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.

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: _____

Respectfully submitted,

SIGNATURE M Christina Thomson

Date 01 June 2004

REGISTRATION NO. 43,190 *(if appropriate)*

Docket Number: 1039.PROV

TYPED or PRINTED NAME Mary Christina Thomson

TELEPHONE 510-748-7150

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.

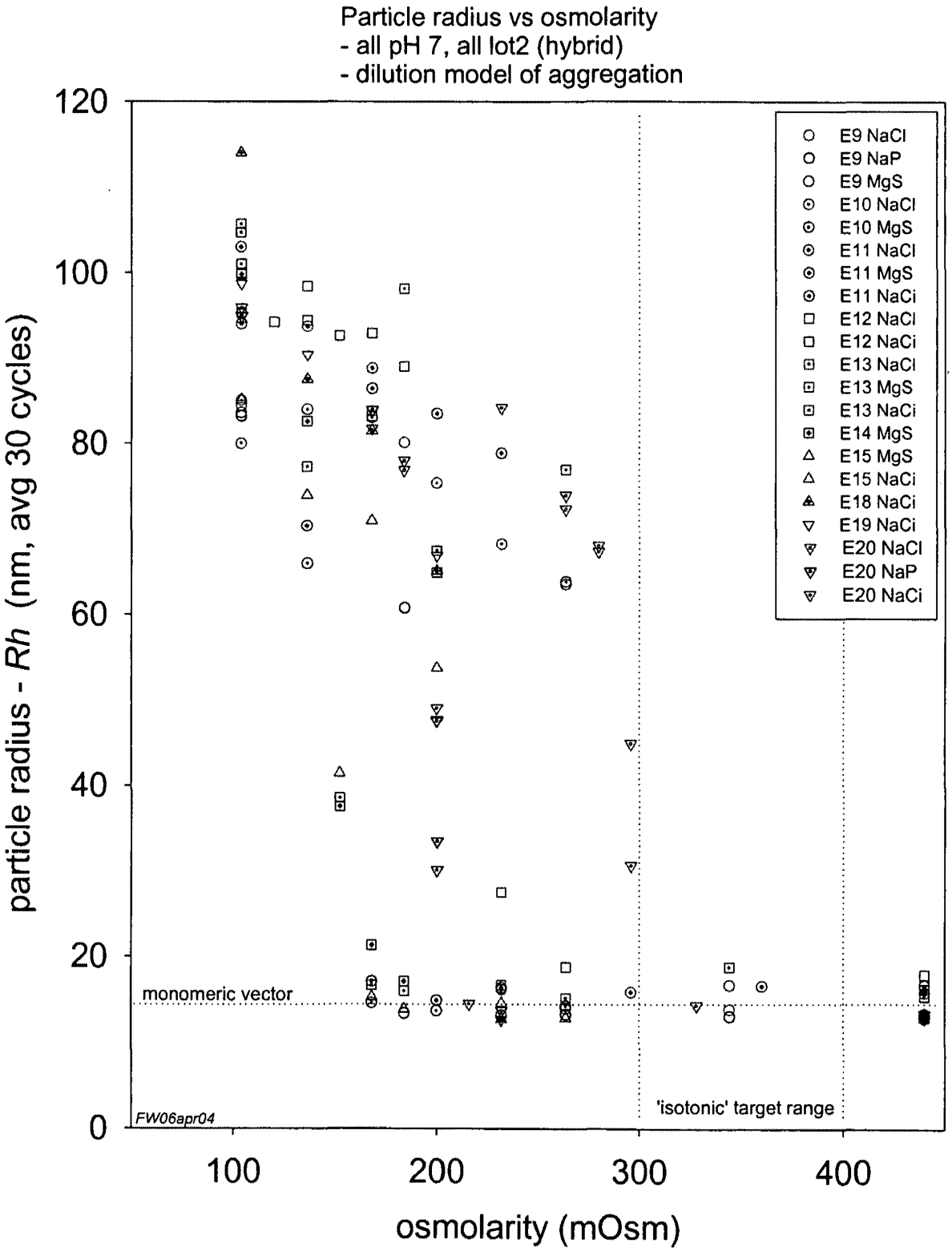
Appendix A

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

