepared in TF2 at a concentration of $6.4x10^{13}$ vg/mL. After being stored for 45 days at 4 °C the preparation has a vector genome to infectious unit ratio (vg/IU) of 13, compared to a value of 16 vg/IU for the reference vector. This difference is not significant given the reported variability of this assay (RSD ~50%).

Transduction efficiency is assessed by measuring the expression of AADC protein by ELISA following transduction of D7/4 cells. FIG. 3 shows no significant difference between vector prepared in TF2 and the reference control for vector input ranging from 10 to 10^5 vg/cell. Together, these data indicate that preparation and storage of AAV2 vectors in high ionic strength TF2 does not have a deleterious effect on vector infectivity or transduction efficiency.

EXAMPLE 1

AAV PURIFICATION METHODS

AAV2 vectors expressing human coagulation factor IX (FIX) or human amino acid decarboxylase (AADC) are produced by triple transfection of HEK293 cells as previously described [25], with modifications. For the large scale preparations, cells are cultured and transfected in 850 mm^2 roller bottles (Corning). Vectors are purified by one of three methods.

In purification Method 1, modified from Matsushita, transfected HEK293 cells in roller bottles are collected by centrifugation (1000 *g*, 15min), resuspended in 10mM sodium phosphate, 500mM sodium chloride, pH 7.2, and lysed by three freeze / thaw cycles (alternating an ethanol / dry ice bath and a 37°C water bath). The cell lysate is clarified by centrifugation (8,000 *g*,

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15 min). The supernatant is then diluted to 200mM NaCl by addition of 10mM sodium phosphate, pH 7.2, and digested with Benzonase (Merck, Purity Grade 1; 200 U/mL, 1h, 37 °C). The lysate is adjusted to 25mM $CaCl_2$ using a 1M stock solution, and incubated at 4°C for one hour.

The mixture is centrifuged (8,000 *g*, 15 min), and the supernatant containing vector is collected. To precipitate virus from the clarified cell lysate, polyethylene glycol (PEG8000) is added to a final concentration of 8%, the mixture incubated at 4°C for three hours, and then centrifuged (8,000 *g*, 15 min). The pellets containing vector are re-suspended with mixing in 0.15M NaCl, 50mM Hepes, 25mM EDTA, pH 8.0 and incubated at 4°C for 16 hours. The resuspended material is pooled, and solid cesium chloride is added to a final density of 1.40 gm/ml. Vector is then banded by ultracentrifugation (SW28, 27,000rpm, 24h, 20°C) using a Beckman model LE-80 centrifuge. The centrifugation tubes are fractionated, and densities from1.38 to 1.42 gm/mL containing vector are pooled. This material is banded a second time by ultracentrifugation (NVT65 rotor, 65,000 rpm, 16h, 20°C), and fractions containing purified AAV2 vectors are pooled. To concentrate vector and to perform buffer exchange, vectors in concentrated cesium chloride solution are subjected to ultrafiltration / diafiltration (UF/DF) by tangential flow filtration as described below (Example 2).

In purification Method 2, cell harvests containing AAV are microfluidized and filtered sequentially through 0.65 and 0.22 μm filters (Sartorius). Virus is purified from the clarified cell lysates by cation exchange chromatography using Poros HS50 resin as previously described [26]. For the nuclease digestion described in FIG. 2, column-purified vectors are incubated (4h, RT) with 100 U/mL Benzonase and 10 U/mL DNAse I (RNAse free, Roche Diagnostics, Indianapolis, Indiana).

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For purification Method 3, AAV2 vectors purified by cation exchange chromatography are subjected to an additional cesium chloride gradient ultracentrifugation step (SW28, 27,000rpm, 20h) to remove empty capsids prior to UF/DF.

Real time quantitative PCR (Q-PCR) is used to quantify AAV preparations as previously described [27]. Vectors purified by each of the three methods are analyzed by SDS-PAGE / silver staining analysis, and in all cases VP1, VP2 and VP3 are present in the expected ratios, with the capsid proteins representing >95% of total proteins as determined by scanning densitometry. However, unlike gradient-purified AAV2 vectors purified using Methods 1 and 3, vectors purified by Method 2 (column chromatography) contain empty capsids, ranging from 3-10 empty capsids per vector genome.

EXAMPLE 2

ULTRAFILTRATION AND DIAFILTRATION TO DETECT AAV AGGREGATION

Disposable hollow fiber tangential flow filtration devices (Amersham BioSciences 8" Midgee, 100 kDa nominal pore size) are used to concentrate and diafilter AAV2 vectors purified by the methods described above, and for the UF/DF experiments described in Table 2. For all UF/DF procedures a volume of diafiltration buffer corresponding to 10x the product volume is used, and it is added in ~1mL increments to approximate continuous diafiltration. Using this method, the calculated residual CsCl after diafiltration is <0.5mM.

The following three formulations were used for UF/DF: Control Formulation (CF: 140mM sodium chloride, 10mM sodium phosphate, 5% sorbitol, pH 7.3); Test Formulation 1 (TF1: 150mM sodium phosphate, pH7.5); and Test Formulation 2 (TF2: 100 mM sodium citrate, 10mM Tris, pH8.0). For Experiment 1 shown in Table 2, diafiltration is performed at a volume

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corresponding to a vector concentration of $1x10^{13}$ vg/mL, and following diafiltration the volume is reduced to a value corresponding to $2.5x10^{13}$ vg/mL (assuming no vector loss).

For Experiment 2, diafiltration is performed at a volume corresponding to a $2x10^{13}$ vg/mL, and the volume is then reduced to a value corresponding to $6.7x10^{13}$ vg/mL.

For Experiment 3 (CF ± Bz), AAV2-AADC (approximately $1.2x10^{14}$ vg) is first diafiltered into TF1 (a formulation compatible with nuclease activity) and then passed through a 0.22 µm filter. The titer of this material is determined, and the volume is adjusted to correspond to a concentration of $1x10^{13}$ vg/mL. To 10 mL of this material, $MgCl_2$ is added to a concentration of 2 mM, and then divided into two equal aliquots. One aliquot is incubated with Benzonase (200 U/mL, 4h, RT), and the second is mock-incubated. Each aliquot is then diafiltered at a volume corresponding to a vector concentration $2x10^{13}$ vg/mL, and then concentrated to a $3.6x10^{13}$ vg/mL target. Following all UF/DF protocols, Pluronic® F-68 (BASF Corp., Mount Olive, NJ) from a 1% stock is added to the vector product to a final concentration of 0.001%, and the solution is passed through a 0.22µm syringe filter (Sartorius). All UF/DF procedures are performed in a laminar flow cabinet.

EXAMPLE 3

MEASUREMENT OF VECTOR AGGREGATION BY DYNAMIC LIGHT SCATTERING

Purified vectors are analyzed for aggregation by dynamic light scattering (DLS) using a Protein Solutions *DynaPro 99* (λ=825.4 nm). Primary data (particle radius – *Rh*, average value measured over 30 cycles, 10 cycles/min) are used for all analyses reported. A "dilution-stress" method is used to assess the effect of varying excipients on vector aggregation. In this method,

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80 µL of test diluent is added to 20 µL of vector solution with mixing in the actual cuvette used for DLS measurement, and data collection is initiated within 10 seconds of mixing. Prior to addition of test diluents, the *Rh* value for AAV2 vector preparations is measured and confirmed to be <15 nm to ensure that the starting material is monomeric. Samples that are not 100% monomeric are passed through a 0.22μm syringe disc filter (Sartorius, low protein binding) to remove aggregates.

The osmolarity and ionic strength values given in FIGS. 1 and 2 are calculated using all excipients present in the mixture (i.e. weighted: test diluent (80%) and starting vector formulation (20%)). Osmolarity is calculated according to the equation: Osmolarity = $\sum c_i$, where c_i is the molar concentration of each solute species. The ionic strength (μ) is calculated according to the equation: $\mu = \frac{1}{2} \sum c_i z_i^2$, where z_i is the charge on each species. In conditions that resulted in vector aggregation (e.g. low μ) a progressive increase in *Rh* is observed over the course of data collection. To validate the use of the average *Rh* measured over the 3 minute interval following dilution as a reliable measure of aggregation, the average rate of increase of *Rh* (ΔRh / Δt) over the same time interval is also determined (not shown). Analysis of ΔRh / Δt gives results concordant with those obtained using the average *Rh* value reported in FIGS. 1 and 2.

EXAMPLE 4

AAV VIRION INFECTIVITY

Infectivity of AAV2-AADC vectors is determined using a highly sensitive assay as previously described [15]. Briefly, samples are serially diluted (10-fold dilutions, 10 replicates / dilution) and added to D7/4 cells (modified HeLa cells expressing AAV *rep* and *cap*) grown in

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96 well tissue culture plates (Falcon, cat. #353227) in DMEM medium containing 10% FBS. Adenovirus (Ad-5, 100 vp/cell) is added to each well to provide helper functions. After 48h, replication of AAV vector in each well is quantified by Q-PCR using transgene-specific primers and probes, and the frequency of infection at limiting dilution is analyzed by the Karber method to calculate the infectivity titer. The test sample is run concurrently with an AAV2-AADC reference previously prepared in CF and stored at -80 °C.

The transduction efficiency of AAV2 vectors is quantified by a whole cell ELISA. D7/4 cells grown in 96 well plates are infected with 10-fold serial dilutions of the test sample and reference vector, corresponding to 10 to 10^5 vg / cell input (5 replicates / dilution). After 48h, the culture medium is removed, and cells are washed twice with 200 μL PBS (10 mM sodium phosphate, 140mM sodium chloride, pH 7.2). Cells are then permeabilized and fixed by addition of 100μL of PBS containing 0.5% Triton X-100 and 4% paraformaldehyde to each well (15 min). The fixing solution is removed, and the cells are washed twice with PBS containing 0.5% Triton X-100. Non-specific sites are blocked by adding PBS containing 3% bovine serum albumin (BSA) and 0.5% Triton X-100 (60min).

After washing, cells are incubated for one hour with rabbit anti-AADC IgG antibody (Chemicon, AB136), and washed. Cells are then incubated for one hour with alkaline phosphatase-conjugated goat anti-rabbit IgG, and washed. Antibodies are diluted 1:1000 in PBS containing 1% BSA, 0.5% Triton X-100. Substrate (PNPP, Pierce, cat. #34047) is then added (1 mg/mL in 1X diethanolamine substrate buffer, Pierce, cat. #34064), and after incubation for 30min the concentration of cleaved substrate is measured spectrophotometrically (λ=405nm). Human AADC expression as a function of vector input is fitted using a spline curve (SigmaPlot). The AAV2-AADC reference vector is measured concurrently with the test sample.

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While preferred illustrative embodiments of the present invention are described, it will be apparent to one skilled in the art that various changes and modifications may be made therein without departing from the invention, and it is intended in the appended claims to cover all such changes and modifications that fall within the true spirit and scope of the invention.

All publications, patents and patent applications referred to herein are hereby incorporated by reference in their entireties.

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REFERENCES

1. Grimm, D., and Kleinschmidt, J. A. (1999). Progress in adeno-associated virus type 2 vector production: promises and prospects for clinical use. *Hum Gene Ther.* **10**: 2445-2450.
2. High, K. A. (2001). AAV-mediated gene transfer for hemophilia. *Ann. N. Y. Acad. Sci.* **953**: 64-67.
3. Pfeifer, A., and Verma, I. M. (2001). Gene therapy: Promises and problems. *Ann. Rev. Genomics Hum. Genet.* **2**: 177-211.
4. Berns, K. I. (1996). Parvoviridae: The viruses and their replication. In *Fields Virology* (B. N. Fields, D. M., Knipe, P. M., Howley, *et. al.* Eds.), pp. 2173-2197. Lippincott-Raven Publishers, Philadelphia.
5. Xie, Q., *et al.* (2002). The atomic structure of adeno-associated virus (AAV-2), a vector for human gene therapy. *Proc. Natl. Acad. Sci. U.S.A.* **99**: 10405-10410.
6. Croyle, M. A., Cheng, X., and Wilson, J. M. (2001). Development of formulations that enhance physical stability of viral vectors for gene therapy. *Gene Therapy* **8**: 1281-1290.
7. Huang, J., *et al.* (2000). Aggregation of AAV vectors, its impact on liver directed gene transfer and development of vectors formulations to prevent and dissolve aggregation and enhance gene transfer efficiency. *Mol. Therapy* **1**: S286.
8. Wright, J. F., Qu, G., Tang, C., and Sommer, J. M. (2003). Recombinant adeno-associated virus: Formulation challenges and strategies for a gene therapy vector. *Curr. Opin. Drug Disc. Dev.* **6**: 174-178.
9. Xie, Q., Hare, J., Turnigan, J., Chapman, M. S. (2004). Large-scale production, purification and crystallization of wild-type adeno-associated virus-2. *J. Virol. Methods.* **122**: 17-27.
10. Braun, A., Kwee, L., Labow, M. A., Alsenz, J. (1997). Protein aggregates seem to play a key role among the parameters influencing the antigenicity of interferon alpha (IFN-alpha) in normal and transgenic mice. *Pharm. Res.* **14**: 1472-1478.

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GENZ_NOV_00149064

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11. Chenuaud, P., *et al.* (2004). Autoimmune anemia in macaques following erythropoietin gene therapy. *Blood* **103**: 3303-3304.

12. Flotte, T. R. (2004). Immune responses to recombinant adeno-associated virus vectors: Putting preclinical findings into perspective. *Human Gene Ther.* **15**: 716-717.

13. Gao, G., et al. (2004). Erythropoietin gene therapy leads to autoimmune anemia in macaques. *Blood* **103**: 3300-3302.

14. High, K. A., *et al.* (2004). Human immune responses to AAV-2 capsid may limit duration of expression in liver-directed gene transfer in humans with hemophilia B. *Blood* **104**: 121a.

15. Zhen, Z., Espinoza, Y., Bleu, T., Sommer, J. M., and Wright, J. F. (2004). Infectious titer assay for adeno-associated virus vectors with sensitivity sufficient to detect single infectious events. *Human Gene Ther.* **15**: 709-715.

16. Chen, B., Arakawa, T., Hsu, E., Harhi, L. O., Tressel, T. J., and Chien, S. L. (1994). Strategies to suppress aggregation of recombinant keratinocyte growth factor during liquid formulation development. *J. Pharm. Sci.* **83**: 1657-1661.

17. Shire, S. J., Shahrokh, Z., and Liu, J. (2004). Challenges in the development of high protein concentration formulations. *J. Pharm. Sci.* **93**: 1390-1402.

18. Wang, W. (1999). Instability, stabilization, and formulation of liquid protein pharmaceuticals. *Int. J. Pharm.* **185**: 129-188.

19. Won, C. M., Molnar, T. E., McKean R. E., Spenlehauer, G. (1998). Stabilizers against heat-induced aggregation of RPR 114849, an acidic fibroblast growth factor (aFGF). *Int. J. Pharm.* **167**: 25-36.

20. Meijer, K. D. F., and Molema, G. (1995). Targeting of drugs to the liver. *Sem. Liver Dis.* **15**: 206.

21. Adadevoh, K., Croyle, M., Malarme, D., Bonfils, E., and Bowe, M. A. (2002). A short-term field use and shipping stability study of a wild type Ad5 adenoviral reference material. *BioProcessing* fall: 62-69.

22. Parenteral excipients website.

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23. Qu, G., Connolly, C., Koblansky, A., Sommer, J. M., McClelland, A., and Wright, J. F. (2003). Evidence that ionic interactions are involved in concentration-induced aggregation of recombinant adeno-associated virus. *Mol. Therapy* **7**: S238.

24. Smith, P., Wright, J. F., Qu, G., Patarroyo-White, S., Parker, A., Sommer, J. M. (2003). Packaging of host cell and plasmid DNA into recombinant adeno-associated virus particles produced by triple transfection. *Mol. Therapy* **7**: S348.

25. Matsushita, T., *et al.* (1998). Adeno-associated virus vectors can be efficiently produced without helper virus. *Gene Therapy* **5**: 938-945.

26. Wright, J. F., and Qu, G. (2003). Large-scale recombinant adeno-associated virus (rAAV) production and purification. United States Patent 6,593,123.

27. Sommer, J. M., *et al.* (2003). Quantification of adeno-associated virus particles and empty capsid by optical density measurement. *Mol. Therapy* **7**: 122-128

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We claim:

1. A method of preventing aggregation of virions in a preparation of virions, comprising treating said preparation of virions with a nuclease.

2. The method of claim 1, wherein the virions are AAV virions.

3. The method of claim 2, wherein the virions are AAV2 virions.

4. The method of claim 1, further comprising adding one or more excipients to the preparation of virions to achieve an ionic strength of greater than 200 mM.

5. The method of claim 4, wherein one or more of the excipients is a compound comprising one or more multivalent ions.

6. A solution for the storage of purified virus particles, comprising:

a pH buffer; and

one or more multivalent ions; wherein the ionic strength of the solution is greater than 200 mM.

7. The solution of claim 6, wherein the purified virus particles are AAV virus particles.

8. The solution of claim 6, wherein one of the one or more multivalent ions is citrate.

9. The solution of claim 6, further comprising Pluronic® F68.

10. The solution of claim 9, wherein the Pluronic® F68 is present at 0.001%.

11. The solution of claim 6, comprising:

10 mM Tris, pH 8.0; and

100 mM sodium citrate.

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ABSTRACT

Compositions and methods are provided for preparation of concentrated stock solutions of AAV virions without aggregation. Formulations for AAV preparation and storage are high ionic strength solutions (e.g. μ ~500mM) that are nonetheless isotonic with the intended target tissue. This combination of high ionic strength and modest osmolarity is achieved using salts of high valency, such as sodium citrate. AAV stock solutions up to $6.4x10^{13}$ vg/mL are possible using the formulations of the invention, with no aggregation being observed even after ten freeze-thaw cycles. The surfactant Pluronic® F68 may be added at 0.001% to prevent losses of virions to surfaces during handling. Virion preparations can also be treated with nucleases to eliminate small nucleic acid strands on virions surfaces that exacerbate aggregation.

