Manufacturing process optimization of ADMA Biologics' intravenous immunoglobulin products, BIVIGAM[®] and ASCENIV[™]

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Humoral immunodeficiency patients require immunoglobulin replacement to prevent infection. Traditional intravenous immunoglobulin manufacturing methods have had the potential for containing impurities caused by physical, chemical and thermal stressors that alter proteins. Two intravenous immunoglobulin products, BIVIGAM[®] and ASCENIV[™], are manufactured by a modified Cohn-Oncley fractionation method followed by chromatographic purification. These products have undergone a systematic quality by design optimization to identify critical manufacturing processes to produce the highest quality product. This data driven, small-scale approach has led to manufacturing enhancements that have yielded consistent product improvements. The systematic approach to optimizing manufacturing has guided process changes, in-process, procedural and engineering controls that have reduced protein shearing and aggregation, and improved purity resulting in products with lot-to-lot consistency.

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Primary immunodeficiency diseases (PIDDs) are a collection of disorders that result in deficient antibody production due to abnormalities of multiple cell lineages in the adaptive and innate immune response. Supplementation with immunoglobulins (IG) is considered to be the standard of care for the treatment of patients with humoral deficiencies that reflect B and T cell abnormalities [1–7]. Within this population, regular infusions with IG prevents most of the serious bacterial infections but often fails to provide sufficient antibody supplementation against viral pathogens to which these patients are susceptible [8,9]. In the USA, there are multiple IG products currently approved that meet the minimum standards for concentration of antibodies set by the US FDA guidance 21 CFR 640 subpart J [10]. These requirements include standards for concentration of antibodies to diphtheria toxoid (≥ 1.21 U/ml), measles (≥ 0.60 CBER reference) and polio (≥ 0.28 CBER reference). Although the FDA defines minimum antibody concentration for these pathogens, standards for antibodies against other common pathogens are not defined, resulting in significant variation among the different commercial IG products in antibody titers to important pathogens [11,12]. Dosing for therapeutic polyclonal intravenous immunoglobulin (IVIG) products is considered to be patient specific, with an increase in dose associated with decreased incidence of infection and improved outcomes [4,5,13,14]. In part due to the variability in antibody composition between IVIG and the lack of standardization requirements for common pathogens, PIDD patients still experience an increased risk of infection while receiving standard-of-care treatment with IVIG.

ADMA Biologics' IVIG products BIVIGAM[®] (intravenous immune globulin, human 10% liquid), and ASCENIV[™] (immune globulin intravenous – slra Human, 10% liquid, formerly known as RI-002) are produced in ADMA's manufacturing facility using similar methodologies; however, these two products have undergone

manufacturing process optimization to increase consistency, purity and stability. BIVIGAM, an IVIG product originally developed by Biotest Pharmaceutical Corporation (Biotest, Dreieich, Germany), was manufactured from source plasma [1]. Over time, it was found to have multiple manufacturing inconsistencies and control problems that led to FDA observations resulting in FDA issuing a warning letter to Biotest Pharmaceuticals Corporation in November 2014, citing observations relating to filter clogging, proteinaceous particulates and overall product robustness among other GMP identified issues. BIVIGAM was voluntarily withdrawn from the commercial market by Biotest in December 2016. ADMA and Biotest announced that the acquisition by ADMA of the Biotest Therapy Business Unit (BTBU) in January 2017 and ADMA committed to address these manufacturing deficiencies and other related FDA concerns. ADMA acquired the BTBU on June 6, 2017 and initiated a data driven approach for optimizing the IVIG manufacturing process that would bring BIVIGAM into complete FDA compliance, allowing its reintroduction into the US commercial marketplace.

ASCENIV is manufactured based on patented methodologies. Plasma donors are selected using proprietary testing and the plasma pools are formed that are differentiated from normal source plasma, which is used to manufacture other commercially available IVIG products. The patents which describe the source plasma requirements, and tailored plasma pooling design was initially developed to address the variability seen in neutralizing antibody titers of respiratory pathogens that had previously been observed in commercial IVIG lots. The plasma pooling approaches that are also used to meet the FDA guidance requirements for therapeutics to treat PIDD patients as defined in 21CFR640 subpart J. Specifically, ASCENIV is manufactured through a patented process using source plasma, which is acquired from donors screened using a microneutralization assay to detect and identify which donors possess naturally occurring neutralizing antibody titers to respiratory syncytial virus (RSV) [ADMA BIOLOGICS, DATA ON FILE]. The resulting IG product is monitored by in-process testing controls to document consistent titers of neutralizing anti-RSV antibodies.