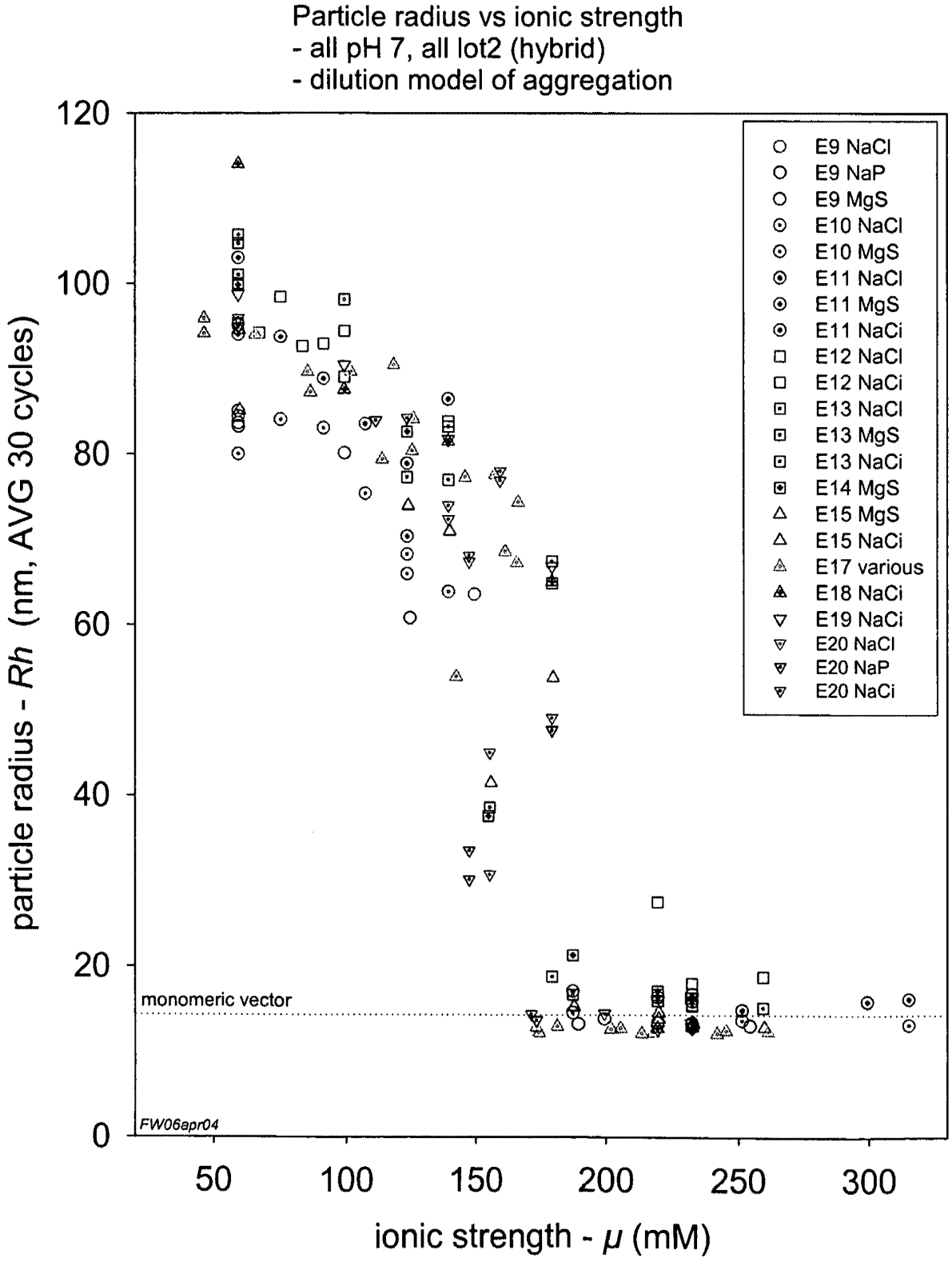
Appendix B

Effect of osmolarity on AAV vector aggregation using various charged species



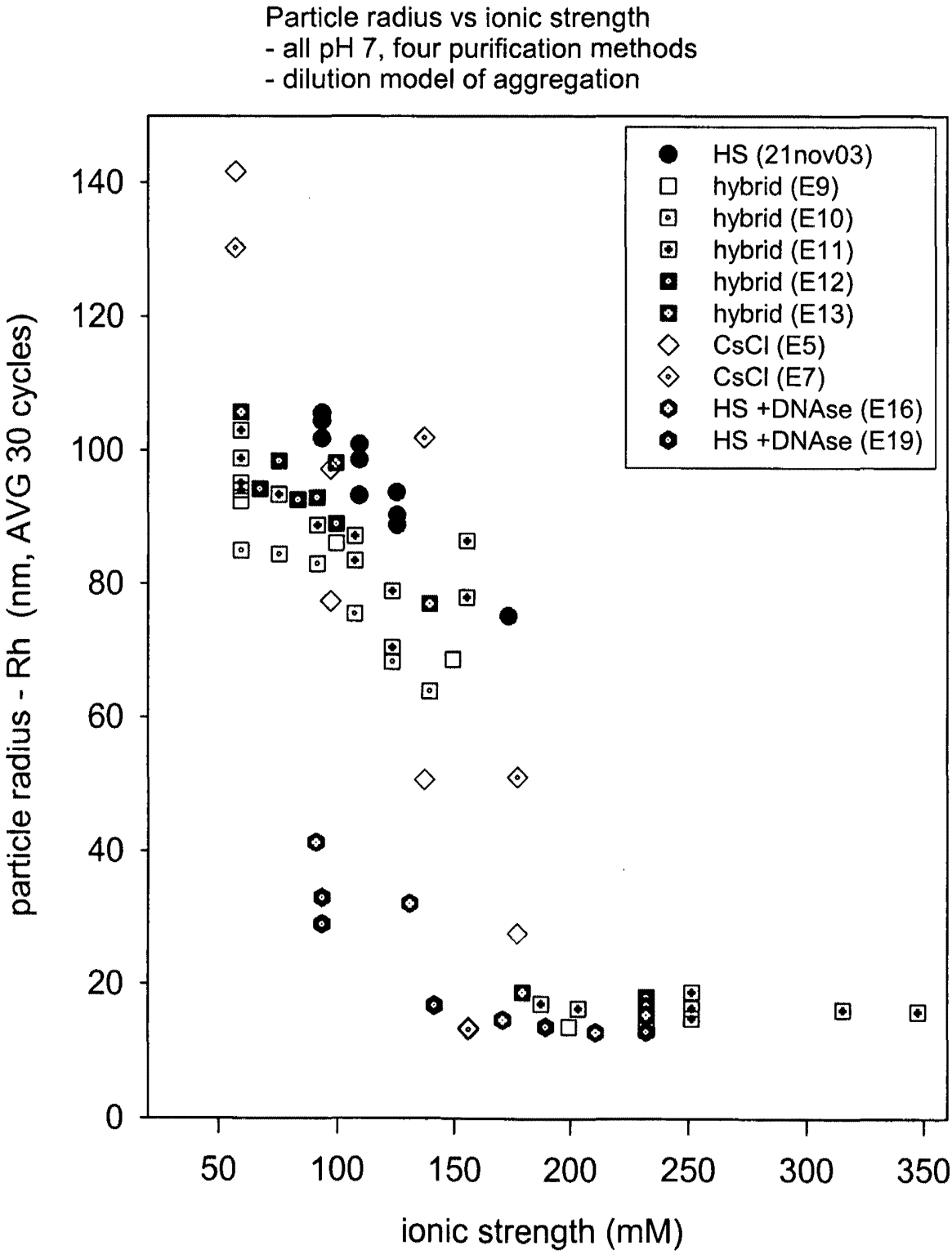
Appendix C

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