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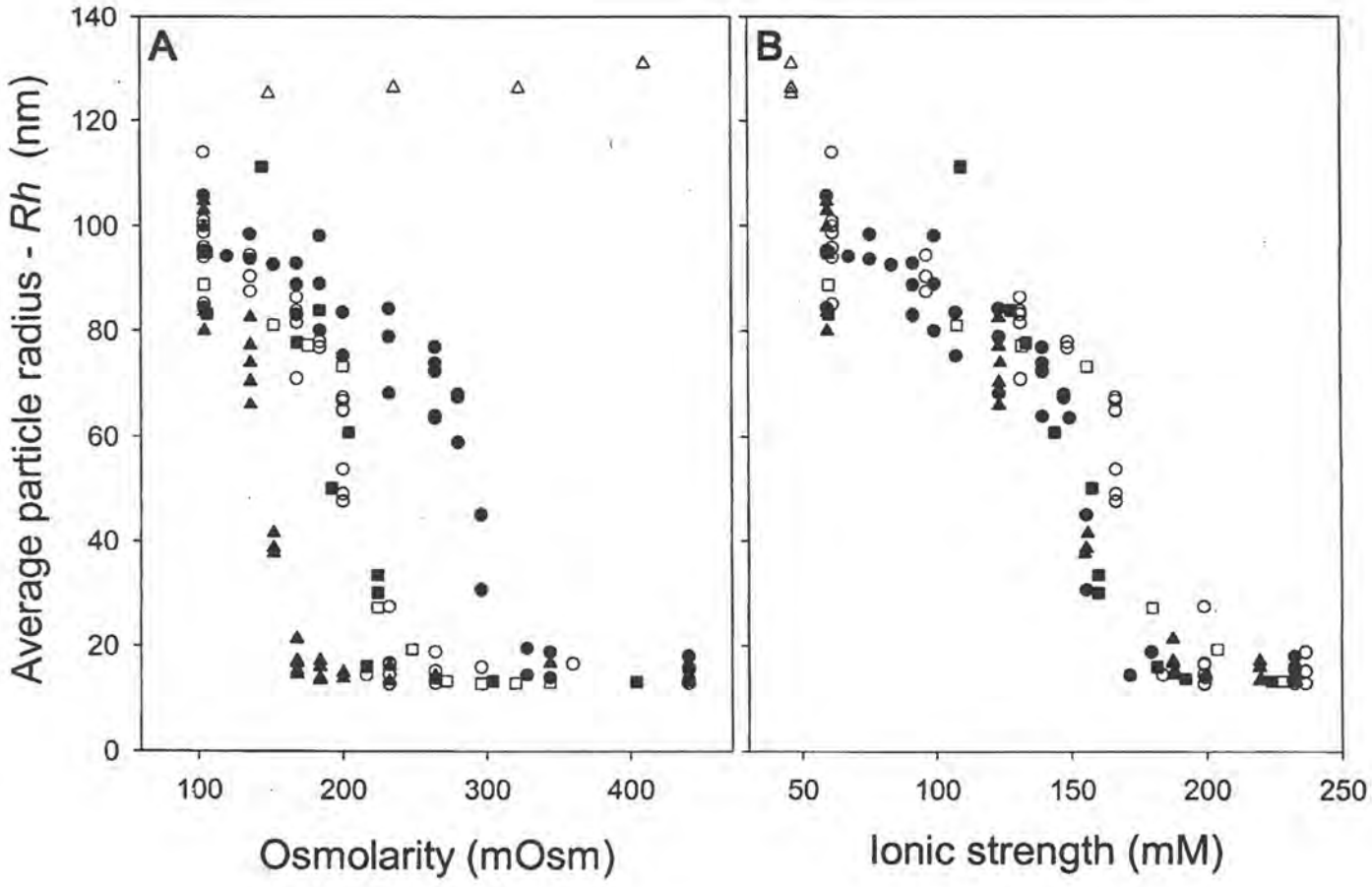


FIGURE 1

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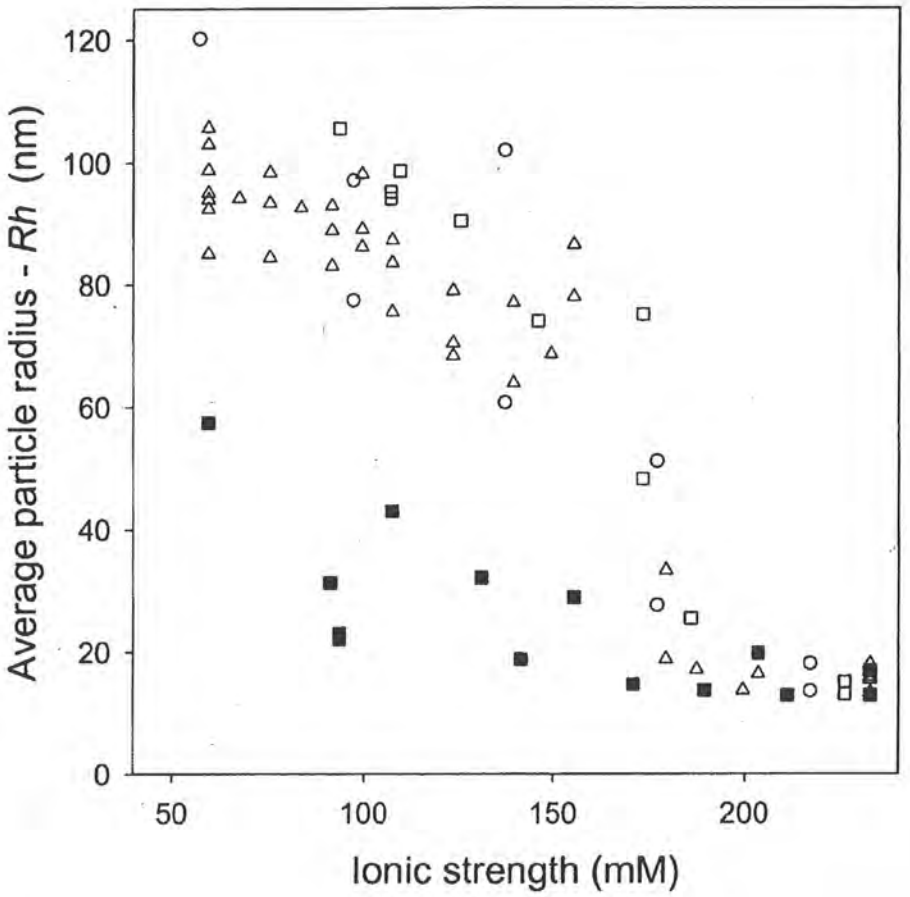
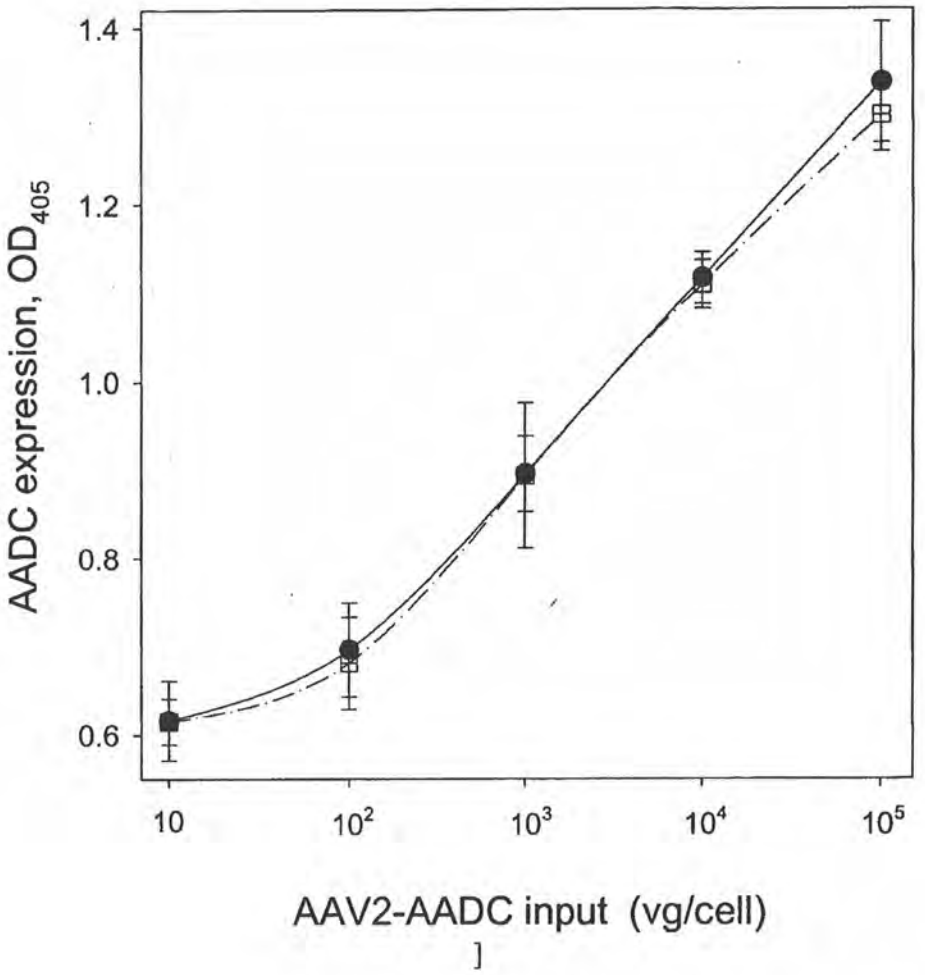


FIGURE 2

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FIGURE 3

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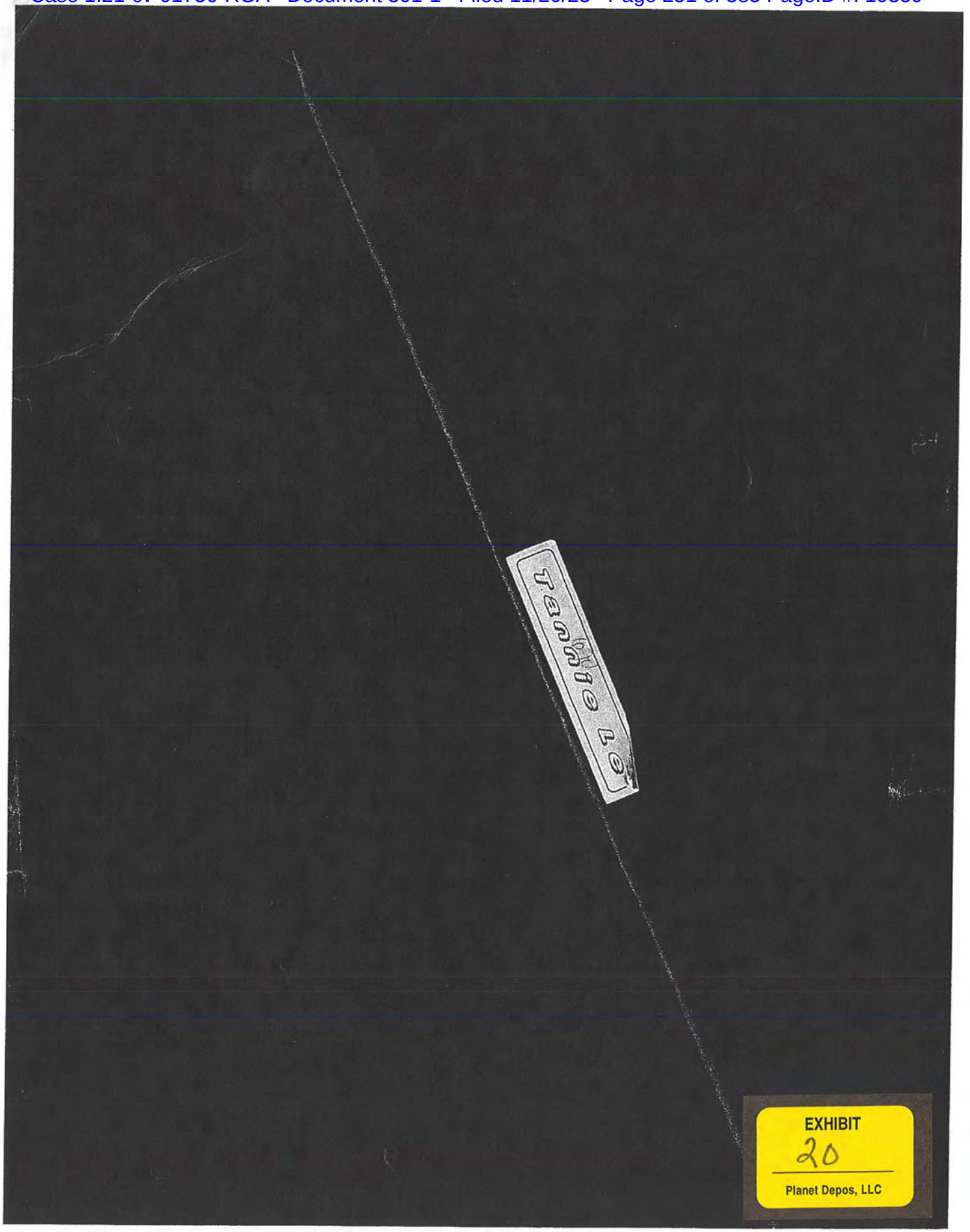
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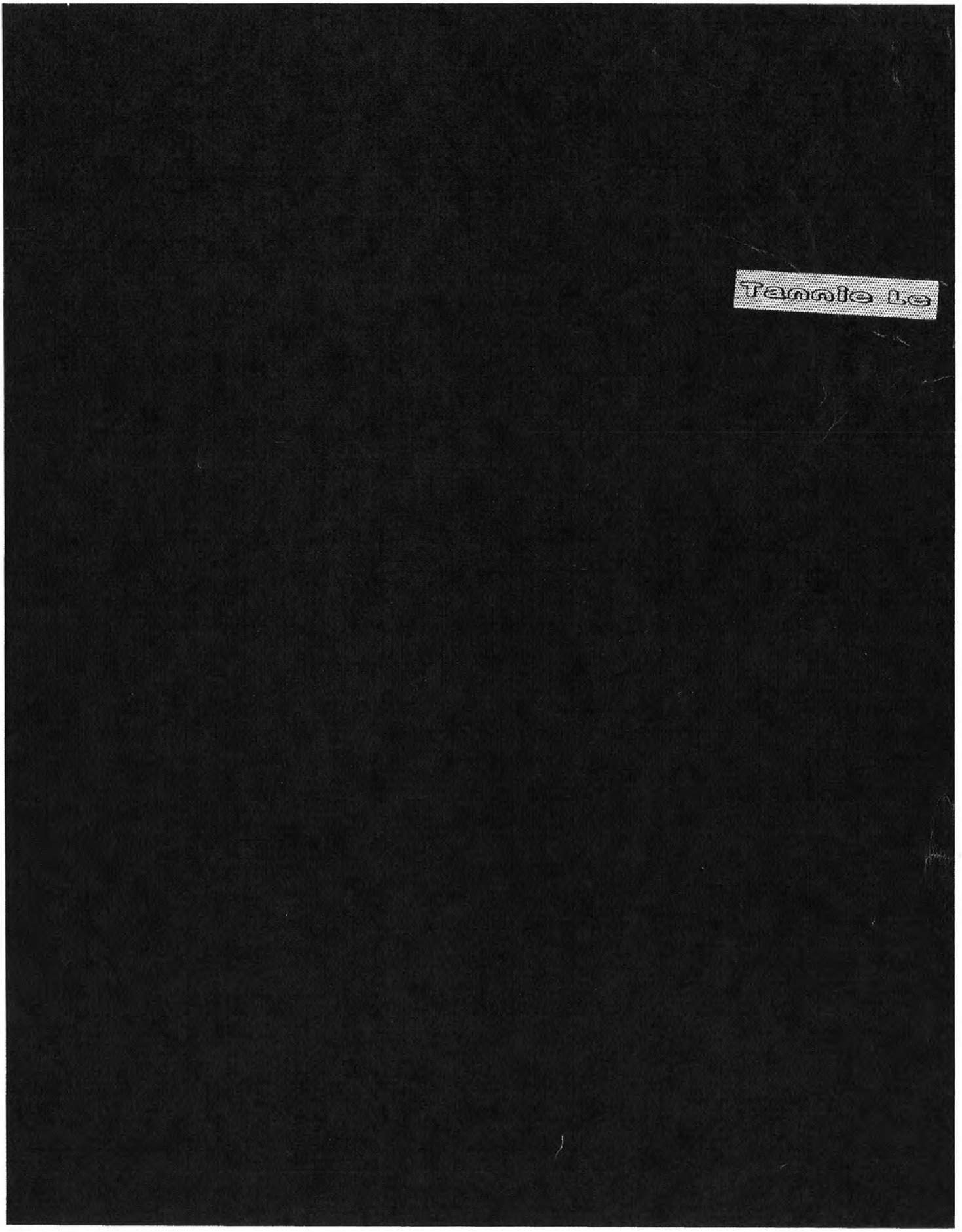
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20
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GUIDELINES

1) This laboratory notebook is intended to be a permanent record of your work.

2) Entries should be made in permanent ink - do not use pencil.

3) The title should be accurately recorded when starting a page.

4) All data is to be recorded directly into the notebook. The inclusion of all elaborate details is preferable. Notes and calculations should not be done on loose paper. In the case of error, draw a single line through the incorrect data. Do not erase or use correction fluid. All corrections should be initialed and dated.

5) After entering your data, sign and date all entries. Witness or witnesses should sign and date each entry in the spaces provided. The witnesses must observe the work that is done and have sufficient knowledge to understand what they have read. Names of operators and witnesses present during any demonstration should be recorded.

6) This notebook, and all information recorded therein, are the exclusive property of the Company. All contents of this book are **strictly confidential.** It must be returned when completed, upon request, or upon termination of employment. It should be kept in a protected place to safeguard against loss. If the book is lost, the supervisor should be notified immediately, and a written report describing the circumstances should be submitted.

TABLE OF CONTENTS

PAGE NO.

Project No. OTT

1

TITLE AAV2 Aggregation study by DLS

Book No. 070

From Page No.

20NOV03

Materials :

[redacted]

procedure :

Perform DLS for following samples (5 min. run) :

[redacted]

Saved format : Date / Sample # / Treatment

20NOV03 Sample 1 : 10mM P_i, pH 7.2

20 NOV03 Sample 2 : AAV2

20 NOV03 Sample 3 : AAV2 1/4
10mM P_i, pH 7.2

20 NOV03 Sample 4 : 1% HSA

20 NOV03 Sample 5 : AAV2 1/4
1% HSA

20 NOV03 sample 6 : 10mM P_i, pH 7.2

20 NOV03 Sample 7 : AAV2

20 NOV03 Sample 8 : AAV 1/4
10mM P_i, pH 7.2

20 NOV03 Sample 9 : 1% HSA

20 NOV03 Sample 10 AAV2 1/4
1% HSA

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Project No. Exp 25

TITLE AAV2 Formulation.

Book No.

From Page No.

Initial UF/DF studies.

See pg 126 for starting material

[redacted]

UF/DF

[redacted]

[redacted]

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Page No.

402 Formulation.

Final UF/DF studies.

prep 3 test formulations
1 control formulation.

See pg 126 for starting material (FU).

UF/DF
Formulation (1) 150 mM HEPES, pH 7.5

prepare 500 mL; use 500 mL (150 mM final / 500 mM stock) = 150 mL 500 mM stock HEPES.

add 350 mL H_2O

Formulation (2) 100 mM HEPES, pH 7.5
1% glycerol

prepare 500 mL; use 500 mL (100 mM final / 500 mM stock) = 100 mL 500 mM stock HEPES.

add 1 gm/100 mL = 5 gm glycerol.

add 395 mL H_2O

Formulation (3) 10 mM Tris pH 8.0
90 mM HCl

prepare 500 mL; use 500 mL (10 mM final / 200 mM stock) = 25 mL 200 mM stock Tris, pH 8

use 500 mL (90 mM target / 500 mM stock) = 90 mL 500 mM stock HCl

Add 385 mL H_2O

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Fraser Wright
08May04

Experiment 25
Midgee Screening of Three Candidate Formulations

Experiment 25 / 08May04 1100
Plan for UF/DF experiments to assess new formulations
Introduction:
Aggregation has been a major problem during production of AAV2 based vectors. Vector aggregation has resulted in significant loss of material (for example, loss of vector during 0.2u filtration), inability to achieve high concentration stocks, and concern about the aggregation state as function of storage time of final products.

[redacted]

Methods:
UF/DF will be performed using TTF on hollowfibers (AGT Midgees, 8").
The protocol that will be used is Avigen BPR: Ultrafilitration of AAV-hAADC-2 Vector, with modifications.

			Approx mOsm	μ
Control formulation		10mM Na Phosphate, pH 7.3 140mM NaCl 5% Sorbitol (275mM) 0.001% F68	580	159
Test formulations:	1	150mM Na Phosphate, pH 7.5 0.001% F68	390	312
	2	100mM Na Phosphate, pH 7.5 1% glycerol (109 mM) 0.001% F68	369	208
	3	10mM Tris, pH 8.0 100mM Na Citrate 0.001% F68	366	516

[redacted]

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Fraser Wright
08May04

Experiment 25
Midgee Screening of Three Candidate Formulations

[redacted]

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Project No. Exp 25 123

TITLE UF/DF experts Book No.

From Page No.

Made on pH and conductivity of control and test formulations

[redacted]

DI H2O used to prepare formulations

Control Formulation
(5% sorbitol, PBS)

Test Formulation (1)
150 mM Pi

Test Formulation (2)
100 mM Pi
1% Sorbitol

Test Formulation (3)
10 mM Tris
90 mM NaCitrate

Repeat → Control Formulation
(5% sorbitol, PBS)

[redacted]

[redacted]

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F/DF experts Book No.

ge No.

work on pH and conductivity of [redacted]

[redacted]

DLD used to prepare formulations

Control Formulation
(5% Sorbitol, PBS)

Test Formulation (1)
150 mM P:

Test Formulation (2)
100 mM P:
1% Sucrose

Test Formulation (3)
10 mM Tris
90 mM NaCitrate

Repeat ↖ Control Formulation
(5% Sorbitol, PBS)

[redacted]

[redacted]

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TITLE

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Peter,

[redacted]

1) **Control Formulation (CF) pre (0.2um) filtration**
2) **CF post filtration**
3) **Test Formulation 1 (TF1) pre filtration**
4) **TF1 post filtration**
5) **TF2 pre filtration**
6) **TF2 post filtration**
7) **TF3 pre filtration**
8) **TF3 post filtration**

Thanks in advance!

Fraser
10May04

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TITLE UF/DF exps Book No.

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pA hek pH7.0 std
pH10 std

Confcd Foundation
Tab Formulation (1)
Tab Formulation (2)
Tab Formulation (3)

[redacted]

Eximio Conductivity

Confcd Formulation
Tab Form (1)
Tab Form (2)
Tab Form (3)

[redacted]

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Process 1B Cesium Purified Materials

Harvest Source	Harvest date	(Ave vp/RB) - Post-MF	CsCl Purif lot #	CsCl Purif Titer (vp/mL)	Vol (mL)	vol net	Total vp net	# of tubes	# RBs/tube	Equiv RBs	vp/RB post CsCl	Comments
[redacted]												

hold in reserve
Meets upstream and downstream criteria
[redacted] used for dilute vector study

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TITLE UF/DF Book No.