ASCENIV & BIVIGAM clinical efficacy & safety

The clinical efficacy and safety of ASCENIV was investigated in a prospective, open-label, single-arm and multicenter clinical trial conducted in 59 (28 male, 31 female) subjects with PIDD and who were receiving a stable dose of IVIG replacement therapy (defined as no change >50% in mean dose) between 300 and 800 mg/kg for at least 3 months prior to study entry and who were 3–74 years of age, inclusive [15]. Subjects were given intravenous infusions of ASCENIV at varying doses ranging from 284 to 1008 mg/kg every 3–4 weeks. Doses were individualized for subjects based on prestudy schedules up to approximately 1 year prior to study entry, and were adjusted to maintain trough IgG concentrations >500 mg/dl throughout the study. The study population included subjects with common variable immunodeficiency, x-linked agammaglobulinemia, antibody deficiencies and other primary immunodeficiency diagnoses.

The primary efficacy end point for this study was the rate of acute serious bacterial infection (aSBI) including bacterial pneumonia, bacteremia/septicemia, osteomyelitis/septic arthritis, visceral abscess and bacterial meningitis during the 12-month treatment period. Clinical success was defined as an aSBI rate less than 1.0 infection per person-year in accordance with FDA guidance. During the trial, zero aSBIs were observed during 55.9 person-years of treatment. Secondary end points included the rate of infections of any kind (3.4 infections per subject per year), number of days lost from school, daycare, or work due to infection (1.7 days per subject per year), unscheduled medical visits due to infections (0.97 visits per subject per year), hospitalizations due to infection (0.018 hospitalizations per subject per year) and antibiotic use due to infection (32.9 days per subject per year). ASCENIV was well tolerated and the AEs reported in ≥5% of subjects included headache, sinusitis, diarrhea, viral gastroenteritis, nasopharyngitis, upper respiratory tract infection, bronchitis and nausea. No study drug-related serious adverse events were reported.

BIVIGAM was previously evaluated by Biotest Pharmaceuticals Corporation for clinical efficacy and safety in a similarly designed prospective, open-label, single-arm and multicenter clinical trial conducted in 63 (31 male and 32 female) subjects with PI and who were receiving a stable dose of IVIG replacement therapy between 300 and 800 mg/kg for at least 3 months prior to study entry and who were 6–75 years of age, inclusive [16,17]. Subjects were given intravenous infusions of BIVIGAM at varying doses ranging from 254 to 1029 mg/kg every 3–4 weeks. During the 12-month duration of the trial, two aSBIs (both bacterial pneumonia) were observed in two subjects for an aSBI rate of 0.037. BIVIGAM was also well tolerated and the AEs reported in ≥5% of subjects included headache, sinusitis, fatigue, upper respiratory tract infection, diarrhea, cough, bronchitis, pyrexia and nausea.

Table 1. Critical process changes between original and optimized intravenous immunoglobulin manufacturing process.

Process step	Original IVIG process	Optimized IVIG process
	Tempering of source plasma	Tempering of source plasma
1	Pooling and thawing of plasma	Pooling and thawing of plasma
	Separation of cryoprecipitate	Separation of cryoprecipitate
2	Precipitation/separation of Fraction I	Precipitation/separation of Fraction I
3	Precipitation/separation of Fraction II + III	Precipitation/separation of Fraction II + III
4	Resuspension of Fraction II + III (731–1192 rpm)	Resuspension of Fraction II + III (200–300 rpm)
	Precipitation/separation of Fraction II + IIIw	Precipitation/separation of Fraction II + IIIw
5 (critical)	Resuspension of Fraction II + IIIw (two lots, 872–1044 rpm)	Resuspension of Fraction II + IIIw (one lot, 200–300 rpm)
	Precipitation/separation of Fraction III at pH 5.20	Precipitation/separation of Fraction III at pH 5.40
6 (critical)	Depth filtration of Fraction III centrifugate	Depth filtration of Fraction III centrifugate
	pH adjustment of Fraction III filtrate	pH adjustment of Fraction III filtrate
7	Ultrafiltration and diafiltration of Fraction III filtrate (UF1)	Ultrafiltration and diafiltration of Fraction III filtrate (UF1)
8 (critical)	Virus inactivation with triton X-100 and TnBP (temperature ramp rate: ≥ 7 h)	Virus inactivation with triton X-100 and TnBP (temperature ramp rate: ≤ 1 h)
9 (critical)	C-18 chromatography	C-18 chromatography
10 (critical)	Q-sepharose chromatography	Q-sepharose chromatography
11 (critical)	Nanofiltration	Nanofiltration
12	Ultrafiltration/diafiltration of nanofiltrate (UF2) 45 l/m²	Ultrafiltration/diafiltration of nanofiltrate (UF2) 20–30 l/m²
13 (critical)	Polysorbate 80 addition	Polysorbate 80 addition
	0.2 μ m bulk filtration	0.2 μ m bulk filtration

The values in bold are the process step numbers for the critical steps to help identify the differences.
IVIG: Intravenous immunoglobulin.