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[redacted]

Inside: the marginal distribution of the conditional

with Yoc (QC), odds of conditioning and

Conditioning Formulation (1)

Formulation (2) (3)

[redacted]

Conditioning Formulation (2)

Formulation (3)

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RAU2 (GRADE) UF/OF

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AAV2 (AADC)
UF/OF

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Project No. Ex 25

TITLE Book No.

From Page No.

From: Peter Smith
Sent: Monday, May 10, 2004 4:16 PM
To: Fraser Wright
Cc: Jurg Sommer
Subject: formulation experiment results

Here's the first batch:

[redacted]

From: Peter Smith
Sent: Tuesday, May 11, 2004 2:30 PM
To: Fraser Wright
Cc: Jurg Sommer
Subject: formulation study

Good afternoon,
Here are the results for the other two test formulations: looks good!

[redacted]

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Project No. E25 135

TITLE 2F/DF Midgaes

Book No.

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D25 analyses on Formulations of Concern

Pre Filtration

Contl Formexcip
vector pre mix
vector post mix

Test Form① excip
vector pre mix
post mix

Test Form② excip
vector pre mix
vector post mix

Test Form③ excip
vector pre mix
vector post mix

[redacted]

Post 0.2μ Filtration

Contl Form Vector 14.46 (10/10)

Test Form① vector 13.76 (10/10)

Test Form② vector 13.85 (10/10)

Test Form③ vector 13.82 (10/10)

[redacted]

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)F/DF Midgets

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D2S analyses on Formulations of Cancelled Vials.

RL

Intensity

[redacted]

l Formexcip
vector pre mix
vector post mix

Form① excip
vector pre mix
post mix

Form② excip
vector pre mix
vector post mix

Form③ excip
vector pre mix
vector post mix

0.2μ Filtration

[redacted]

Form Vector 14.46 (10/ω)

Form① vector 13.76 (10/ω)

Form② vector 13.85 (10/ω)

Form③ vector 13.82 (10/ω)

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Project No.

TITLE

Book No.

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Project No

TITLE Book No.

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[redacted]

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Project No. E25

TITLE _____ Book No. _____

From Page No.

Today is 14 May 04

Performed day 7 post UF/DF filtration on E25 TF1, TF2, TF3 (0.5 mL each filtered)

DLS analyses on the post filtration samples:

Sample	Rh	Ct Rt.
TF1 (post filt, d7)	14.66 (10/10)	1637
TF2 "	13.95 (10/10)	1481
TF3 "	13.73 (10/10)	1482

d7 post UF/DF

To Page No.

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	14 May 04		

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Project No. Exp 25

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LE UF/DF Hydrogels

Book No.

am Page No.

DLS Analyses on CF and TF 1-3

10 May 04

Sample

2-8°C (2 days)

TF1 except.

CF vials
TF1 vials
TF2 vials
TF3 vials

-20°C (1x F/T)

CF vials
TF1 vials
TF2 vials
TF3 vials

-80°C (1x F/T)

CF vials
TF1 vials
TF2 vials
TF3 vials

[redacted]

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	10 May 04		

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Project No. Exp 25

139

IF/DF Hydrogels

Book No.

ige No.

DLS Analyses on CF & TF 1-3

2 days 04

angle

°C (2 days)

:1 exp 1.

F vesicles
F1 vesicles
F2 vesicles
F3 vesicles

19C (1x F/T)

: vesicles
F1 vesicles
F2 vesicles
F3 vesicles

0 °C (1x F/T)

: vesicles
F1 vesicles
F2 vesicles
F3 vesicles

[redacted]

To Page No.

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Project No.

TITLE

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Project No. Exp 25

UF/DF Undyed.

Book No.

From Page No.

D25 Analysis on CF1 & TF1-3.

11 Aug 04.

Samples

2-8 °C (3 days)

CF vials
TF1 vials
TF2
TF3

-20 °C (3 F/T cycles)

CF vials
TF1
TF2
TF3

80 °C (3 F/T cycles)

CF vials
TF1
TF2
TF3

[redacted]

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CONFIDENTIAL

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Project No

Book No

From Page No

Fraser Wright
11May04 1100

Experiment 26
Midgee UF/DF of TF3 to >5E13 vg/mL target

[redacted]

Introduction:
Aggregation has been a major problem during production of AAV2 based vectors. Vector aggregation has resulted in significant loss of material (for example, loss of vector during 0.2u filtration), inability to achieve high concentration stocks, and concern about the aggregation state as function of storage time of final products.

[redacted]

To Page No

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Project No.

TITLE

Book No.

From Page No.

Fraser Wright
11May04 1100

Experiment 26
Midgee UF/DF of TF3 to >5E13 vg/mL target

[redacted]

Fraser Wright
11May04 1100

Experiment 26
Midgee UF/DF of TF3 to >5E13 vg/mL target

[redacted]

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[redacted]

[redacted]

Infection D25 assessment.

	RC
Re	
Ut last.	
1 day post UF/DF	13.59 (10/10)
2 day post UF/DF	13.05 (10/10)
oC (5 days)	26.95 (7/10)
	18.60 (9/10)
	16.30 (9/10)
	42.37 (2/10)
	14.41 (10/10)
(6 F/T)	
	56.10 (7/10)
	ND
	59.17 (7/10)
	14.03 (10/10)
(6 F/T cycles)	
	57.49 (9/10)

[redacted]

[redacted]

Sample. | LC

E25 t day post UF/DF | 13.59 (10/10)
E26 2 day post UF/DF | 13.05 (10/10)

E25 2-8 °C (5 days)

CF	26.95	(7/10)
TF1	18.60	(10/10)
TF2	16.30	(10/10)
TF3	42.37	(2/10)
	14.41	(10/10)

-20 °C (6 F/T)

CF	56.10	(7/10)
TF1	ND	
TF2	59.17	(7/10)
TF3	14.03	(10/10)

-80 °C (6 F/T cycles)

CF	57.49	(9/10)
TF1	ND	
TF2	44.91	(5/10)
TF3	27.89	(10/10)
	21.32	(10/10)

[redacted]

SIGNATURE (Approved) | DATE | WITNESS

13 May 04

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Update 13may04

List of samples requested for vg quantification by Q-PCR

Peter

[redacted]

Thanks in advance!
Fraser
13May04

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[redacted]

-20 °C (20 ~~F~~ 10 F/T cycles)
CF 94.52 (4/10)
TF1 TNTC
TF2 37.86 (10/10)
TF3 14.11 (10/10)

-80 °C (10 F/T cycles)
CF 140.72 (9/10)
TF1 ND
TF2 83.54 (10/10)
TF3 50.85 (10/10)

[redacted]

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[redacted]

[redacted]

C (20F 10 F/T cycles)
94.52 (4/10)
TNTC
37.86 (10/10)
14.11 (10/10)

(10 F/T cycles)
140.72 (9/10)
ND
83.54 (10/10)
50.85 (10/10)

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Project No. E25

TITLE UF/DF

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[signature]	13 May 04.		

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Project No. Dep of FTF 3 for 147
RVC (Dave Brunzheim).

TITLE JF/DF

Book No.

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	12 May 04		

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Project No:

TITLE

Book No

From Page No

From: Peter Smith
Sent: Friday, May 14, 2004 4:49 PM
To: Fraser Wright
Cc: Jurg Sommer
Subject: Formulation study

Good afternoon,

[redacted]

[redacted]

SIGNATURE DATE WITNESSED & UNDERSTOOD BY DATE

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Project No. E26 stab 149

TITLE UF/DF Book No.

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TF3

Project No E26

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[redacted]

[redacted]

[redacted]

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CF

Project No. E27

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TITLE OF/DF hyperconcern

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today is [redacted]

[redacted]

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Project No

TITLE

Book No

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Fraser Wright
14May04 1100

Experiment 27
Midgee UF/DF of CF to >5E13 vg/mL target

[redacted]

Introduction:
Aggregation has been a major problem during production of AAV2 based vectors. Vector aggregation has resulted in significant loss of material (for example, loss of vector during 0.2u filtration), inability to achieve high concentration stocks, and concern about the aggregation state as function of storage time of final products.

[redacted]

[redacted]

SIGNATURE

DATE 17 May 04

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DATE

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Updated 24Jun04

List of samples requested for vg quantification by Q-PCR

Abbreviated summary for results received from Peter

QPCR vg quantitation for Experiment 25, 26, and 27 (Assessment of Control (CF) and Test Formulations (TF1-3) by Midgee UF/DF)



24Jun04

Fraser Wright
08May04

Experiment 25
Midgee Screening of Three Candidate Formulations

[redacted]

Introduction:
Aggregation has been a major problem during production of AAV2 based vectors. Vector aggregation has resulted in significant loss of material (for example, loss of vector during 0.2u filtration), inability to achieve high concentration stocks, and concern about the aggregation state as function of storage time of final products.

[redacted]



Process 1B Cesium Purified Materials

Harvest Source	Harvest date	(Ave vg/RB) - Post-MF	CsCl Purif lot #	CsCl Purif Titer (vg/mL)	Vol (mL)	vol net*	Total vg net	# of tubes	# RBs/tube	Equiv RBs	vg/RB post CsCl	Comments
[redacted]												

[redacted] hold in reserve
Meets upstream and downstream criteria
[redacted] used for dilute vector study
[redacted]

GENZ_NOV_00290705

HIGHLY CONFIDENTIAL

Exhibit 7

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CONTAINS HIGHLY CONFIDENTIAL INFORMATION

Transcript of Guang Qu, Ph.D.

Date: June 22, 2023
Case: Genzyme Corporation, et al. -v- Novartis Gene Therapies, Inc., et al.

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Email: transcripts@planetdepos.com
www.planetdepos.com

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IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

GENZYME CORPORATION :
and AVENTIS INC., :
Plaintiffs, :
v. : C.A. No.
NOVARTIS GENE THERAPIES, INC.; : 21-1736 (RGA)
NOVARTIS PHARMACEUTICALS :
CORPORATION; and NOVARTIS AG, :
Defendants. :

CONTAINS HIGHLY CONFIDENTIAL INFORMATION

Videotaped Deposition of GUANG QU, Ph.D.
Conducted Virtually
Thursday, June 22, 2023
9:41 a.m. EDT

Job No.: 496642
Pages 1 - 238
Reported by: Debra A. Whitehead

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Videotaped Deposition of GUANG QU, Ph.D., conducted virtually.

Pursuant to notice, before Debra Ann Whitehead, E-Notary Public in and for the State of Maryland.

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ALSO PRESENT:
JACK DUNN, Video Specialist
JACKSON SCHUELER, A/V Technician

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By Mr. Weingarten 9

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PROCEEDINGS

VIDEO SPECIALIST: Here begins Media Number 1 in the videotaped deposition of Guang Qu in the matter of Genzyme Corporation, et al., v Novartis Gene Therapies, Incorporated, et al.; in the United States District Court for the District of Delaware; Case Number 21-1736 (RGA).

Today's date is June 22nd, 2023. The time on the video monitor is 9:41. The remote videographer today is Jack Dunn, representing Planet Depos. All parties of this deposition are attending remotely.

Would counsel please voice-identify themselves and state whom they represent.

MR. WEINGARTEN: Good morning. David Weingarten from Finnegan, Henderson, Farabow, Garrett & Dunner, on behalf of Defendants Novartis, and joined with me is Marta Daneshvar, also from Finnegan.

MS. HELM: Katherine Helm from Dechert, representing Plaintiffs Genzyme Corporation and Aventis, as well as the witness.

VIDEO SPECIALIST: The court reporter today is Debbie Whitehead, representing Planet Depos.

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The witness will now be sworn.
GUANG QU, Ph.D.,
having been duly sworn, testified as follows:
EXAMINATION BY COUNSEL FOR DEFENDANTS
BY MR. WEINGARTEN:
Q Good morning, Dr. Qu.
Can you hear me okay?
A Yes, I can hear you well. Thank you.
Q Okay.
A Good morning.
Q Can you please state your name and address, for the record.
A Yes. My name is Guang Qu, G-U-A-N-G, Q-U as my last name. [redacted]
Q And where are you physically located right now for your deposition?
A This is the conference room in law firm Dechert --
Q In --
A -- in Philadelphia areas.
Q Thank you very much. Appreciate that.
That actually gives a segue into some of the ground rules that we'll have during this deposition. And the ground rules will be for both

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of our benefits as well as the court reporter's benefit. But first let me ask, have you been deposed before?
A No.
Q Well, basically the simple rules, and I'm sure that your counsel has provided you some of this guidance, but the rules will be that you're under oath. I'll ask some questions, and, you know, you will be obligated to answer those questions unless you're instructed not to by counsel.
When I ask a question, I'm going to assume that you understand my question if you answer the question. So if you have any concerns or need clarification, just ask. You know, I mean, my goal is to make sure my questions are clear, but sometimes they won't be, and I'll expect that you'll ask for clarification.
In addition to that, when I'm asking questions, you know, please wait for me to finish asking the question before you start to answer. I'll try to do the same for you. In part that's because the court reporter is not able to sort of document both of us talking at the same time.
When I do ask questions, I'm going to ask

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for verbal responses. So, you know, yes, no, or whatever the response might be. But try to refrain from a, you know, shaking your head yes or no or sounds that may in the normal conversation may be understood to mean something, but for the purposes of this proceeding only the oral response. Is that okay?
A Yes, it is okay.
Q Thank you. Now, you know, the day is going to get a little bit long. And, you know, we're cognizant of that. But we're going to do our best to give you breaks throughout the day.
I would expect that the breaks will be every hour or so. And so, you know, really what that comes down to is is that if we're not right at the hour and you want a break, just ask for a break. We'll do our best to accommodate you.
We may take a break a little bit longer than that, maybe every hour and 15 minutes. We'll see how it goes. In part it depends upon you. It depends on whether you or counsel or the court reporter or we want a break. And those breaks will often be short breaks, and then we'll also, obviously, have a break for lunch.
So we'll discuss those when the time

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comes. But I want you to be comfortable asking for a break if you need one. The only rule that we have in place for that is that if there is a question pending, then, you know, we ask that you answer the question that's pending. And there might be a short series of questions that I want to get finished before we take that break. But we'll certainly try our best to accommodate.
Now, I know that this deposition is being conducted remotely, which means that I'm not sitting in the room there with you. Which would be, you know, I guess practiced back in the olden days. And what I ask is that both counsel have agreed to certain ground rules, and that includes that the witness will be by themselves.
And so what I ask you to do is confirm that you are by yourself, nobody else is in the room with you is what I mean by that. And then if somebody does come in the room, absent some technical issue they're trying to solve, that you, you know, let us know so we can pause the deposition, if necessary, and go from there.
Do you understand?
A Yes, I do. Thank you.
Q Yes. I also understand that you have a

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'99.
Do you see that?
A Yes, I do.
Q Is that the promotion you are referring to?
A Yes, that's right.
Q So let's turn back to the page ending in 875, and where it says Research Specialist. For that, basically, I want to say, nine-month period there, during that period of time it says that you worked on optimizing triple transfection methods for rAAV production.
A Yes.
Q And it also says that you participated in assay development, including the development of real-time Q-PCR methods and other assays.
Do you see that?
A Yes, I do.
Q What kind of assays did you develop as a research specialist from 1997 September to July 1998?
A Yes. So I say "development." I think, you know, particularly for AAV part, there is several different methodologies you can use to analyze AAV vectors, particularly about the AAV

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titer. We -- you know, titer refer to, you know, quantities or units, if you want, how you define your vectors.
The technique at that time, you know, you probably can use something so-called hybridization techniques. Either, you know, whatever the quantitation method you can use, you know, standard procedure and then you do your hybridizations and then, you know, to see where you are in the vector titer.
Specifically for the real-time Q-PCR, that's the -- I think, you know, regarding that technique, it is a very early stage. Not a lot of lab, you know, are using this techniques. I think I learned this technologies from my previous postdoc work. Probably I introduced that, you know, technique into Dr. Jim Wilson's lab.
And at the beginning, you know, how to use it, you know, how to set up the standard curves and how to interpret the data, I did involved in those work based on my memories at this -- this moment, yes. That's one of the major assays I involved specifically reasonably, you know, extensively.
The other assays I may involved, I don't

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remember exactly how much work I did. However, as a specialist I do, you know, do a lot of, you know, the other things or instruct my team to several other, you know, analytical work, such as run SDS-PAGE, to different -- you know, to identify, you know, the vectors, you know, in the vector prep, even so for some other assays, probably I can, like ELISA assays, you know, those kind of things.
Q Now, you indicated -- and if I get this wrong, correct me. You said "titers." You used the word "titers." And then you said it's quantities.
Is that what you -- well, let me ask you a different way. When you refer to titers or quantities, it's the quantities of vector?
A Yes, that's right.
Q And is it -- by an assay are you measuring the actual number, sort of the real number of the vectors in the solutions?
MS. HELM: Objection. Form.
A When I talk about the titer and the quantitative amount, yes. You know, this assay specifically, you know, the assay you perform to understand your vector prep, that's the way to

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refer.
So, you know, how much of vector particles contains in certain volume, that's the -- you know, the titer was defined as. Therefore I think, you know, when I mention "titer," I probably refer that.
Q In your practice, you've been preparing rAAV vectors for quite a while, you said, purifying them, I should say. In your practice is obtaining the actual number, getting a titer of the -- of the vector standard?
MS. HELM: Objection to form.
A Please repeat the question. Let me digest a little bit about your question, make sure I understand it right, and then I can provide my answer.
Q Of course.
In your practice, and you've been preparing rAAV vectors for quite a while, you said, purifying them, I should say. In your practice, is obtaining the actual number, getting the titer of the vector standard?
MS. HELM: Same objection.
A I think that the vector titer need certain technique. It's not a trivial job, for

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vectors.
Is that right?
A That's correct.
Q And we both agree that it's concentration-dependent.
Is that right?
A Yes.
Q And we both agree that pH can affect that. Right?
A More or less. I think Aristophanes is probably the most important factor to affect that.
Q But pH can affect that. Right?
A True.
Q And one of the -- one of the goals here or the abstract said -- well, just look at the title of the invention. If you go back to Page Bates 190. It says, "Compositions and methods to prevent AAV vector aggregation."
Right?
A That's right.
Q And what I'm asking is, are you aware of any compositions that have an ionic strength greater than 200 and vector particle concentration above 1 times 10 to the 13 VGs per M L, up to 6.4 times 10 to the 13th VGs per M L where an

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aggregation was not prevented upon storage?
A I think I do aware that.
Q And can you describe what those formulations that you're aware of, what -- the basis of your awareness.
A Based on my awareness, I think the phosphate formulation is one of them.
Q And so phosphate formulation is not always stable -- I'm sorry. Go ahead.
A I'm sorry. Let me just finish this, and then we can discuss further if you need.
I think, you know, we had to connect it with a different conditions in the experiments. I think the phosphate formulations I refer to, and based on my awareness, I refer to testing formulation -- let me just make sure I do it right.
Q And right now my question -- go ahead and look. My question simply is are you aware. And you said yes. And the basis of your awareness.
A Yes.
Q Is the basis of your awareness looking at the first provisional application?
A No. Based on this, you know, the '542.
Q And so what in the '542 are you looking

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at to say that the phosphate formulation isn't a good formulation for storage?
A It's not a good formulation if you went to say the TF1 in Table 3.
Q And that's because it shows significant aggregation?
A Yes.
Q Dr. Qu, you know, we threw some names at you earlier. I'm going to throw a few additional names at you, and I'd like to know if you had any interaction or discussions with any of these individuals after you left Avigen.
You've already indicated that you don't know Gregory Bellomy.
Is that right?
A That's correct.
Q What about Jenny Buchbinder?
A No.
Q What about Susan T. Evans?
A No.
Q What about Dana Pasternak? That's spelled P-A-S-T-E-R-N-A-K.
A No.
Q What about Roberta L. Robbins?
A The name sounds familiar, but I don't

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remember now. Roberta. Roberta. No, I don't remember.
Q So it's possible you had discussions with her?
A That's a woman? No.
Q Roberta. I can't say for sure, I can find out in a couple of minutes.
A No. No. Let me say no.
Q Do you know who Mary Christina Thompson is?
A Mary Christina Thompson probably was the lawyer, junior lawyer, at Avigen. She is a -- I mean a female lawyer. Tall young lady.
Q And did you interact with Mary Christina Thompson related to the inventions disclosed in the applications leading to the '542 patent?
A No. No.
MR. WEINGARTEN: All right. We need a five-minute break. We may be out of questions here. But I just want to confer with my colleagues. So if we can take a five-minute -- actually, let's make it ten minutes so we have sufficient time.
THE WITNESS: Okay. Sure. Thank you.
VIDEO SPECIALIST: Please stand by.

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We are going off the record at 17:28.
(A recess was taken.)
VIDEO SPECIALIST: We are going back on the record. The time is 17:39.
MR. WEINGARTEN: Dr. Qu, subject to your attorney having any questions, we have no more questions.
MS. HELM: Dr. Qu, I have no questions for you.
THE WITNESS: Thank you.
MR. WEINGARTEN: All right. Dr. Qu, thank you very much for your time today. I really appreciate it. We thank you for your time, and have a great day.
VIDEO SPECIALIST: Stand by.
This marks the end of the deposition of Guang Qu. We are going off the record at 17:39.
(Off the record at 5:39 p.m. EDT)

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CERTIFICATE OF SHORTHAND REPORTER - NOTARY PUBLIC

I, Debra A. Whitehead, the officer before whom the foregoing proceedings were taken, do hereby certify that the foregoing transcript is a true and correct record of the proceedings; that said proceedings were taken by me stenographically and thereafter reduced to typewriting under my supervision; that reading and signing was not discussed; and that I am neither counsel for, related to, nor employed by any of the parties to this case and have no interest, financial or otherwise, in its outcome.

IN WITNESS WHEREOF, I have hereunto set my hand and affixed my notarial seal this 24th day of June, 2023.

My commission expires:
April 30, 2027



E-NOTARY PUBLIC IN AND FOR THE STATE OF MARYLAND

Exhibit 8

20427 060105 U.S. PTO

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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

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First Inventor	Wright et al.
Title	COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION
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APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

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1. ☐ **Fee Transmittal Form** (e.g., PTO/SB/17) *(Submit an original and a duplicate for fee processing)*
2. ☒ **Applicant claims small entity status.** See 37 CFR 1.27.
3. ☒ **Specification** [*Total Pages* 30] Both the claims and abstract must start on a new page *(For information on the preferred arrangement, see MPEP 608.01(a))*
4. ☒ **Drawing(s)** *(35 U.S.C.113)* [*Total Sheets* 3]
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6. ☒ **Application Data Sheet.** See 37 CFR 1.76 (4 pages)
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ACCOMPANYING APPLICATION PARTS

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0800-0045
PATENT

UTILITY PATENT APPLICATION

COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

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0800-0045
PATENT

COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims benefit under 35 U.S.C. § 119(e) of provisional applications 60/575,997 filed June 1, 2004 and 60/639,222 filed December 22, 2004, which applications are hereby incorporated by reference in their entireties.

FIELD OF THE INVENTION

The present invention relates to compositions and methods of preparing and storing AAV virions that prevent aggregation.

BACKGROUND

Recombinant adeno-associated virus (rAAV) is a promising vector for human gene transfer. Grimm, D., and Kleinschmidt, J. A. (1999) *Hum Gene Ther.* 10: 2445-2450; High, K. A. (2001) *Ann. N. Y. Acad. Sci.* 953: 64-67; Pfeifer, A., and Verma, I. M. (2001) *Ann. Rev. Genomics Hum. Genet.* **2**: 177-211. AAV is a member of the Dependovirus genus of the parvoviruses. AAV serotype 2 (AAV2) is composed of a single-strand DNA molecule of 4680 nucleotides encoding replication (*rep*) and encapsidation (*cap*) genes flanked by inverted terminal repeat (ITR) sequences. Berns, K. I. (1996) in *Fields Virology* (B. N. Fields *et. al.* Eds.), pp. 2173-2197. Lippincott-Raven Publishers, Philadelphia. The genome is packaged by three capsid proteins (VP1, VP2 and VP3), which are amino-terminal variants of the *cap* gene

product. The resulting icosahedral virus particle has a diameter of ~26 nm. A high resolution crystal structure of AAV2 has been reported. Xie, Q. *et al.* (2002) *Proc. Natl. Acad. Sci. U.S.A.* **99**: 10405-10410.

The solubility of purified AAV2 virus particles is limited, and aggregation of AAV2 particles has been described as a problem. Croyle, M. A. *et al.* (2001) *Gene Therapy* 8: 1281-1290; Huang, J. *et al.* (2000) *Mol. Therapy* 1: S286; Wright, J. F. *et al.* (2003) *Curr. Opin. Drug Disc. Dev.* 6: 174-178; Xie, Q. *et al.* (2004) *J. Virol. Methods* 122: 17-27. In commonly used buffered-saline solutions, significant aggregation occurs at concentrations of 10^{13} particles/mL, and aggregation increases at higher concentrations. Huang and co-workers reported that AAV vectors undergo concentration-dependent aggregation. Huang, J. *et al.* (2000) *Mol. Therapy* 1: S286. Xie and coworkers (Xie, Q. *et al.* (2004) *J. Virol. Methods* 122: 17-27) similarly reported that at concentrations exceeding 0.1mg/mL, AAV2 vectors require elevated concentrations of salt to prevent aggregation. Aggregation of AAV2 vectors occurs at particle concentrations exceeding 10^{13} particles/mL in commonly used neutral-buffered solutions such as phosphate- and Tris-buffered saline. This corresponds to a protein concentration of ~0.06 mg/mL, and emphasizes the low solubility of AAV2 under these conditions. The effective vector concentration limit may be even lower for vectors purified using column chromatography techniques because excess empty capsids are co-purified and contribute to particle concentration.

Particle aggregation is a significant and not fully resolved issue for adenovirus vectors as well. Stability of a recently established adenovirus reference material (ARM) was recently reported. Adadevoh, K. *et al.* (2002) *BioProcessing* 1(2): 62-69. Aggregation of the reference material, formulated in 20mM Tris, 25 mM NaCl, and 2.5% glycerol at pH 8.0, was assessed by dynamic light scattering, photon correlation spectroscopy and visual appearance. A variable

level of vector aggregation following either freeze-thaw cycling or non-frozen storage was observed, resulting in restrictive protocols for the use of the ARM.

Aggregation can lead to losses during purification and inconsistencies in testing of purified vector preparations. The *in vivo* administration of AAV2 vectors to certain sites, such as the central nervous system, may require small volumes of highly concentrated vector, and the maximum achievable dose may be limited by low vector solubility.

Vector aggregation is also likely to influence biodistribution following *in vivo* administration, and cause adverse immune responses to vectors following their administration. As has been reported for proteins (Braun, A. *et al.* (1997) *Pharm. Res.* 14: 1472-1478), aggregation of vector may increase immunogenicity by targeting the vector to antigen presenting cells, and inducing enhanced immune responses to the capsid proteins and transgene product. The reports of immune responses to AAV vectors in pre-clinical (Chenuaud, P. *et al.* (2004) *Blood* 103: 3303-3304; Flotte, T. R. (2004) *Human Gene Ther.* 15: 716-717; Gao, G. *et al.* (2004) *Blood* 103: 3300-3302) and clinical (High, K. A. *et al.* (2004) *Blood* 104: 121a) studies illustrate the need to address all factors that may contribute to vector immunogenicity.

Testing protocols to characterize purified vectors are also likely to be affected by vector aggregation. Determination of the infectivity titer of vector was reported to be highly sensitive to vector aggregation. Zhen, Z. *et al.* (2004) *Human Gene Ther.* 15: 709-715. An important concern is that vector aggregates may have deleterious consequences following their *in vivo* administration because their transduction efficiency, biodistribution and immunogenicity may differ from monomeric particles. For example, intravascular delivery of AAV vectors to hepatocytes requires that the vectors pass through the fenestrated endothelial cell lining of hepatic sinusoids. These fenestrations have a radius ranging from 50 to 150 nm (Meijer, K. D.

F., and Molema, G. (1995) *Sem. Liver Dis.* 15: 206) that is predicted to allow the passage of monomeric AAV vectors (diameter ~26 nm), but prevent the passage of larger vector aggregates. In biodistribution studies in mice, aggregated AAV2 vectors labeled with the fluorescent molecule Cy3 were sequestered in liver macrophages following vascular delivery. Huang, J. *et al.* (2000) *Mol. Therapy* 1: S286.

Formulation development for virus-based gene transfer vectors is a relatively recent area of investigation, and only a few studies have been reported describing systematic efforts to optimize AAV vector formulation and stability. Croyle, M. A. *et al.* (2001) *Gene Therapy* 8: 1281-1290; Wright, J. F. *et al.* (2003) *Curr. Opin. Drug Disc. Dev.* 6: 174-178; Xie, Q. *et al.* (2004) *J. Virol. Methods* 122: 17-27. Defining formulations compatible with pre-clinical and clinical applications that minimize changes in vector preparations is an important requirement to achieve consistently high vector safety and functional characteristics. As is well established for protein therapeutics (Chen, B. *et al.* (1994) *J. Pharm. Sci.* 83: 1657-1661; Shire, S. J. *et al.* (2004) *J. Pharm. Sci.* 93: 1390-1402; Wang, W. (1999) *Int. J. Pharm.* 185: 129-188; Won, C. M. *et al.* (1998) *Int. J. Pharm.* 167: 25-36), an important aspect of vector stability is solubility during preparation and storage, and vector aggregation is a problem that needs to be fully addressed. Vector aggregation leads to losses during vector purification, and while aggregates can be removed by filtration, the loss in yield results in higher costs and capacity limitations when producing vector for pre-clinical and clinical studies. Even after filtration to remove aggregates, new aggregates can form in concentrated preparations of AAV2 vector in buffered-saline solutions.

The need exists for improved formulations and methods for purification and storage of AAV vectors, such as rAAV2, that prevent aggregation of virus particles.

SUMMARY OF THE INVENTION

These and other needs in the art are met by the present invention, which provides high ionic strength solutions for use in preparing and storing AAV vectors that maintain high infectivity titer and transduction efficiency, even after freeze-thaw cycles.

In one aspect the invention relates to methods of preventing aggregation of virions in a preparation of virions by adding excipients to achieve an ionic strength high enough to prevent aggregation. In another aspect the invention relates to compositions of virions having an ionic strength high enough to prevent aggregation.

In some embodiments of the invention, the ionic strength is at least about 150mM, 200mM, 250mM, 300mM, 350mM, 400mM, 450mM, 500mM, 600mM, 700mM or more. In some embodiments this ionic strength is accomplished using excipients comprising one or more multivalent ions, for example citrate, sulfate, magnesium or phosphate.

In additional embodiments, the osmolarity of the preparation of virions is maintained at near isotonic levels, for example 200mOsm, 250mOsm, 280mOsm, 300mOsm, 350mOsm or 400mOsm, even though the ionic strength is high enough to prevent virion aggregation.

In some embodiments the virions are adeno-associated virus (AAV) virions, for example AAV-2.

In other embodiments of the methods of the present invention preparations of virions are treated with a nuclease, for example Benzonase®. In further embodiments, nuclease treatment is combined with addition of excipients that achieve an ionic strength high enough to prevent aggregation.

In some embodiments of the present invention, the surfactant Pluronic® F68 is added to a preparation of virions, for example to 0.001%. In one embodiment, the composition comprises purified virus particles, 10 mM Tris pH 8.0, 100mM sodium citrate and 0.001% Pluronic® F68.

In one embodiment, AAV vectors can be stored as compositions of the present invention at concentrations exceeding $1x10^{13}$ vg/mL, for example $2x10^{13}$, $3x10^{13}$, $4x10^{13}$, $5x10^{13}$ and up to $6.4x10^{13}$ vg/mL, without significant aggregation. In some embodiments, AAV vectors stored using the methods and compositions of the invention do not exhibit significant aggregation when stored at 4°C for five days. In other embodiments, AAV vectors that are stored as such compositions do not exhibit significant aggregation after one, five, ten or more freeze-thaw cycles at -20°C or at -80°C.

In some embodiments, preparations of virions stored according to the methods and compositions of the invention exhibit an average particle radius (*Rh*), as measured by dynamic light scattering, indicating that no significant aggregation of virions has taken place. In some embodiments, preparations of virions stored according to the methods and compositions of the invention exhibit an average particle radius (*Rh*) greater than about 15nm, 20nm, or 30nm.

In some embodiments, recovery of virions from preparations of virions stored according to the methods and compositions of the invention is greater than about 85%, 90% or 95% following filtration through a 0.22μm filter.

In yet another aspect, the invention relates to kits comprising the high ionic strength formulations of the invention. In one embodiment the kit comprises a pre-mixed solution of excipients. In another embodiment the kit comprises two or more separate components of a high ionic strength composition of the present invention to be mixed by a user. In some embodiments the kit comprises sodium citrate, Tris® and Pluronic® F68. In other embodiments, the kit further

comprises instructions for making a composition or performing a method of the present invention.

DESCRIPTION OF THE DRAWINGS

FIGS. 1A and 1B present data showing aggregation of AAV2-FIX particles as a function of osmolarity (FIG. 1A) or ionic strength (FIG. 1B) for various buffer compositions. AAV2-FIX vectors are prepared by Method 2 of Example 1. Average particle radius is measured by dynamic light scattering (DLS) following vector dilution in varying concentrations of excipients buffered with 10 mM sodium phosphate at pH 7.5. Excipients include sodium chloride (●), sodium citrate (○), sodium phosphate (■), sodium sulfate (□), magnesium sulfate (▲), and glycerol (Δ).

FIG. 2 presents data on AAV2-FIX aggregation as a function of the method of purification. The average particle radius is measured by DLS following vector dilution in varying concentrations of sodium chloride buffered with10mM sodium phosphate at pH 7.5. Vectors are purified by Method 1 (double CsCl gradient) (○); Method 2 (cation exchange chromatography) (□); Method 2 plus nuclease digestion (■); or Method 3 (chromatography plus one CsCl gradient) (Δ). Purification Methods 1-3 are described in Example 1.

FIG. 3 presents data on transgene expression from D7/4 cells transduced with rAAV2-AADC virions prepared and stored in high ionic strength formulation (□) or in a control formulation (●). The concentration of AADC was measured by ELISA (in triplicate for each data point) 72 hours post-transduction. Error bars represent standard deviations.

DETAILED DESCRIPTION OF THE INVENTION

AAV2 vector aggregation is frequently observed in concentrated preparations of vectors and can affect purification recovery, and *in vivo* potency and safety. Hence, an important objective for the development AAV2 vectors is to identify methods and formulations that prevent aggregation of vectors when concentrated stocks are prepared.

Unless otherwise indicated, the term "vector" as used herein refers to a recombinant AAV virion, or virus particle, regardless of the frequent use of "vector" to also refer to non-viral DNA molecules, such as plasmids, in other contexts.

The present invention is based in part on the observation that solution ionic strength is an important parameter in AAV vector aggregation, implicating the involvement of ionic interactions between virus particles in the aggregation process. The observation that elevated ionic strength increases AAV2 vector solubility regardless of the identity of the charged excipient supports the hypothesis that ionic strength of solution *per se*, rather than interactions involving a specific ionic species, is the relevant physico-chemical parameter. A threshold ionic strength of at least 200mM is required to prevent aggregation at vector particle concentrations examined herein.

Of practical concern, commonly used buffered saline solutions have insufficient ionic strength to prevent AAV2 vector aggregation at concentrations exceeding 10^{13} particles/mL. It is known that high salt concentrations increase AAV2 vector solubility (e.g. highly concentrated AAV2 vectors recovered from gradients generally remain soluble in concentrated CsCl). However, optimal formulations for pre-clinical and clinical studies should be close to isotonic (280-400 mOsm), especially for *in vivo* administration of vector to sites where dilution of hypertonic solutions may be slow. In embodiments of the present invention the exponential

relationship of ionic strength with charge valency is used to develop isotonic formulations with high ionic strengths. Salt species with multiple charge valencies (e.g. salts of sulfate, citrate, and phosphate) that are commonly used as excipients in human parenteral formulations can provide the level of ionic strength needed to prevent AAV2 vector aggregation when used at isotonic concentrations. While isotonic (150mM) sodium chloride has an ionic strength of 150mM, a value insufficient to maintain AAV2 solubility at high vector concentrations, isotonic sodium citrate, with an ionic strength of ~500mM, can support AAV2 vector concentrations of at least 6.4×10^{13} vg/mL without aggregation.

Without intending to be limited by theory, the low solubility of AAV2 particles may be caused by their highly symmetrical nature in conjunction with the stabilizing effect of complementary charged regions between neighbouring particles in aggregates. The surface charge density based on the crystal structure of AAV2 (Xie, Q. *et al.* (2002) *Proc. Natl. Acad. Sci. U.S.A.* 99: 10405-10410) reveals a pattern of positive and negative charges on the virus surface. Previous reports have shown that AAV2 vector aggregation is pH dependent, and hypothesized that amino acids with charged side groups are involved in inter-particle binding. Qu, G. *et al.* (2003) *Mol. Therapy* **7**: S238. These reports hypothesized that if charged amino acid side chains are involved in vector aggregation, high concentrations of free amino acids could block vector particle interactions. However, we have found that amino acids with charged side chains are not effective in preventing AAV2 vector aggregation beyond their contribution to ionic strength.

Vector aggregation at low ionic strength was also found to be reduced but not prevented by efficient nuclease treatment of purified vector particles. Digestion at an earlier stage of the purification process (clarified HEK cell lysate) did not reduce aggregation following vector

purification. It is likely that digestion of already purified virions is more efficient because of a higher enzyme to nucleic acid substrate ratio. One mechanism to explain these results is that residual nucleic acid impurities (e.g. host cell and plasmid DNA) bound to the vector surface can bridge to binding sites on neighbouring virus particles and thus cause aggregation. Purified AAV2 vectors (empty capsid free) have been reported to contain approximately 1% non-vector DNA. Smith, P. *et al.* (2003) *Mol. Therapy* 7: S348. While >50% of this non-vector DNA was reported to be nuclease resistant and was packaged within capsid particles, some impurity DNA was nuclease resistant and appeared to be associated with the surface of purified vector particles. The observation that efficient nuclease treatment can reduce vector aggregation suggests that nucleic acids associated with the vector surface at an average level not greater than ~25 nucleotides per vector particle can contribute to AAV vector aggregation.

In summary, the use of high ionic strength solutions during AAV2 vector purification and final formulation, and efficient removal of residual vector surface DNA are two effective strategies to achieve highly concentrated solutions of AAV2 vectors for use in pre-clinical and clinical studies. High ionic strength solutions and nuclease treatment can be used in combination or separately. Although data were obtained using AAV2 vectors, the composition and methods of the present invention may also be useful with other AAV serotypes / variants, or other viral vectors such as adenoviruses, lentiviruses and retroviruses.

AAV Aggregation as a Function of Excipient Concentration

Initial screening experiments are performed to elucidate the mechanism of AAV vector aggregation and to identify classes of excipients that can reduce / prevent aggregation. Vector aggregation can be caused by dilution (5-fold) of vector in neutral-buffered saline with low

concentration buffer (20mM sodium phosphate, pH 7.2). Excipients are screened using this "dilution-stress" method to identify excipients that are able to prevent vector aggregation when included in the diluent. For screening, aggregation is measured by dynamic light scattering (DLS). Classes of excipients examined included selected inorganic salts, amino acids, uncharged carbohydrates, and surfactants. Results are presented in Table 1.

TABLE 1

SCREENING FOR EXCIPIENTS THAT PREVENT AAV2 VECTOR AGGREGATION USING DILUTION-STRESS METHOD

Excipient	Osm required to prevent aggregation (max tested)
Magnesium sulfate	180 mOsm
Sodium citrate	220 mOsm
Sodium chloride	320 mOsm
Sodium phosphate	220 mOsm
Sodium sulfate	220 mOsm
Arginine	NIA (200 mOsm)
Aspartic acid	320 mOsm
Glutamic acid	320 mOsm
Glycine	NIA (200 mOsm)
Histidine	NIA (200 mOsm)
Lysine	300 mOsm
Glycerol	NIA (5% w/v, 543 mOsm)
Iodixanol	NIA (5% w/v, 32 mOsm)
Mannitol	NIA (5% w/v, 275 mOsm)
Sorbitol	NIA (5% w/v, 275 mOsm)
Sucrose	NIA (5% w/v, 146 mOsm)
Trehalose	NIA (5% w/v, 146 mOsm)
Pluronic® F68	NIA (10% w/v, 12 mOsm)
Polysorbate 80	NIA (1% w/v)

NIA: No inhibition of aggregation

As illustrated in Table 1, charged excipients (inorganic salts and amino acids) prevent aggregation when present at sufficient concentrations. However, salt concentrations required to prevent vector aggregation vary, ranging from 180 mOsm for magnesium sulfate, to 320 mOsm for sodium chloride. The amino acids arginine, aspartic acid, glutamic acid, glycine, histidine, and lysine do not prevent aggregation at 200 mOsm, but lysine, aspartic acid, and glutamic acid prevent aggregation at 300-320 mOsm. Arginine, glycine and histidine were not tested at concentrations other than 200 mOsm. Selected carbohydrates have no effect on vector particle aggregation when present at concentrations up to 5% w/v. For example, 5% w/v glycerol (543 mOsm) does not prevent aggregation. The surfactants Polysorbate80 (1% w/v) and Pluronic® F68 (10% w/v) similarly have no effect on aggregation using the "dilution-stress" method.