The original IVIG process for BIVIGAM & ASCENIV (pre-optimization)

ASCENIV was approved by the FDA for treatment of primary humoral immunodeficiency (PI) in adults and adolescents in April 2019 [15]. Following the acquisition of the BTBU by ADMA Biologics, ASCENIV manufacturing was aligned with the robust, optimized IVIG manufacturing process developed and validated by ADMA for the production of BIVIGAM, resulting in improvements throughout the manufacturing sequence for both drug products. The steps, which were taken to improve overall product quality of these two commercial IVIG products, are discussed below.

The sole difference in production of these two IVIG drug substances is that ASCENIV is produced from using unselected source plasma pooled with plasma from donors identified and selected as having sufficient RSV neutralizing antibody titers, while BIVIGAM is produced from only unselected source plasma. While the plasma pool requirements differ for BIVIGAM and ASCENIV, the IVIG manufacturing process used by ADMA to produce ASCENIV and BIVIGAM is identical. ADMA's IVIG manufacturing process is based on a modified Cohn-Oncley cold fractionation method followed by chromatographic purification procedures. The process incorporates a minimum of four validated viral inactivation steps to remove or inactivate any blood-borne pathogens. Overall, the process consists of 13 steps grouped into fractionation, upstream processes including filtration and viral inactivation, and downstream processes including chromatography and further filtration (Table 1).

The original process for production of BIVIGAM was associated with several manufacturing problems including filter clogging, excessive foaming, impurities, protein aggregation and particulates that were found in process intermediates as well as the final drug product. Impurities identified in BIVIGAM included fibrinogen (Factor I), Factor XI (FXI), Factor XIa (FXIa), prekallikrein activator (PKA) and complement protein C3 [ADMA BIOLOGICS, DATA ON FILE]. Additionally, proteolytic activity was observed in the product during the fractionation process. The impurities resulted in protein aggregation and visible particle formation, and unacceptable lot-to-lot variability. These concerns, both with drug substance and drug product, were not able to be resolved through relevant changes within the FDA approved Biological License Application for BIVIGAM and required more intensive investigation that ultimately led ADMA to optimize the overall manufacturing process. The revised, optimized process was submitted to FDA for prior approval.

Table 2. Critical and noncritical quality attributes tested for BIVIGAM drug substance and drug product.

Safety	Potency	Regulatory requirement	Step efficiency, formulation and purity	Noncritical
Bioburden	IgG	Anti-A activity by hemagglutination	Residual TnBP	IgM
Endotoxin (LAL)	Chromatographic purity (IgG monomer and dimer)	Anti-B activity by hemagglutination	Residual Triton X-100	Identity (human)
Pyrogenicity	Total	Anti-D activity by hemagglutination	Residual ethanol pH (formulation)	Chloride (formulation)
Sterility	Protein	Electrophoretic purity	Polysorbate 80 (formulation)	Glycine (formulation)
Appearance	IVIG potency (anti-HBs ortho vitros ECI)	IVIG potency (diphtheria by neutralization)	Plasmin	Residual albumin
Subvisible particulate matter		IVIG potency (measles by neutralization)	Plasminogen	
IgA		IVIG potency (polio by neutralization)		
Prekallikrein activator		Osmolality		
Procoagulant activity (Factor Xla)				
Anti-complement activity				
Chromatographic purity (aggregates)				
Chromatographic purity (fragments)				
IVIG: Intravenous immunoglobulin.				

Process optimization: a methodical & systematic approach

A systematic, quality by design approach was undertaken through multiple small-scale development studies with the aim of increasing control of the manufacturing process, increasing purity of the drug substance and the drug product, reducing filter clogging and particle formation, and minimizing physical, chemical and thermal stress [ADMA BIOLOGICS, DATA ON FILE]. Process changes and controls were identified with the intent of being applied to the relevant steps in the IVIG manufacturing processes. In addition to guiding impactful process changes, engineering and procedural controls were also evaluated to improve production consistency and product purity.