AAV Aggregation as a Function of Osmolarity and Ionic Strength

FIGS. 1A and 1B show the results of a more detailed analysis of vector aggregation as a function of the concentration of various salts. FIG. 1A shows vector aggregation as a function of the osmolarity of selected excipients. For charged species a concentration-dependent inhibition of AAV2 vector aggregation is observed. Salts with multivalent ions achieve a similar degree of inhibition of aggregation at lower concentrations than monovalent sodium chloride. For example, magnesium sulfate prevents aggregation at $\geq$ 200 mOsm whereas sodium chloride requires $\geq$ 350 mOsm to achieve a similar effect. Sodium citrate, sodium sulfate, and sodium phosphate are intermediate in their potency to prevent vector aggregation.

Although the results in FIG. 1A and Table 1 show no effect of glycerol and certain sugars at concentrations up to 5% on AAV2 vector aggregation induced by low ionic strength, the data

cannot rule out improvement of AAV2 solubility at glycerol concentrations above 5%. For example, Xie and co-workers reported that 25% (w/v) glycerol enabled concentration of AAV2 to very high concentrations (4.4 to 18 x 10^{14} particles/ml) in low ionic strength solutions. Xie, Q. *et al.* (2004) *J. Virol. Methods* 122: 17-27.

FIG. 1B shows the data of FIG. 1A plotted as a function of the calculated ionic strength, rather than osmolarity, for each excipient. FIG. 1B demonstrates that vector aggregation is prevented when ionic strength is ~200 mM or greater regardless of which salt is used. These data suggested that the ionic strength (μ) of a solution, a parameter that depends on both solute concentration and charge valency, is the primary factor affecting aggregation.

Ionic strengths useful to prevent aggregation in embodiments of the present invention include, for example, 250 mM, 300 mM, 350 mM, 400 mM, 450 mM, 500 mM, 600 mM, 700 mM or higher ionic strengths. Multivalent ions are preferred to achieve these ionic strengths in methods and formulations of the present invention, such as divalent, trivalent, tetravalent, pentavalent ions and ions of even higher valency. The pH buffer in solutions and formulations of the present invention may be phosphate, Tris, or HEPES (or other Good's buffers), but any other suitable pH buffer may be used. In preferred embodiments, the multivalent ions and buffer are selected to be compatible with the target tissue for the vector being prepared.

Use of multivalent ions in the methods and compositions of the invention makes it possible to create compositions of high ionic strength but relatively low osmolarity. High ionic strength compositions of the present invention may be nearly isotonic, and may be, for example, about 200mOsm, 250mOsm, 280mOsm, 300mOsm, 350mOsm or 400mOsm, although other osmolarities may be acceptable for some uses of the compositions.

AAV Aggregation as a Function of the Method of AAV Purification

Recombinant AAV2 purified using different methods (e.g. density gradient purification versus ion-exchange chromatography) would be expected to have different impurity profiles. FIG. 2 shows vector aggregation as a function of ionic strength for several preparations of AAV differing in the purification method. Purification methods are described in Example 1. Sodium chloride is used to vary the ionic strength. AAV2-FIX vectors purified by double cesium chloride gradient ultracentrifugation (Method 1), by cation exchange column chromatography (Method 2), or by combined column and cesium chloride gradient ultracentrifugation (Method 3) each demonstrate similar aggregation responses as ionic strength is decreased. In contrast, AAV2-FIX purified by the column method and then subjected to a nuclease digestion step (Method 2 + nuclease) shows reduced aggregation at low ionic strength.

AAV Aggregation at Preparative Scale

The data in Table 1 and FIGS. 1A, 1B and 2 involve vector aggregation *at an analytical scale*, employing DLS to measure aggregation. Table 2, in contrast, shows the effects of elevated ionic strength and nuclease treatment on AAV2 vector aggregation at a larger scale, using methods to induce and quantify vector aggregation that are relevant to *preparative scale* vector purification. Experimental details are provided in Example 2. Purified AAV vectors are diafiltered into solutions of various ionic strengths, the volume is reduced to achieve high vector concentrations, and aggregation is then assessed by measuring vector recovery after filtration through a 0.22μm filter. Aliquots from a single pool of AAV2-AADC vector purified by Method 1 through the second CsCl gradient centrifugation step ($1.8x10^{15}$ vg in 91mL, $1.8x10^{13}$ vg/mL, in ~3M CsCl) are used as starting material in the diafiltration experiments. Tangential

flow filtration using hollow fibers is used for diafiltration because it is scalable and yet it still enables preparation of volumes (min. 1.4mL), and thus AAV concentrations, at which aggregation would be expected in neutral buffered saline.

In Experiment 1, three hollow fiber units are used to diafilter AAV2-AADC vector in formulations CF, TF1, or TF2, and the volume is reduced to a target of $2.5x10^{13}$ vg/mL. *See* Example 2. The samples are then filtered through a 0.22μm filter. Results are shown in Table 2. Vector recovery ("Yield %") for both elevated ionic strength formulations TF1 (95 ± 7.4%) and TF2 (93 ± 7.4%) are significantly higher than the recovery using the control formulation CF (77 ± 6.6%).

TABLE 2
AAV VECTOR RECOVERY AT PROCESS SCALE

Experiment	Formulation	μ (mM)	Target (vg/mL)	Actual (vg/mL)	Yield % (RSD)
1	CF	160	2.5E13	1.93E13	77 (6.6)
1	TF1	310	2.5E13	2.38E13	95 (7.4)
1	TF2	510	2.5E13	2.33E13	93 (7.4)
2	CF	160	6.7E13	3.98E13	59 (6.0)
2	TF2	510	6.7E13	6.42E13	96 (4.4)
3	CF (-Bz)	160	3.6E13	2.46E13	68 (11)
3	CF (+Bz)	160	3.6E13	3.29E13	91 (12)

In Experiment 2, AAV2-AADC is concentrated to a higher target value ($6.7x10^{13}$ vg/mL) in CF or TF2. Vector recovery using TF2 (96 ± 4.4%) is again significantly higher than recovery using CF (59 ± 6.0%). Within the variability of the assays used, vector was recovered fully at both target concentrations using TF2, indicating that aggregation was prevented. In contrast, significant aggregation was observed at both target concentrations using CF, and the extent of

aggregation (i.e. loss following 0.22μm filtration) was higher at the higher target vector concentration. In an additional experiment (not shown), 50 μL samples of AAV2 vector are taken following concentration but prior to the 0.22μm filtration step of Experiment 2, and examined by light microscopy. Vector concentrated in CF contains obvious amounts of visible material (not shown), while no such material is seen in vector concentrated in TF2.

Experiment 3 examines the effect of prior nuclease digestion of purified vector on aggregation. In the absence of nuclease digestion recovery of AAV2-AADC in CF is 68 ± 11%, similar to the recoveries in Experiments 1 and 2. In contrast, purified vector treated with nuclease and then concentrated in CF gives higher recovery (91 ± 12%). These prep scale results reflect the same effect of nuclease digestion shown in FIG. 2 using the "dilution-stress" (analytical scale) method.

The results presented in Table 2 demonstrate that the methods and compositions of the present invention increase the recovery of AAV vector recovery. For example, in various embodiments of the present invention, recovery is improved from less than about 80% to at least about 85%, 90%, 95% or more.

AAV Stability and Activity Following Storage or Freeze-Thaw Cycling

Croyle and coworkers reported a significant loss of titer of AAV and adenovirus following multiple freeze-thaw cycling in sodium phosphate buffer, and demonstrated that the better pH buffering provided by potassium phosphate during freeze-thaw cycling prevented titer loss. Croyle, M. A. *et al.* (2001) *Gene Therapy* 8: 1281-1290. Results of our freeze-thaw stability study using sodium phosphate support these findings. We find that while 150mM sodium phosphate provides sufficient ionic strength to prevent aggregation during preparation

and non-frozen storage of concentrated AAV2-AADC vector, even a single freeze-thaw cycle at -20 or -80 °C results in aggregation.

AAV stability after storage or freeze-thaw (F/T) cycling is assessed in buffers of the present invention as follows. The concentrated vectors prepared in CF, TF1, and TF2 (Table 2, Experiment 1) are subjected to a short stability study to investigate whether aggregation will occur during refrigerated storage, or following multiple freeze-thaw (F/T) cycles. Aggregation is assessed by DLS using undiluted samples, and *Rh* values >20nm are deemed to indicate the occurrence of some level of aggregation.

TABLE 3

STABILITY OF AAV2 VECTORS

	Particle radius – Rh (nm)							
Formulation	Pre	4 °C	- 20 °C			- 80 °C		
		5d	1 F/T	5 F/T	10 F/T	1 F/T	5 F/T	10 F/T
CF	14.5	27.0	22.4	56.1	94.5	20.6	57.5	141
TF1	13.8	16.3	TH	TH	TH	TH	TH	TH
TF2	13.8	14.4	14.2	14.0	14.1	13.8	21.3	50.9

Pre: DLS radius measured immediately following 0.2μm filtration.

Vector concentrations (vg/mL): CF: 1.93E13, TF1: 2.38E13, TF2: 2.33E13.

TH: signal intensity is too high to measure because of extensive aggregation.

As shown in Table 3, AAV2-AADC vector prepared in CF shows some aggregation after 5 days of storage at 4 °C, as well as following one or more F/T cycles at -20 or -80°C. For vector prepared in TF1, no aggregation occurs after 5 days at 4°C, but aggregation occurs following a single F/T cycle at -20 or -80 °C as indicated by a DLS signal intensity that is too

high to measure. Visual inspection of these samples reveals slight cloudiness, which is consistent with aggregation. For vector prepared in TF2, no aggregation is observed at 4 °C, or following up to 10 F/T cycles at -20 °C. Some aggregation is observed following 5 and 10 F/T cycles at -80 °C.

AAV activity after storage or F/T cycling in TF2 is assessed as follows. As described above, the high ionic strength, isotonic formulation TF2 effectively prevents vector aggregation during concentration and storage, and therefore represents a promising candidate for further study. An important question is whether preparation and storage of the vector in high ionic strength TF2 would adversely affect its functional activity. To assess this, assays are performed to measure the infectious titer and the transduction efficiency of vectors prepared and stored for an extended period of time in TF2.

For infectivity, a highly sensitive infectivity assay capable of detecting single infectious events is used. Zhen, Z. *et al.* (2004) *Human Gene Ther.* 15: 709-715. AAV2-AADC is prepared in TF2 at a concentration of $6.4x10^{13}$ vg/mL. After being stored for 45 days at 4 °C the preparation has a vector genome to infectious unit ratio (vg/IU) of 13, compared to a value of 16 vg/IU for the reference vector. This difference is not significant given the reported variability of this assay (RSD ~50%).

Transduction efficiency is assessed by measuring the expression of AADC protein by ELISA following transduction of D7/4 cells. FIG. 3 shows no significant difference between vector prepared in TF2 and the reference control for vector input ranging from 10 to 10^5 vg/cell. Together, these data indicate that preparation and storage of AAV2 vectors in high ionic strength TF2 does not have a deleterious effect on vector infectivity or transduction efficiency.

Conclusion

The effect of ionic strength (μ) on virus particle interactions is determined to elucidate the mechanism of vector aggregation. The ionic strength of neutral-buffered isotonic saline (μ = 150mM) is insufficient to prevent aggregation of AAV2 vectors purified by gradient ultracentrifugation or by cation exchange chromatography at concentrations exceeding ~10^{13} particles/mL. Inclusion of sugars (sorbitol, sucrose, mannitol, trehalose, glycerol) at concentrations up to 5% (w/v) or of surfactants Tween80® (1%) or Pluronic® F68 (10%) does not prevent aggregation of vector particles.

In contrast, vector particles remain soluble when elevated ionic strength solutions (μ > 200mM) are used during purification and for final vector formulation. Elevated ionic strength solutions using isotonic excipient concentrations for *in vivo* administration are prepared with salts of multivalent ions, including sodium citrate, sodium phosphate, and magnesium sulfate. An isotonic formulation containing 10mM Tris, 100mM sodium citrate, 0.001% Pluronic® F68, pH 8.0 (μ ~500mM) enables concentration of AAV2-AADC vectors to 6.4x10^{13} vg/mL with no aggregation observed during preparation and following ten freeze-thaw cycles at -20 °C. *See* Table 3, below, and accompanying discussion. AAV2-AADC vectors prepared and stored for an extended period in elevated ionic strength formulation retain high infectivity titer (13 IU/vg) and transduction efficiency.

Nuclease treatment of purified AAV2 vectors reduces the degree of vector aggregation, implicating vector surface nucleic acid impurities in inter-particle interactions. Hence, purification methods to efficiently remove vector surface residual nucleic acids, coupled with the use of elevated ionic strength isotonic formulations, are useful methods to prevent AAV2 vector aggregation.

EXAMPLE 1

AAV PURIFICATION METHODS

AAV2 vectors expressing human coagulation factor IX (FIX) or human amino acid decarboxylase (AADC) are produced by triple transfection of HEK293 cells as previously described (Matsushita, T. *et al.* (1998) *Gene Therapy* **5**: 938-945), with modifications. For the large scale preparations, cells are cultured and transfected in 850 mm^2 roller bottles (Corning). Vectors are purified by one of three methods.

In purification Method 1, modified from Matsushita, transfected HEK293 cells in roller bottles are collected by centrifugation (1000g, 15min), resuspended in 10mM sodium phosphate, 500mM sodium chloride, pH 7.2, and lysed by three freeze / thaw cycles (alternating an ethanol / dry ice bath and a 37°C water bath). The cell lysate is clarified by centrifugation (8,000g, 15 min). The supernatant is then diluted to 200mM NaCl by addition of 10mM sodium phosphate, pH 7.2, and digested with Benzonase® (Merck, Purity Grade 1; 200 U/mL, 1h, 37 °C). The lysate is adjusted to 25mM $CaCl_2$ using a 1M stock solution, and incubated at 4°C for one hour.

The mixture is centrifuged (8,000g, 15 min), and the supernatant containing vector is collected. To precipitate virus from the clarified cell lysate, polyethylene glycol (PEG8000) is added to a final concentration of 8%, the mixture incubated at 4°C for three hours, and then centrifuged (8,000g, 15 min). The pellets containing vector are re-suspended with mixing in 0.15M NaCl, 50mM Hepes, 25mM EDTA, pH 8.0 and incubated at 4°C for 16 hours. The resuspended material is pooled, and solid cesium chloride is added to a final density of 1.40

gm/ml. Vector is then banded by ultracentrifugation (SW28, 27,000rpm, 24h, 20°C) using a Beckman model LE-80 centrifuge. The centrifugation tubes are fractionated, and densities from 1.38 to 1.42 gm/mL containing vector are pooled. This material is banded a second time by ultracentrifugation (NVT65 rotor, 65,000 rpm, 16h, 20°C), and fractions containing purified AAV2 vectors are pooled. To concentrate vector and to perform buffer exchange, vectors in concentrated cesium chloride solution are subjected to ultrafiltration / diafiltration (UF/DF) by tangential flow filtration as described below (Example 2).

In purification Method 2, cell harvests containing AAV are microfluidized and filtered sequentially through 0.65 and 0.22 μm filters (Sartorius). Virus is purified from the clarified cell lysates by cation exchange chromatography using Poros HS50 resin as previously described. U.S. Pat. No. 6,593,123. For the nuclease digestion described in FIG. 2, column-purified vectors are incubated (4h, RT) with 100 U/mL Benzonase and 10 U/mL DNAse I (RNAse free, Roche Diagnostics, Indianapolis, Indiana).

For purification Method 3, AAV2 vectors purified by cation exchange chromatography are subjected to an additional cesium chloride gradient ultracentrifugation step (SW28, 27,000rpm, 20h) to remove empty capsids prior to UF/DF.

Real time quantitative PCR (Q-PCR) is used to quantify AAV preparations as previously described. Sommer, J. M. *et al.* (2003) *Mol. Therapy* **7**: 122-128. Vectors purified by each of the three methods are analyzed by SDS-PAGE / silver staining analysis, and in all cases VP1, VP2 and VP3 are present in the expected ratios, with the capsid proteins representing >95% of total proteins as determined by scanning densitometry. However, unlike gradient-purified AAV2 vectors purified using Methods 1 and 3, vectors purified by Method 2 (column chromatography) contain empty capsids, ranging from 3-10 empty capsids per vector genome.

EXAMPLE 2

ULTRAFILTRATION AND DIAFILTRATION TO DETECT AAV AGGREGATION

Disposable hollow fiber tangential flow filtration devices (Amersham BioSciences 8" Midgee, 100 kDa nominal pore size) are used to concentrate and diafilter AAV2 vectors purified by the methods described above, and for the UF/DF experiments described in Table 2. For all UF/DF procedures a volume of diafiltration buffer corresponding to 10x the product volume is used, and it is added in ~1mL increments to approximate continuous diafiltration. Using this method, the calculated residual CsCl after diafiltration is <0.5mM.

The following three formulations were used for UF/DF: Control Formulation (CF: 140mM sodium chloride, 10mM sodium phosphate, 5% sorbitol, pH 7.3); Test Formulation 1 (TF1: 150mM sodium phosphate, pH7.5); and Test Formulation 2 (TF2: 100 mM sodium citrate, 10mM Tris, pH8.0). For Experiment 1 shown in Table 2, diafiltration is performed at a volume corresponding to a vector concentration of $1x10^{13}$ vg/mL, and following diafiltration the volume is reduced to a value corresponding to $2.5x10^{13}$ vg/mL (assuming no vector loss).

For Experiment 2, diafiltration is performed at a volume corresponding to a $2x10^{13}$ vg/mL, and the volume is then reduced to a value corresponding to $6.7x10^{13}$ vg/mL.

For Experiment 3 (CF ± Bz), AAV2-AADC (approximately $1.2x10^{14}$ vg) is first diafiltered into TF1 (a formulation compatible with nuclease activity) and then passed through a 0.22 μm filter. The titer of this material is determined, and the volume is adjusted to correspond to a concentration of $1x10^{13}$ vg/mL. To 10 mL of this material, $MgCl_2$ is added to a concentration of 2 mM, and then divided into two equal aliquots. One aliquot is incubated with Benzonase (200 U/mL, 4h, RT), and the second is mock-incubated. Each aliquot is then

diafiltered at a volume corresponding to a vector concentration $2x10^{13}$ vg/mL, and then concentrated to a $3.6x10^{13}$ vg/mL target. Following all UF/DF protocols, Pluronic® F-68 (BASF Corp., Mount Olive, NJ) from a 1% stock is added to the vector product to a final concentration of 0.001%, and the solution is passed through a 0.22μm syringe filter (Sartorius). All UF/DF procedures are performed in a laminar flow cabinet.

EXAMPLE 3

MEASUREMENT OF VECTOR AGGREGATION BY DYNAMIC LIGHT SCATTERING

Purified vectors are analyzed for aggregation by dynamic light scattering (DLS) using a Protein Solutions *DynaPro 99* (λ=825.4 nm). Primary data (particle radius – *Rh*, average value measured over 30 cycles, 10 cycles/min) are used for all analyses reported. A "dilution-stress" method is used to assess the effect of varying excipients on vector aggregation. In this method, 80 μL of test diluent is added to 20 μL of vector solution with mixing in the actual cuvette used for DLS measurement, and data collection is initiated within 10 seconds of mixing. Prior to addition of test diluents, the *Rh* value for AAV2 vector preparations is measured and confirmed to be <15 nm to ensure that the starting material is monomeric. Samples that are not 100% monomeric are passed through a 0.22μm syringe disc filter (Sartorius, low protein binding) to remove aggregates.

The osmolarity and ionic strength values given in FIGS. 1 and 2 are calculated using all excipients present in the mixture (i.e. weighted: test diluent (80%) and starting vector formulation (20%)). Osmolarity is calculated according to the equation: Osmolarity = $\sum c_i$, where c_i is the molar concentration of each solute species. The ionic strength (μ) is calculated

according to the equation: $\mu = \frac{1}{2} \sum c_i z_i^2$, where z_i is the charge on each species. In conditions that resulted in vector aggregation (e.g. low μ) a progressive increase in *Rh* is observed over the course of data collection. To validate the use of the average *Rh* measured over the 3 minute interval following dilution as a reliable measure of aggregation, the average rate of increase of *Rh* ($\Delta Rh / \Delta t$) over the same time interval is also determined (not shown). Analysis of $\Delta Rh / \Delta t$ gives results concordant with those obtained using the average *Rh* value reported in FIGS. 1 and 2.

EXAMPLE 4

AAV VIRION INFECTIVITY

Infectivity of AAV2-AADC vectors is determined using a highly sensitive assay as previously described. Zhen, Z. *et al.* (2004) *Human Gene Ther.* 15: 709-715. Briefly, samples are serially diluted (10-fold dilutions, 10 replicates / dilution) and added to D7/4 cells (modified HeLa cells expressing AAV *rep* and *cap*) grown in 96 well tissue culture plates (Falcon, cat. #353227) in DMEM medium containing 10% FBS. Adenovirus (Ad-5, 100 vp/cell) is added to each well to provide helper functions. After 48h, replication of AAV vector in each well is quantified by Q-PCR using transgene-specific primers and probes, and the frequency of infection at limiting dilution is analyzed by the Karber method to calculate the infectivity titer. The test sample is run concurrently with an AAV2-AADC reference previously prepared in CF and stored at -80 °C.

The transduction efficiency of AAV2 vectors is quantified by a whole cell ELISA. D7/4 cells grown in 96 well plates are infected with 10-fold serial dilutions of the test sample and reference vector, corresponding to 10 to 10^5 vg / cell input (5 replicates / dilution). After 48h,

the culture medium is removed, and cells are washed twice with 200 µL PBS (10 mM sodium phosphate, 140mM sodium chloride, pH 7.2). Cells are then permeabilized and fixed by addition of 100µL of PBS containing 0.5% Triton X-100 and 4% paraformaldehyde to each well (15 min). The fixing solution is removed, and the cells are washed twice with PBS containing 0.5% Triton X-100. Non-specific sites are blocked by adding PBS containing 3% bovine serum albumin (BSA) and 0.5% Triton X-100 (60min).

After washing, cells are incubated for one hour with rabbit anti-AADC IgG antibody (Chemicon, AB136), and washed. Cells are then incubated for one hour with alkaline phosphatase-conjugated goat anti-rabbit IgG, and washed. Antibodies are diluted 1:1000 in PBS containing 1% BSA, 0.5% Triton X-100. Substrate (PNPP, Pierce, cat. #34047) is then added (1 mg/mL in 1X diethanolamine substrate buffer, Pierce, cat. #34064), and after incubation for 30min the concentration of cleaved substrate is measured spectrophotometrically (λ=405nm). Human AADC expression as a function of vector input is fitted using a spline curve (SigmaPlot). The AAV2-AADC reference vector is measured concurrently with the test sample.

While preferred illustrative embodiments of the present invention are described, it will be apparent to one skilled in the art that various changes and modifications may be made therein without departing from the invention, and it is intended in the appended claims to cover all such changes and modifications that fall within the true spirit and scope of the invention.

All publications, patents and patent applications referred to herein are hereby incorporated by reference in their entireties.

We claim:

1. A method of preventing aggregation of virions in a preparation of virions, comprising adding one or more excipients to the preparation of virions to achieve an ionic strength of at least about 200 mM.

2. The method of claim 1, wherein the virions are AAV virions.

3. The method of claim 1, further comprising treating said preparation of virions with a nuclease.

4. The method of claim 3, wherein the nuclease is Benzonase®.

5. The method of claim 1, wherein one or more of the excipients comprises a multivalent ion.

6. The method of claim 5, wherein the multivalent ion is citrate.

7. The method of claim 1, wherein the osmolarity of the preparations of virions after addition of the one or more excipients is no greater than about 280mOsm.

8. The method of claim 1, wherein, after addition of the one or more excipients, the average particle radius (*Rh*) of the virions in the preparation of virions is less than about 20nm as measured by dynamic light scattering.

9. The method of claim 1, wherein, after addition of the one or more excipients, recovery of the virions is at least about 90% following filtration of the preparations of virions through a 0.22μm filter.

10. A composition for the storage of purified virus particles, comprising:

purified virus particles;

a pH buffer; and

excipients comprising one or more multivalent ions; wherein the ionic strength of the composition is greater than about 200 mM.

11. The composition of claim 10, wherein the purified virus particles are AAV virus particles.

12. The composition of claim 10, wherein one of the one or more multivalent ions is citrate.

13. The composition of claim 10, further comprising Pluronic® F68.

14. The composition of claim 13, wherein the Pluronic® F68 is present at 0.001%.

15. The composition of claim 10, wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate.

16. The composition of claim 10, wherein the average particle radius (*Rh*) of the purified virus particles is less than about 20nm as measured by dynamic light scattering.

17. The composition of claim 10, wherein recovery of the purified virus particles is at least about 90% following filtration of the composition of virions through a 0.22μm filter.

18. A method of preventing aggregation of virions in a preparation of virions, comprising treating said preparation of virions with Benzonase®.

19. The method of claim 18, wherein, after Benzonase® treatment, the average particle radius (*Rh*) of the virions in the preparation of virions is less than about 20nm as measured by dynamic light scattering.

20. The method of claim 18, wherein, after Benzonase® treatment, recovery of the virions is at least about 90% following filtration of the preparation of virions through a 0.22μm filter.

ABSTRACT

Compositions and methods are provided for preparation of concentrated stock solutions of AAV virions without aggregation. Formulations for AAV preparation and storage are high ionic strength solutions (e.g. μ ~500mM) that are nonetheless isotonic with the intended target tissue. This combination of high ionic strength and modest osmolarity is achieved using salts of high valency, such as sodium citrate. AAV stock solutions up to $6.4x10^{13}$ vg/mL are possible using the formulations of the invention, with no aggregation being observed even after ten freeze-thaw cycles. The surfactant Pluronic® F68 may be added at 0.001% to prevent losses of virions to surfaces during handling. Virion preparations can also be treated with nucleases to eliminate small nucleic acid strands on virions surfaces that exacerbate aggregation.

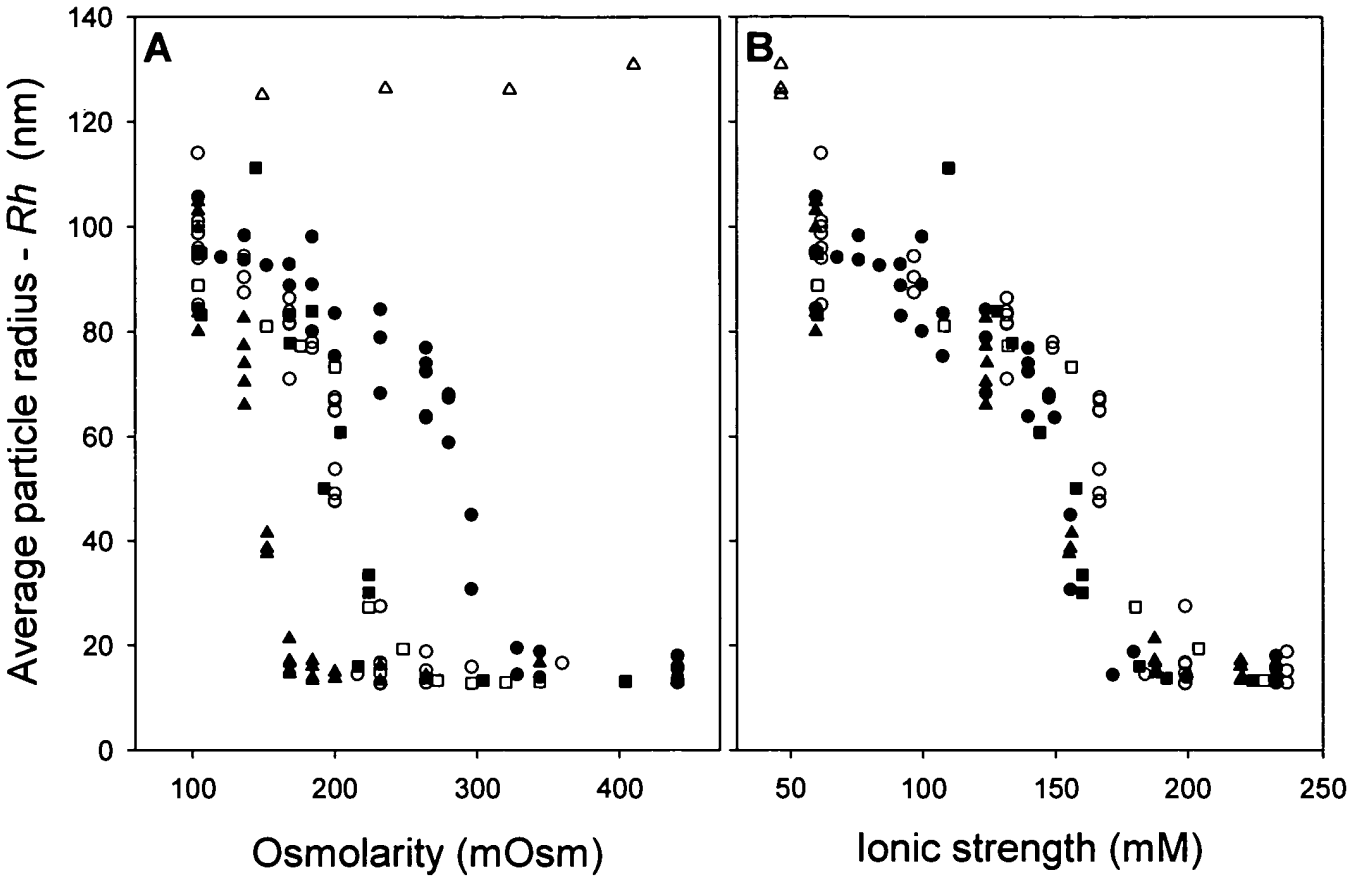

A
B
Average particle radius - Rh (nm)
140
120
100
80
60
40
20
0
100
200
300
400
50
100
150
200
250
Osmolarity (mOsm)
Ionic strength (mM)

FIGURE 1

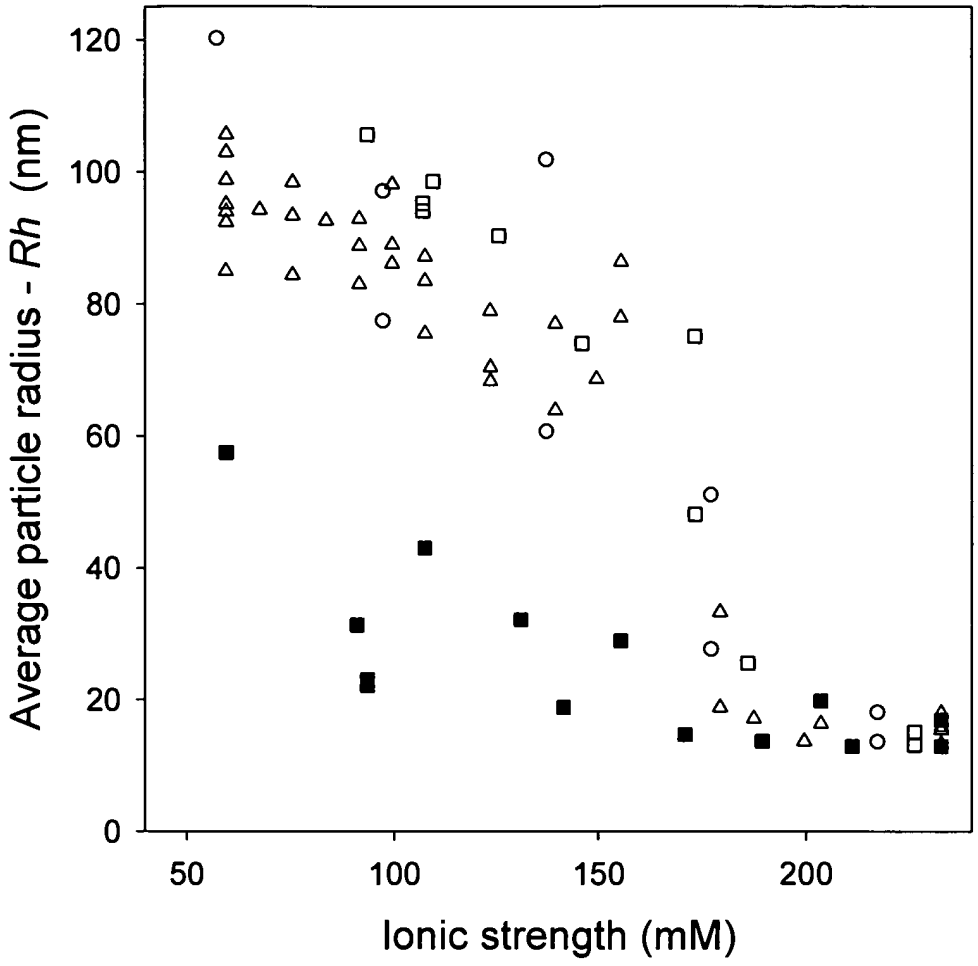

Average particle radius - Rh (nm)
120
100
80
60
40
20
0
50
100
150
200
Ionic strength (mM)

FIGURE 2

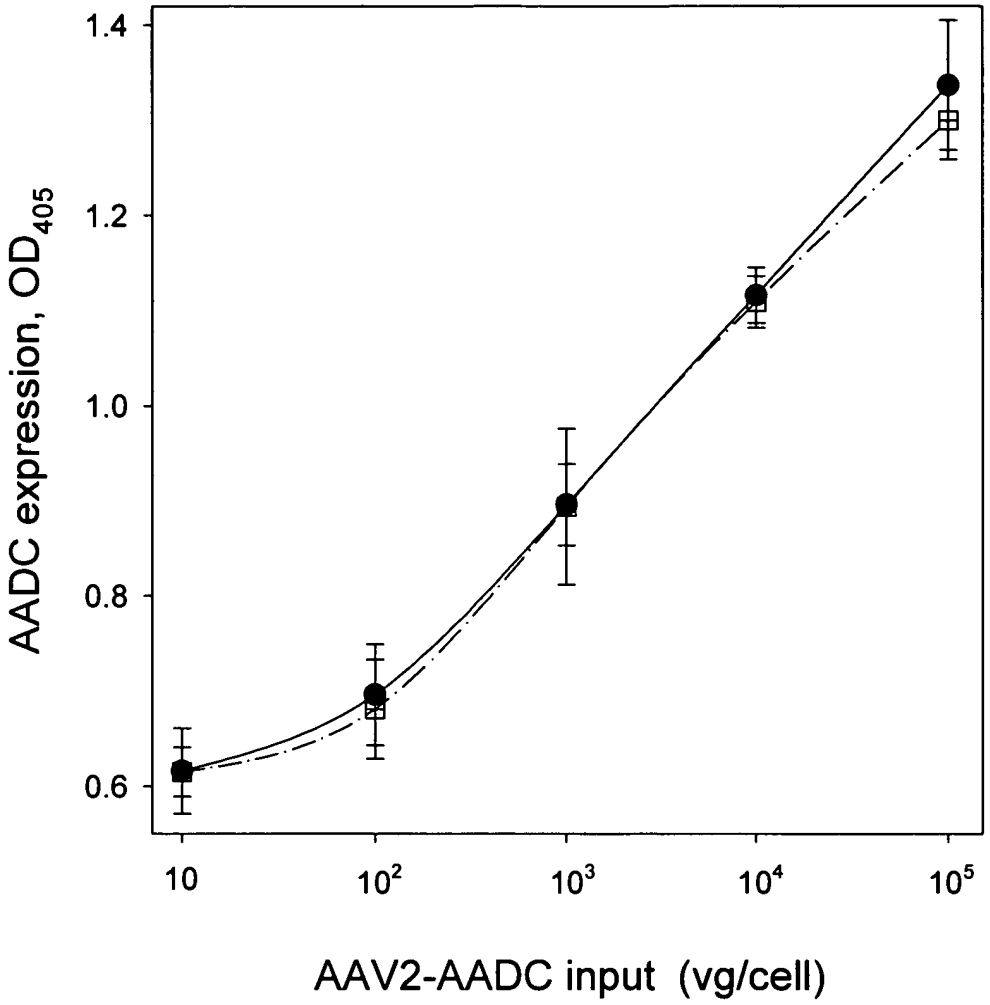

AADC expression, OD405
1.4
1.2
1.0
0.8
0.6
10
10^2
10^3
10^4
10^5
AAV2-AADC input (vg/cell)

FIGURE 3

Application Data Sheet

Application Information

Application Number::	
Filing Date::	
Application Type::	Regular
Subject Matter::	Utility
Suggested classification::	
Suggested Group Art Unit::	
CD-ROM or CD-R?::	None
Number of CD disks::	
Number of copies of CDs::	
Sequence submission?::	
Computer Readable Form (CRF)?::	No
Number of copies of CRF::	
Title::	COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION
Attorney Docket Number::	0800-0045
Request for Early Publication?::	No
Request for Non-Publication?::	No
Suggested Drawing Figure::	
Total Drawings Sheets::	3
Small Entity?::	Yes
Latin Name::	
Variety denomination name::	
Petition included?::	No
Petition Type::	
Licensed US Govt. Agency::	
Contract or Grant Numbers::	
Secrecy Order in Parent Appl.?::	No

Applicant Information

Applicant Authority Type::	Inventor
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Family Name::	Wright
Name Suffix::	
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Status::	Full
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Middle Name::	
Family Name::	Qu
Name Suffix::	
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DOMESTIC PRIORITY INFORMATION

Application::	Continuity Type::	Parent Application::	Parent Filing Date::
This Application	Claims the benefit under 35 USC 119(e) of	60/575,997	June 1, 2004
and	Claims the benefit under 35 USC 119(e) of	60/639,222	December 22, 2004

FOREIGN PRIORITY INFORMATION

Country::	Application Number::	Filing Date::	Priority Claimed::

ASSIGNEE INFORMATION

Assignee name::

Street of mailing address::

City of mailing address::

State of mailing address::

Country of mailing address::

Zip Code of mailing address::

PTO/SB/06 (12-04)
Approved for use through 7/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 11141996

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY FEE ($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	20 minus 20 =	* —	X =		OR	X =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	3 minus 3 =	* —	X =			X =	
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))			N/A			N/A	
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	150		TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT A		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY ADDITIONAL FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X =		OR	X =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X =		OR	X =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))					N/A		OR	N/A	
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

AMENDMENT B		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)	OR	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X =		OR	X =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X =		OR	X =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))					N/A		OR	N/A	
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

JUN 20 2005

1FW

PTO/SB/21 (09-04)

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	5

Application Number	11/141,996
Filing Date	June 1, 2005
First Named Inventor	John Fraser Wright
Art Unit	Unassigned
Examiner Name	Unassigned
Attorney Docket Number	0800-0045

ENCLOSURES *(Check all that apply)*

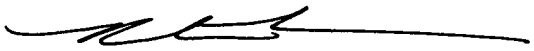
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  - ☐ Fee Attached
- ☐ Amendment/Reply
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Reply to Missing Parts/ Incomplete Application
  - ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation Change of Correspondence Address
- ☐ Terminal Disclaimer
- ☐ Request for Refund
- ☐ CD, Number of CD(s) _____
  - ☐ Landscape Table on CD
- ☐ After Allowance Communication to TC
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- ☐ Proprietary Information
- ☐ Status Letter
- ☒ **Other Enclosure(s) (please identify below):**

Supplemental ADS (3 pages)
Return Receipt Postcard (1 page)

Remarks: The Commissioner is authorized to charge any additional fees to Deposit Account 18-1648.

A supplemental ADS is submitted to correct the spelling of the second inventor's name.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	Robins & Pasternak LLP		
Signature			
Printed name	Roberta L. Robins		
Date	6/16/05	Reg. No.	33,208

CERTIFICATE OF TRANSMISSION/MAILING

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Signature			
Typed or printed name	Anne Currier Carr	Date	6/16/05

JUN 2 0 2005
PATENT & TRADEMARK OFFICE

Application Data Sheet

Application Information

Application Number::	11141996
Filing Date::	06/01/05
Application Type::	Regular
Subject Matter::	Utility
Suggested classification::	
Suggested Group Art Unit::	
CD-ROM or CD-R?::	None
Number of CD disks::	
Number of copies of CDs::	
Sequence submission?::	
Computer Readable Form (CRF)?::	No
Number of copies of CRF::	
Title::	COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION
Attorney Docket Number::	0800-0045
Request for Early Publication?::	No
Request for Non-Publication?::	No
Suggested Drawing Figure::	
Total Drawings Sheets::	3
Small Entity?::	Yes
Latin Name::	
Variety denomination name::	
Petition included?::	No
Petition Type::	
Licensed US Govt. Agency::	
Contract or Grant Numbers::	
Secrecy Order in Parent Appl.?::	No

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DOMESTIC PRIORITY INFORMATION

Application::	Continuity Type::	Parent Application::	Parent Filing Date::
This Application	Claims the benefit under 35 USC 119(e) of	60/575,997	June 1, 2004
and	Claims the benefit under 35 USC 119(e) of	60/639,222	December 22, 2004

FOREIGN PRIORITY INFORMATION

Country::	Application Number::	Filing Date::	Priority Claimed::

ASSIGNEE INFORMATION

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Country of mailing address::

Zip Code of mailing address::

Atty Dkt No: 0800-0045
PATENT

CERTIFICATE OF TRANSMISSION PURSUANT TO 37 CFR § 1.8

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Date 8/19/08 Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

JOHN FRASER WRIGHT et al. Confirmation No.: 5399

Application No.: 11/141,996 Art Unit: 1657

Filing Date: June 1, 2005 Examiner: Satyendra K. Singh

Title: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

AMENDMENT UNDER 37 CFR 1.111

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

This paper is responsive to the Office Action mailed February 19, 2008, with a shortened statutory period of three months for response. Accordingly, a three-month extension of time in which to respond is requested and a petition and fee therefor accompany this response. Reconsideration of the application is requested in view of the following amendments and remarks.

A **listing of claims** begins on page 2 of this paper.

Remarks begin on page 4 of this paper.

I. AMENDMENT

Amendments to the Claims:

The following listing reflects amendments to the claims and replaces all prior versions and listings of claims in this application.

1. (Previously presented) A method of preventing aggregation of recombinant adeno-associated virus (rAAV) virions in a preparation of rAAV virions, comprising:

1) providing a lysate comprising rAAV virions;

2) purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography, wherein said virions are purified; and

3) adding one or more excipients to said purified virions to produce a preparation of virions with ~~achieve~~ an ionic strength of at least 200 mM, wherein the concentration of purified rAAV virions in said preparation exceeds $1x10^{13}$ vg/ml.

2. (Canceled)

3. (Previously presented) The method of claim 1, further comprising treating said purified virions with a nuclease.

4. (Previously presented) The method of claim 3, wherein the nuclease is an endonuclease from *Serratia marcescens* (Benzonase®).

5. (Original) The method of claim 1, wherein one or more of the excipients comprises a multivalent ion.

6. (Currently amended) The method of claim ~~1~~ 5, wherein the multivalent ion is citrate.

7. (Currently amended) The method of claim 1, wherein the osmolarity of the preparations of virions after addition of the one or more excipients is no greater than about 280mOsm.

8. (Original) The method of claim 1, wherein, after addition of the one or more excipients, the average particle radius (Rh) of the virions in the preparation of virions is less than about 20nm as measured by dynamic light scattering.

9. (Currently amended) The method of claim 1, wherein, after addition of the one or more excipients, recovery of the virions is at least about 90% following filtration of the preparations of virions through a 0.22μm filter.

10-20. (Canceled)

21. (Previously presented) The method of claim 3, wherein rAAV virions are purified from the lysate using cesium chloride gradient ultracentrifugation.

22. (Previously presented) The method of claim 3, wherein rAAV virions are purified from the lysate using cation exchange chromatography.

23. (Previously presented) The method of claim 3, wherein rAAV virions are purified from the lysate using cation exchange chromatography and cesium chloride gradient ultracentrifugation.

24. (Currently amended) The method of claim 3, further comprising diafiltering the purified rAAV virions to achieve an ionic strength of at least ~~about~~ 200 mM.

II. REMARKS

Introductory Comments

Claims 1, 3-9 and 21-24 were examined in the Office Action under reply and stand variously rejected under (1) 35 U.S.C. §112, second paragraph (claim 24); and (2) 35 U.S.C. §103(a) (claims 1, 3-9 and 21-24). These grounds of rejection are believed to be overcome by this response and are otherwise traversed for reasons discussed in detail below.

Applicants note with appreciation the withdrawal of the previous rejections under 35 U.S.C. §112, second paragraph and 35 U.S.C. §102(b).

Overview of the Above Amendments

Claim 1 has been amended to provide proper antecedent basis for dependent claims 7-9 and recites that a "preparation of virions" is produced in step 3. Additionally, the preparation is characterized by having a concentration of purified rAAV virions that exceeds $1x10^{13}$ vg/ml. Support for this recitation can be found throughout the specification at, for example, page 6, line 5 and in the examples.

Claim 6 has been amended to depend from claim 5 rather than claim 1 and claim 24 has been amended to delete the term "about."

Claims 7 and 9 have been amended to correct a typographical error.

The foregoing amendments are made without prejudice, without intent to abandon any originally claimed subject matter, and without intent to acquiesce in any rejection of record. Applicants expressly reserve the right to file one or more continuing applications containing the unamended claims.

Claim Objections

The Office objected to the previous claim amendments, noting that claim 6 had been amended to depend from claim 1 but was indicated as being an original claim. Claim 6 has been amended to correct this typographical error which was introduced in the previous response. Applicants apologize for any confusion this inadvertent error caused.

Rejection Under 35 U.S.C. §112, Second Paragraph

Claim 24 was rejected as indefinite based on the use of the term "about." This term has been deleted from claim 24. Thus, this basis for rejection has been overcome and withdrawal thereof is respectfully requested.

Rejections Under 35 U.S.C. §103(a)

Claims 1, 3-9 and 21-24 were rejected under 35 U.S.C. §103(a) as being unpatentable over U.S. Patent No. 6,146,874 to Zolotukhin et al. ("Zolotukhin"), taken with U.S. Patent No. 6,566,118 to Atkinson et al. ("Atkinson") and Drittanti et al, *J. Gene Med.* (2001) 3:59-71 ("Drittanti'); in view of Qu et al., *Molec. Ther.* (2003) 5:S348, Abstract 901 ("Qu") and Chen et al., *J. Pharm. Sci.* (1994) 83:1657-1661 ("Chen"), and further in view of U.S. Patent No. 6,593,123 to Wright et al. ("Wright").

The Office argues each of the references of Zolotukhin, Atkinson and Drittanti teach a method step for the treatment of rAAV virions with a nuclease and that process steps such as ultracentrifugation, chromatography and diafiltration are used and disclosed by each of these references. Office Action, page 6. Qu is cited for disclosing problems associated with concentration-induced aggregation of rAAV virions and Chen is cited for disclosing strategies to suppress aggregation of proteins. Office Action, page 7. Finally, Wright is cited for teaching a method for large scale production of rAAV virions and the use of a nuclease. However, applicants submit the Office has failed to establish a prima facie case of obviousness.

All pending claims pertain to methods of preventing aggregation of rAAV virions in a **purified** preparation. The rAAV virions are purified from a lysate using ultracentrifugation and/or chromatography. One or more excipients are then added to achieve an ionic strength of at least 200 mM and the concentration of purified rAAV virions in the preparation exceeds $1x10^{13}$ vg/ml. In certain embodiments, the purified preparation is treated with a nuclease. None of the cited art, either alone or in combination, teaches or suggests adding one or more excipients to a **purified** rAAV virion preparation to achieve an ionic strength of at least 200 mM wherein the preparation includes more than $1x10^{13}$ vg/ml, as claimed. For this reason alone, the cited combination fails to render the claims obvious.

In particular, Zolotukhin (1) does not recognize that rAAV virions self-aggregate and (2) therefore does not provide suggestions regarding a method for preventing aggregation of rAAV virions as claimed. This is particularly noteworthy in view of Zolotukhin's recognition (see column 15, lines 22-35) that AAV aggregates with **proteins** in the cell lysate wherein high salt in the **first step** of the iodixanol gradient is used to destabilize these AAV-lysate protein interactions. Moreover, applicants assert that this passage expressly teaches away from applicants' invention because Zolotukhin purposely eliminates high salt concentrations from the remainder of the iodixanol gradient, including the gradient from which the rAAV is collected after centrifugation, because the elimination of high salt is important for subsequent purification steps.

Applicants further note that, even though Zolotukhin recognizes AAV virion-lysate protein aggregation as a problem, it does not teach or suggest that self-aggregation of rAAV virions is a problem at all, let alone a problem in **purified**, concentrated AAV preparation. In fact, absent applicants' teaching regarding this problem, there is no recognition that rAAV virion self-aggregation is a concern that may be addressed by manipulating ionic strength in any of the cited art.

Furthermore, in each of the Zolotukhin passages cited by the Examiner to evidence the teaching of excipients greater than 200 mM ionic strength, the rAAV is in the process of being purified. See, for example, Figure 1 where all of the steps using such buffers are purification steps. High salt concentrations are not used in the final preparation. Rather, the final AAV product is formulated in Lactated Ringer's buffer. See, column 12, lines 49-54, where virus is concentrated by centrifugation through a BIOMAX 100K filter and desalted into Lactated Ringer's. Only after this step is the virus considered "purified" in the disclosure and, as explained in the previous responses, Lactated Ringer's has an ionic strength of 138.5 mM. Applicants' purified product, on the other hand, has an ionic strength of at least 200 mM. There is absolutely no suggestion in Zolotukhin or any of the other cited references that a final preparation with an ionic strength of at least 200 mM and more than $1x10^{13}$ vg/ml is desirable or necessary.

Similarly, Atkinson fails to provide the requisite teaching or suggestion to arrive at the instant invention. The Office references Figures 13, 19, 32, 36 and 37 to evidence that excipients

are added to a preparation of AAV virions to achieve an ionic strength of at least 200 mM. However, as in Zolotukhin, there is no disclosure in Atkinson regarding adding excipients to a **purified** preparation of rAAV virions to achieve an ionic strength of at least 200 mM where the purified preparation with more than $1x10^{13}$ vg/ml. Indeed, as with Zolotukhin, **all** of the steps cited by the Office, wherein the buffers utilized may have an ionic strength of greater than 200mM, are steps mid-purification. Furthermore, as with all of the references cited by the Office, the purified AAV preparations disclosed in Atkinson are formulated in a final buffer consisting of Modified Ringer's Solution with 5% glycerol (for example, see Atkinson at column 54, lines 33-44). As noted before, Modified Ringer's Solution does not have an ionic strength of at least 200 mM.

Finally, the Office cites Figures 13, 32, 36 and 37 of Atkinson as relevant to the instant invention. These Figures pertain to the adjustment of ionic strength in cell culture media, well before purification, in order to promote release of the virus into the cell culture without cell lysis. See, columns 41-42 of Atkinson. Thus, Atkinson, as with Zolotukhin, fails to teach or suggest a method where excipients are added to a **purified** preparation of rAAV virions to achieve an ionic strength of at least 200 mM in order to prevent aggregation and to result in a purified preparation with more than $1x10^{13}$ vg/ml.

Drittanti also fails to teach or suggest these elements of the claimed invention. The Office argues rAAV virions are recovered/eluted by adding 300 mM NaCl through a 0-1M NaCl linear gradient in 100 mM HEPES buffer. However, as with Zolotukhin and Atkinson, this step is not a final purification step. Rather, as explained at page 61, second column, third full paragraph, the final preparation is in Ringer-lactate buffer, and as explained above, this buffer does not have an ionic strength of at least 200 mM. Drittanti, as with Zolotukhin and Atkinson, does not teach or suggest a method where excipients are added to a **purified** preparation of rAAV virions to achieve an ionic strength of at least 200 mM in order to prevent aggregation and to result in a purified preparation with more than $1x10^{13}$ vg/ml. Thus, none of the primary references teaches or suggests the claimed invention.

The secondary references of Qu and Chen fail to cure the defects of the primary references. Qu merely proposes a mechanism for AAV vector aggregation. The Examiner asserts Qu suggests that ion bridges between charged amino acids on the surface of vector

particles contribute to inter-particle interactions. At best, this teaching might lead one of skill in the art to adjust the pH of the final formulation, which Qu expressly teaches results "in reversal of concentration-induced vector aggregation". Alternatively, the skilled artisan might postulate that high concentrations of free amino acids could block vector particle interactions. However, as explained at page 9, lines 18-20 of the specification, it has been found that amino acids with charged side chains are not effective in preventing AAV2 vector aggregation beyond their contribution to ionic strength. Additionally, Qu specifically states that other types of interactions may play a role in aggregation and concludes: "In conjunction with further elucidation of the mechanism(s) of AAV vector aggregation, these observations will facilitate formulation development for optimal large-scale vector purification and clinical use." Thus, Qu explicitly states that further work is needed in order to provide a viable purification procedure.

Chen also does not make up for the failures of the primary references and Qu. Chen does not even relate to viruses, but rather pertains to methods for preventing aggregation of keratinocyte growth factor (KGF). There is absolutely no reason to believe that art directed to growth factors is in any way pertinent to virion production. Moreover, Chen used sulfated polysaccharides in combination with citrate to prevent aggregation. There is no suggestion in Chen to use citrate alone, or to carry out a purification protocol as claimed in order to prevent aggregation of rAAV virions and to result in a purified preparation with more than $1x10^{13}$ vg/ml of rAAV virions.

Finally, Wright does not provide the elements missing from Zolotukhin, Atkinson, Drittanti, Qu and Chen. Wright nowhere mentions that self-aggregation of rAAV virions occurs and hence does not teach or suggest methods for preventing aggregation of rAAA virions as claimed. Wright uses high salt concentrations initially to promote release of rAAV virions from cells and in some mid-purification steps. The only discussion in Wright regarding adding excipients to the final purified product is in Example 9. However, unlike applicants' purification method, the excipient added is phosphate-buffered saline, pH 7.1, containing 5% sorbitol. This buffer does not have an ionic strength of at least 200 mM.

Thus, Wright, as with all of the references discussed above, does not teach or suggest a method for preventing aggregation of rAAV virions where excipients are added to a purified

preparation of rAAV virions to achieve an ionic strength of at least 200 mM and to result in a purified preparation with more than $1x10^{13}$ vg/ml.

Applicants' process, on the other hand, provides a commercially viable method for producing high amounts of rAAV virions. As explained at pages 16-17 of the specification and in Table 2, vector recovery using applicants' claimed methods results in yields of more than 90%.

The combination cited by the Office does not provide evidence that the claimed invention is a "predictable use of prior art elements according to their established functions." *KSR Int'l Co. v. Teleflex, Inc.*, 82 USPQ2d 1385, 1396 (U.S. 2007). Rather, as explained above, the evidence is to the contrary.

Applicants submit the Examiner has chosen bits and pieces of the cited references to arrive at the allegation that this combination of references suggests the claimed invention. The very fact that the Examiner relies on six references in making the present obviousness rejection is evidence of the above. This is improper. As stated in *KSR*, "a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." *KSR*, page 1396. The Federal Circuit has consistently reversed a finding of obviousness, even when all claimed elements are individually present in the references. See, e.g., *In re Kotzab*, 55 USPQ2d 1313, 1317 (Fed. Cir. 2000). Thus, a rejection cannot be predicated on the mere identification of individual components of claimed limitations. Rather, particular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner.

For at least these reasons, withdrawal of the rejections under 35 U.S.C. § 103(a) is respectfully requested.

III. CONCLUSION

Applicants respectfully submit that the claims are now in condition for allowance and request early notification to that effect. The Examiner is encouraged to contact the undersigned if the Examiner notes any further matters which might be resolved by a telephone interview.

Respectfully submitted,

Date: 8/19/08

By: _____
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PTO/SB/30 (09-04)

Request for Continued Examination (RCE) Transmittal

Address to:
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Alexandria, VA 22313-1450

Application Number	11/141,996
Filing Date	June 1, 2005
First Named Inventor	John Fraser Wright et al.
Art Unit	1657
Examiner Name	Satyendra K. Singh
Attorney Docket Number	0800-0045

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. See Instruction Sheet for RCEs (not to be submitted to the USPTO) on page 2.

1. Submission required under 37 CFR 1.114 Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

   a. ☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.
      i. ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____
      ii. ☐ Other _____

   b. ☒ Enclosed
      i. ☒ Amendment/Reply
      ii. ☐ Affidavit(s)/ Declaration(s)
      iii ☐ Information Disclosure Statement (IDS)
      iv. ☐ Other _____

2. Miscellaneous

   a. ☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of ___months. (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

   b. ☐ Other _____

3. Fees The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.

   a. ☒ The Director is hereby authorized to charge the following fees, or credit any overpayments, to Deposit Account No. 18-1648. **I have enclosed a duplicate copy of this sheet.**
      i. ☒ RCE fee required under 37 CFR 1.17(e)
      ii. ☒ Extension of time fee (37 CFR 1.136 and 1.17)
      iii. ☒ Other **any fees not already included in the attached check**

   b. ☐ Check in the amount of $ _____ enclosed

   c. ☒ Payment by credit card

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization of PTO-2038.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

Signature		Date	December 8, 2009
Name (Print /Type)	Roberta L. Robins	Registration No.	33,208

CERTIFICATE OF MAILING OR TRANSMISSION

I hereby certify pursuant to 37 CFR §1.8 that this correspondence is being transmitted via EFS to the United States Patent and Trademark Office on the date shown below.

Signature			
Name (Print /Type)	Tanisha Lawrence-Caceres	Date	December 8, 2009

Atty Dkt No: 0800-0045
PATENT

CERTIFICATE OF TRANSMISSION PURSUANT TO 37 CFR § 1.8

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Date 12/06/2009 Signature 

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

JOHN FRASER WRIGHT et al. Confirmation No.: 5399

Application No.: 11/141,996 Art Unit: 1657

Filing Date: June 1, 2005 Examiner: Satyendra K. Singh

Title: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

SUBMISSION UNDER 37 CFR 1.114

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

This Submission under 37 C.F.R. §1.114 is being filed in response to the Final Office Action mailed November 18, 2008. In lieu of filing an Appeal Brief, a Request for Continued Examination is being filed concurrently herewith. A request for an extension of time, as well as the requisite fee accompanies this submission. Applicants request reconsideration of the above-referenced patent application in view of the following amendments and remarks.

A **listing of claims** begins on page 2 of this paper.

Remarks begin on page 4 of this paper.

I. AMENDMENT

Amendments to the Claims:

The following listing reflects amendments to the claims and replaces all prior versions and listings of claims in this application.

1. (Currently amended) A method of preventing aggregation of recombinant adeno-associated virus (rAAV) virions in a preparation of rAAV virions, comprising:

1) providing a lysate comprising rAAV virions;

2) purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography, wherein said virions are purified; and

3) adding one or more excipients to said purified virions to produce a preparation of virions with an ionic strength of at least 200 mM, wherein the concentration of purified rAAV virions in said preparation exceeds $1x10^{13}$ vg/ml up to $6.4x10^{13}$ vg/ml.

2. (Canceled)

3. (Previously presented) The method of claim 1, further comprising treating said purified virions with a nuclease.

4. (Previously presented) The method of claim 3, wherein the nuclease is an endonuclease from *Serratia marcescens* (Benzonase®).

5. (Original) The method of claim 1, wherein one or more of the excipients comprises a multivalent ion.

6. (Previously presented) The method of claim 5, wherein the multivalent ion is citrate.

7. (Previously presented) The method of claim 1, wherein the osmolarity of the preparation of virions after addition of the one or more excipients is no greater than about 280mOsm.

8. (Original) The method of claim 1, wherein, after addition of the one or more excipients, the average particle radius (Rh) of the virions in the preparation of virions is less than about 20nm as measured by dynamic light scattering.

9. (Previously presented) The method of claim 1, wherein, after addition of the one or more excipients, recovery of the virions is at least about 90% following filtration of the preparation of virions through a 0.22μm filter.

10-20. (Canceled)

21. (Previously presented) The method of claim 3, wherein rAAV virions are purified from the lysate using cesium chloride gradient ultracentrifugation.

22. (Previously presented) The method of claim 3, wherein rAAV virions are purified from the lysate using cation exchange chromatography.

23. (Previously presented) The method of claim 3, wherein rAAV virions are purified from the lysate using cation exchange chromatography and cesium chloride gradient ultracentrifugation.

24. (Previously presented) The method of claim 3, further comprising diafiltering the purified rAAV virions to achieve an ionic strength of at least 200 mM.

II. REMARKS

Introductory Comments

Claims 1, 3-9 and 21-24 were examined in the Final Office Action dated November 18, 2008 and stand rejected under 35 U.S.C. §112, first paragraph and 35 U.S.C. §103(a). These rejections are believed to be overcome by this response and are otherwise traversed for reasons discussed in detail below.

Overview of the Above Amendments

Claim 1 has been amended to recite that the concentration of purified rAAV virions in the preparation exceeds $1x10^{13}$ vg/ml "up to $6.4x10^{13}$ vg/ml." Support for this recitation can be found throughout the specification at, for example, page 6, lines 5-6.

The foregoing amendment is made without prejudice, without intent to abandon any originally claimed subject matter, and without intent to acquiesce in any rejection of record. Applicants expressly reserve the right to file one or more continuing applications containing the unamended claims.

Rejection Under 35 U.S.C. §112, First Paragraph

Claims 1, 3-9 and 21-24 were rejected under 35 U.S.C. §112, first paragraph as failing to comply with the written description requirement. In particular, the Office asserts the concentration of purified AAV virions in the preparation can exceed "$1x10^{13}$ vg/ml and up to infinity." Office Action, page 3. Solely in an effort to advance prosecution, claim 1 has been amended to recite that the concentration of purified rAAV virions in the preparation exceeds $1x10^{13}$ vg/ml up to $6.4x10^{13}$ vg/ml. Thus, this basis for rejection has been overcome and withdrawal thereof is respectfully requested.

Rejections Under 35 U.S.C. §103(a)

Claims 1, 3-9 and 21-24 remain rejected under 35 U.S.C. §103(a) as being unpatentable over U.S. Patent No. 6,146,874 to Zolotukhin et al. ("Zolotukhin"), taken with U.S. Patent No. 6,566,118 to Atkinson et al. ("Atkinson") and Drittanti et al, *J. Gene Med.* (2001) 3:59-71

("Drittanti'); in view of Qu et al., *Molec. Ther.* (2003) 5:S348, Abstract 901 ("Qu") and Chen et al., *J. Pharm. Sci.* (1994) 83:1657-1661 ("Chen"), and further in view of U.S. Patent No. 6,593,123 to Wright et al. ("Wright").

The Office argues each of the references of Zolotukhin, Atkinson and Drittanti teach a method step for the treatment of rAAV virions with a nuclease and that process steps such as ultracentrifugation, chromatography and diafiltration are used and disclosed by each of these references. Office Action, page 5. The Examiner acknowledges methods of preventing aggregation by adding a citrate are not disclosed by the primary references. Office Action, page 6. Qu is cited for disclosing problems associated with concentration-induced aggregation of rAAV virions and Chen is cited for describing strategies to suppress aggregation of proteins. Office Action, page 6. Finally, Wright is cited for teaching a method for large scale production of rAAV virions and the use of a nuclease. Office Action, page 9. However, Applicants submit the cited combination does not render the claimed invention obvious.

The Office disputes Applicants' previous arguments alleging "the combined teachings in the cited prior art references of record disclose all the elements, including the recognition of the problem of 'self aggregation' of rAAV virions associated with purified preparations of said virions." Office Action, page 11. However, this statement is in error. Applicants submit that all elements of the claimed invention are not taught or suggested by the cited combination. To reiterate, all pending claims pertain to methods of preventing aggregation of rAAV virions in a **purified** preparation. The rAAV virions are purified from a lysate using ultracentrifugation and/or chromatography. One or more excipients are then added to achieve an ionic strength of at least 200 mM and the concentration of purified rAAV virions in the preparation exceeds $1x10^{13}$ up to $6.4x10^{13}$ vg/ml. In certain embodiments, the purified preparation is treated with a nuclease. None of the cited art, either alone or in combination, teaches or suggests adding one or more excipients to a **purified** rAAV virion preparation to achieve an ionic strength of at least 200 mM wherein the preparation includes more than $1x10^{13}$ up to $6.4x10^{13}$ vg/ml, as claimed. For this reason alone, the cited combination fails to render the claims obvious.

As previously explained, Zolotukhin does not recognize that rAAV virions self-aggregate and therefore does not provide any suggestions regarding a method for preventing aggregation of rAAV virions as claimed. This is particularly noteworthy in view of Zolotukhin's recognition

(see column 15, lines 22-35) that AAV aggregates with **proteins** in the cell lysate wherein high salt in the **first step** of the iodixanol gradient is used to destabilize these AAV-lysate protein interactions. Moreover, Applicants assert that this passage expressly teaches away from Applicants' invention because Zolotukhin purposely eliminates high salt concentrations from the remainder of the iodixanol gradient, including the gradient from which the rAAV is collected after centrifugation, because the elimination of high salt is important for subsequent purification steps.

Applicants further note that even though Zolotukhin recognizes AAV virion-lysate protein aggregation as a problem, the reference does not teach or suggest that self-aggregation of rAAV virions is a problem at all, let alone a problem in **purified**, concentrated AAV preparations. In fact, absent Applicants' teaching regarding this problem, there is no recognition that rAAV virion self-aggregation is a concern that may be addressed by manipulating ionic strength in any of the cited art.

Furthermore, in each of the Zolotukhin passages cited by the Examiner to evidence the teaching of excipients greater than 200 mM ionic strength, the rAAV is in the process of being purified. See, for example, Figure 1 where all of the steps using such buffers are purification steps. High salt concentrations are not used in the final preparation. Rather, the final AAV product is formulated in Lactated Ringer's buffer. See, column 12, lines 49-54, where virus is concentrated by centrifugation through a BIOMAX 100K filter and desalted into Lactated Ringer's. Only after this step is the virus considered "purified" in the disclosure and, as explained in the previous responses, Lactated Ringer's has an ionic strength of 138.5 mM. Applicants' purified product, on the other hand, has an ionic strength of at least 200 mM. There is absolutely no suggestion in Zolotukhin or any of the other cited references that a final preparation with an ionic strength of at least 200 mM and more than $1x10^{13}$ up to $6.4x10^{13}$ vg/ml is desirable or necessary.

Similarly, Atkinson fails to provide the requisite teaching or suggestion to arrive at the instant invention. The Office points to Figures 13, 19, 32, 36 and 37 to evidence that excipients are added to a preparation of AAV virions to achieve an ionic strength of at least 200 mM. Office Action, page 5. However, these figures are not relevant to the present invention. These Figures pertain to the adjustment of ionic strength in cell culture media, well before purification,

in order to promote release of the virus into the cell culture without cell lysis. See, columns 41-42 of Atkinson. Additionally, as in Zolotukhin, there is no disclosure in Atkinson regarding adding excipients to a **purified** preparation of rAAV virions to achieve an ionic strength of at least 200 mM where the concentration of virions in the purified preparation is more than $1x10^{13}$ vg/ml up to $6.4x10^{13}$. Indeed, as with Zolotukhin, **all** of the steps cited by the Office where the buffers utilized may have an ionic strength of greater than 200mM, are steps mid-purification. Furthermore, as with all of the references cited by the Office, the purified AAV preparations disclosed in Atkinson are formulated in a final buffer consisting of Modified Ringer's Solution with 5% glycerol (for example, see Atkinson at column 54, lines 33-44). As noted before, Modified Ringer's Solution does not have an ionic strength of at least 200 mM.

Thus, Atkinson, as with Zolotukhin, fails to teach or suggest a method where excipients are added to a **purified** preparation of rAAV virions to achieve an ionic strength of at least 200 mM in order to prevent aggregation and to result in a purified preparation with more than $1x10^{13}$ up to $6.4x10^{13}$ vg/ml.

Drittanti also fails to teach or suggest these elements of the claimed invention. The Office argues rAAV virions are recovered/eluted by adding 300 mM NaCl through a 0-1M NaCl linear gradient in 100 mM HEPES buffer. Office Action, page 5. However, as with Zolotukhin and Atkinson, this step is not a final purification step. Rather, as explained at page 61, second column, third full paragraph, the final preparation is in Ringer-lactate buffer, and as explained above, this buffer does not have an ionic strength of at least 200 mM. Drittanti, as with Zolotukhin and Atkinson, does not teach or suggest a method where excipients are added to a **purified** preparation of rAAV virions to achieve an ionic strength of at least 200 mM in order to prevent aggregation and to result in a purified preparation with more than $1x10^{13}$ up to $6.4x10^{13}$ vg/ml. Thus, none of the primary references teaches or suggests the claimed invention.

The secondary references of Qu and Chen fail to cure the defects of the primary references. The Examiner states that Qu in view of Chen provides the motivation for using high salt concentration in purified vector stocks. Office Action, page 11. However, Qu merely proposes a mechanism for AAV vector aggregation. The Examiner further asserts Qu suggests that ion bridges between charged amino acids on the surface of vector particles contribute to inter-particle interactions. Office Action, page 6. At best, this teaching might lead one of skill in

the art to adjust the pH of the final formulation, which Qu expressly teaches results "in reversal of concentration-induced vector aggregation". Alternatively, the skilled artisan might postulate that high concentrations of free amino acids could block vector particle interactions. However, as explained at page 9, lines 18-20 of the instant specification, it has been found that amino acids with charged side chains are not effective in preventing AAV2 vector aggregation beyond their contribution to ionic strength. Additionally, Qu specifically states that other types of interactions may play a role in aggregation and concludes: "In conjunction with further elucidation of the mechanism(s) of AAV vector aggregation, these observations will facilitate formulation development for optimal large-scale vector purification and clinical use." Thus, Qu explicitly states that further work is needed in order to provide a viable purification procedure.

As for Chen, this reference does not relate to viruses, but rather pertains to methods for preventing aggregation of keratinocyte growth factor (KGF). There is absolutely no reason to believe that art directed to growth factors is in any way pertinent to virion production. Moreover, Chen used sulfated polysaccharides in combination with citrate to prevent aggregation. There is no suggestion in Chen to use citrate alone, or to carry out a purification protocol as claimed in order to prevent aggregation of rAAV virions and to result in a purified preparation with more than $1x10^{13}$ up to $6.4x10^{13}$ vg/ml of rAAV virions.

Finally, although Wright discusses nuclease treatment, Wright does not provide the other elements missing from Zolotukhin, Atkinson, Drittanti, Qu and Chen. Wright nowhere mentions that self-aggregation of rAAV virions occurs and hence does not teach or suggest methods for preventing aggregation of rAAA virions as claimed. Wright uses high salt concentrations initially to promote release of rAAV virions from cells and in some mid-purification steps. The only discussion in Wright regarding adding excipients to the final purified product is in Example 9. However, unlike Applicants' purification method, the excipient added is phosphate-buffered saline, pH 7.1, containing 5% sorbitol. This buffer does not have an ionic strength of at least 200 mM.

Thus, Wright, as with all of the references discussed above, does not teach or suggest a method for preventing aggregation of rAAV virions where excipients are added to a purified preparation of rAAV virions to achieve an ionic strength of at least 200 mM and to result in a purified preparation with more than $1x10^{13}$ up to $6.4x10^{13}$ vg/ml .

Applicants' process, on the other hand, provides a commercially viable method for producing high amounts of rAAV virions. As explained at pages 16-17 of the specification and in Table 2, vector recovery using Applicants' claimed methods results in yields of more than 90%.

Applicants continue to submit the Examiner has chosen bits and pieces of the cited references to arrive at the allegation that this combination of references suggests the claimed invention. It is axiomatic that statements in the prior art must be considered in the context of the teaching of the entire reference, and that rejection of claims **cannot** be predicated on mere identification in a reference of individual components of claimed limitations. In this regard, the Federal Circuit has consistently reversed a finding of obviousness, even when all claimed elements are individually present in the references. *See, e.g., In re Kotzab*, 55 USPQ2d 1313, 1317 (CAFC 2000, emphasis added):

> While the test for establishing an implicit teaching, motivation or suggestion is what the combination of these two statements [in the reference] would have suggested to those of ordinary skill in the art, the two statements cannot be viewed in the abstract. Rather, they must be considered in the context of the teaching of the entire reference. Further, a rejection **cannot** be predicated on the mere identification [in the reference] of individual components of claimed limitations. Rather, particular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed.

Virtually all inventions are combinations of elements that can be individually identified in multiple references. See, e.g., *In re Rouffet*, 47 USPQ2d 1453 (Fed. Cir. 1998), noting that the Office cannot rely on a high level of skill in the art to overcome the differences between the selected elements in the references, it cannot rely on a high level of skill in the art to provide the necessary motivation; *In re Lee*, 61 USPQ2d 1430 (Fed. Cir. 2002), affirming that common knowledge and common sense are not the specialized knowledge and expertise necessary to establish a motivation to arrive at the claimed invention.

Thus, the requirement is not whether each claimed element can be identified individually in a reference but, rather, whether the Examiner can show "reasons that the skilled artisan, confronted with the same problem as the inventor, and with no knowledge of the claimed invention, would select the elements from the cited prior art reference for combination in the

manner claimed." *In re Rouffet*, 47 USPQ2d at 1458. In the pending case, the Office has not met this burden.

As explained in Section 2143.01 of the MPEP, the mere fact that references can be combined or modified does not render the resultant combination obvious, unless the prior art also suggests the desirability of the combination. Since the suggestion or motivation to combine the references to arrive at the claimed invention is not in the references, the Examiner is required to cite to some knowledge generally available to one of ordinary skill in the art for the motivation to combine the references. It is respectfully submitted that the Examiner has not provided such knowledge. Instead, the Examiner has merely asserted that it would have been obvious to combine the various methods of the cited art to arrive at Applicants' specifically claimed method for preventing aggregation of rAAV virions in a purified preparation.

For at least these reasons, withdrawal of the rejections under 35 U.S.C. § 103(a) is respectfully requested.

III. CONCLUSION

Applicants respectfully submit that the claims are now in condition for allowance and request early notification to that effect. The Examiner is encouraged to contact the undersigned if the Examiner notes any further matters which might be resolved by a telephone interview.

Respectfully submitted,

Date: 12/8/09

By: _____
Roberta L. Robins
Registration No. 33,208

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UNITED STATES PATENT AND TRADEMARK OFFICE

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NOTICE OF ALLOWANCE AND FEE(S) DUE

20855 7590 12/18/2009

ROBINS & PASTERNAK
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SUITE 230
PALO ALTO, CA 94303

EXAMINER	
SINGH, SATYENDRA K	
ART UNIT	PAPER NUMBER
1657	

DATE MAILED: 12/18/2009

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/141,996	06/01/2005	John Fraser Wright	0800-0045	5399

TITLE OF INVENTION: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	YES	$755	$300	$0	$1055	03/18/2010

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the SMALL ENTITY status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is the same, pay the TOTAL FEE(S) DUE shown above.

B. If the status above is to be removed, check box 5b on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above, or

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check box 5a on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and 1/2 the ISSUE FEE shown above.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
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INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

20855 7590 12/18/2009

ROBINS & PASTERNAK
1731 EMBARCADERO ROAD
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PALO ALTO, CA 94303

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)

(Signature)

(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/141,996	06/01/2005	John Fraser Wright	0800-0045	5399

TITLE OF INVENTION: COMPOSITIONS AND METHODS TO PREVENT AAV VECTOR AGGREGATION

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	YES	$755	$300	$0	$1055	03/18/2010

EXAMINER	ART UNIT	CLASS-SUBCLASS
SINGH, SATYENDRA K	1657	435-239000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____

2 _____

3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:

❑ Issue Fee

❑ Publication Fee (No small entity discount permitted)

❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**

❑ A check is enclosed.

❑ Payment by credit card. Form PTO-2038 is attached.

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)

❑ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.

❑ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/141,996	06/01/2005	John Fraser Wright	0800-0045	5399

20855 7590 12/18/2009

ROBINS & PASTERNAK
1731 EMBARCADERO ROAD
SUITE 230
PALO ALTO, CA 94303

EXAMINER	
SINGH, SATYENDRA K	
ART UNIT	PAPER NUMBER
1657	

DATE MAILED: 12/18/2009

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 0 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 0 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

Notice of Allowability	Application No.	Applicant(s)	
	11/141,996	WRIGHT ET AL.	
	Examiner	Art Unit	
	SATYENDRA K. SINGH	1657	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *12/8/09*.

2. ☒ The allowed claim(s) is/are *1,3,4,6-9 and 21-24*.

3. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☐ All b) ☐ Some* c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____.
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

4. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
      1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
   (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
3. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
5. ☐ Notice of Informal Patent Application
6. ☒ Interview Summary (PTO-413), Paper No./Mail Date *12/14/09*.
7. ☒ Examiner's Amendment/Comment
8. ☒ Examiner's Statement of Reasons for Allowance
9. ☒ Other *See Continuation Sheet*.

Continuation of Attachment(s) 9. Other: A DRAFT copy of the faxed Exam. Amend. is attached..

Examiner-Initiated Interview Summary	Application No.	Applicant(s)	
	11/141,996	WRIGHT ET AL.	
	Examiner	Art Unit	
	SATYENDRA K. SINGH	1657	

All Participants:

(1) *SATYENDRA K. SINGH*.

(2) *ROBERTA L. ROBINS (ATTORNEY OF RECORD)*.

Status of Application: *AFTER RCE*

(3) _____.

(4) _____.

Date of Interview: *10 December 2009*

Time: *4:45 PM*

Type of Interview:
- ☒ Telephonic
- ☐ Video Conference
- ☐ Personal (Copy given to: ☐ Applicant ☐ Applicant's representative)

Exhibit Shown or Demonstrated: ☒ Yes ☐ No
If Yes, provide a brief description: *see attached DRAFT exam. amendment faxed to applicants on 12/10/09.*

Part I.

Rejection(s) discussed:
N/A

Claims discussed:
1,5,6

Prior art documents discussed:
N/A

Part II.

SUBSTANCE OF INTERVIEW DESCRIBING THE GENERAL NATURE OF WHAT WAS DISCUSSED:
See Continuation Sheet

Part III.

☒ It is not necessary for applicant to provide a separate record of the substance of the interview, since the interview directly resulted in the allowance of the application. The examiner will provide a written summary of the substance of the interview in the Notice of Allowability.

☐ It is not necessary for applicant to provide a separate record of the substance of the interview, since the interview did not result in resolution of all issues. A brief summary by the examiner appears in Part II above.

/Satyendra K. Singh/
Examiner, Art Unit 1657

(Applicant/Applicant's Representative Signature – if appropriate)

Continuation of Substance of Interview including description of the general nature of what was discussed: Applicant's representative of record Miss Roberta L. Robins (phone- 650-493-3400; fax no. 650-493-3440) was telephonically contacted by the examiner, and provided with a proposed Examiner's Amendment (sent by fax on 12/10/09; see attached DRAFT Exam. Amend. attached) to the pending claims (that have been found to be allowable through an in house patentability conference with the Primary examiner, Sandy Saucier and SPE, Michael Wityshyn) for applicant's considerations. Applicant's attorney of record called the examiner (on 12/14/09; at 11:45 AM) and informed that applicant's have accepted the proposed Examiner's Amendment to the claims.

/Satyendra K. Singh/
Examiner, AU1657

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on **12/08/2009** has been entered.

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Roberta L. Robbins (ATTORNEY OF RECORD) on December 14th 2009.

The application has been amended as follows:

In The Claims

Claim 5 has been canceled by this Examiner's amendment.

Claims 1, 3, 4, 6-9 and 21-24 have been allowed by this Examiner's amendment. Claims 1, 4, 6, 7, 8 and 9 have been amended as follows:

1. A method of preventing aggregation of recombinant adeno-associated virus (rAAV) virions in a purified preparation of rAAV virions, comprising:
1) providing a lysate comprising rAAV virions;

2) purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography, wherein said virions are purified; and
3) adding one or more ~~excipients~~ salts of multivalent ions selected from the group consisting of citrate, phosphate, sulfate and magnesium to said purified virions to produce a preparation of virions with an ionic strength of at least 200 mM, wherein the concentration of purified rAAV virions in said preparation exceeds $1x10^{13}$ vg/ml up to $6.4x10^{13}$ vg/ml; and wherein the pH of the purified preparation of rAAV virions is between 7.5 and 8.0.

4. The method of claim 3, wherein the nuclease is an endonuclease from *Serratia marcescens* ~~(Benzonase®)~~.

6. The method of claim ~~5~~ 1, wherein the multivalent ion is citrate.

7. The method of claim 1, wherein the osmolarity of the preparation of virions after addition of the one or more ~~excipients~~ salts of multivalent ions is no greater than about 280mOsm.

8. The method of claim 1, wherein, after addition of the one or more ~~excipients~~ salts of multivalent ions, the average particle radius (Rh) of the virions in the preparation of virions is less than about 20nm as measured by dynamic light scattering.

9. The method of claim 1, wherein, after addition of the one or more ~~excipients~~ salts of multivalent ions, recovery of the virions is at least about 90% following filtration of the preparation of virions through a 0.22μm filter.

The following is an examiner's statement of reasons for allowance:

The amended claims (see instant claim 1 as amended and allowed) were found to be unobvious over the cited art as the combined teachings of the prior art references do not suggest the use of salts of multivalent ions such as citrate, phosphate, sulfate or magnesium at a pH range of 7.5 to 8.0, and an ionic strength of at least 200 mM for preventing aggregation of purified rAAV vector genome and/or rAAV particle preparations. As also argued by applicants (see remarks, pages 5-6, in particular), the cited prior art wants to reduce the high salt concentrations in purified viral stock solutions, and therefore do not suggest the use of multivalent ions at an ionic strength of

at least 200mM at pH 7.5-8.0, which is the desired pH for normal physiological or clinical use of such viral vector preparations. Applicants have provided superior results and appropriate evidentiary data in the instant specification (see table 1, figures 1-2, in particular) to support the amendments in the instant claims, which are not made obvious by the combined teachings in the cited prior art.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SATYENDRA K. SINGH whose telephone number is (571)272-8790. The examiner can normally be reached on 9-5MF.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Manjunath N. Rao can be reached on 571-272-0939. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Sandra Saucier/
Primary Examiner, Art Unit 1651

/Satyendra K. Singh/
Examiner, Art Unit 1657