Utilizing a scale-down approach in the process development laboratory, a criticality assessment was performed on the BIVIGAM manufacturing process to define the critical process parameters (CPPs) for IVIG manufacture. Quality attributes were assessed for criticality based on their potential to impact safety, purity and efficacy of the drug product. Quality attributes that were considered at high risk of potential harm or that were expected to impact efficacy were designated as critical quality attributes (CQAs). Table 2 provides a list of the CQAs that were identified. A cause and effect matrix was generated to relate each of the process steps at the small scale with CQAs that could affect the safety and quality of the product. Each process parameter was assessed and determined to be either critical or noncritical based on their impact on in-process acceptance criteria for relevant CQAs. The physical, chemical or biological characteristics that should be within an appropriate limit, range or distribution to ensure the desired product safety, quality and purity at the process step level and for the process overall were evaluated. Out of the 13 primary process steps, seven were designated as critical due to their impact on product quality (Table 1).

The IVIG fractionation process consists of a series of precipitations at varying pH, temperature, alcohol concentration, ionic strength and protein concentration. Within each alcohol fractionation step, pH, alcohol concentration, protein concentration and temperature parameters were designated as CPPs or in-process controls due to their direct impact on product quality. Determining optimum pH conditions for reduction of impurities, such as IgA, IgM, fibrinogen, FXI and FXII, is critical to achieving appropriate drug substance filterability and drug product stability. A development study was designed and executed to evaluate the impact of pH change during Fraction III precipitation on product yield and impurities in the IVIG manufacturing process [ADMA BIOLOGICS, DATA ON FILE]. The scope of the study was to identify the target pH range for the Fraction III precipitation step that showed the greatest impact in reducing impurity levels in the product as well as to improve the process variability at that step. In the pre-optimized IVIG manufacturing process, Fraction III precipitation was performed at pH

5.20 ± 0.05. The study used Fraction II + IIIw paste as a starting material and the Fraction III precipitation step was performed at pH 5.2, 5.3, 5.4 and 5.5. Product quality attributes from each pH run were evaluated at the Fraction III ultrafiltration/diafiltration (UF/DF) step, which showed an improved product quality and insignificant yield loss with increase in Fraction III precipitation pH [ADMA BIOLOGICS, DATA ON FILE].

Upstream processes, which refer to all operations performed prior to completion of the viral inactivation step, are followed by depth filtration to remove impurities and clarify the product, ultrafiltration and diafiltration to eliminate alcohol and achieve the desired protein concentration and solvent-detergent treatment for the primary purpose of inactivation of enveloped viruses. Celite® (silica filtration supplement) amount and product temperature are CPPs during depth filtration. Control of product temperature, crossflow rate, transmembrane pressure (TMP), filtrate flow control and membrane surface area are critical during the UF/DF process steps. For viral inactivation, solvent concentration, detergent concentration, incubation temperature and time are critical for the inactivation of enveloped viruses.

Design and operational challenges for UF/DF systems, if not adequately controlled, greatly influence quality attributes of both drug substance and drug product stability. The higher the crossflow rate, the higher the flux at equal TMP. A higher crossflow rate increases the sweeping action across the membrane, which reduces the concentration gradient toward the membrane surface. However, higher crossflow rates cause the product to experience more passes through the pump in a given amount of time, which can lead to excess foaming, particle generation and product quality degradation. Further, higher crossflow rates require larger pumps and larger diameter piping, which increases the system deadspace, increasing product losses due to unrecoverable product. One of the most important optimization steps for performance characterization required understanding and optimizing the flux versus TMP curve that is essential to reducing protein shear and maintaining native protein structure.

TMP is mathematically related to flux. Determining an appropriate combination of feed flow rate and TMP to maximize flux is critical. It is essential to reduce process time while minimizing the required membrane area, thus allowing for achievement of optimal membrane area and pump sizing (minimal holdup due to pump sizing as well as pump passes) to be determined. Small scale studies were performed to determine optimum UF/DF processing parameters aimed at understanding the critical relationship between TMP versus a flux curve. A TMP versus flux curve was generated. The first part of the curve shows where the flux increases with increasing TMP and is referred to as the pressure-dependent point. The level part of the curve (knee of the curve) is known as the pressure-independent point where there is no gain in flux made by increasing pressure. The point at which flux begins to plateau determines the optimum process TMP operating point. Determination of this point resulted in achieving high flux without exerting excessive pressure, reaching exceedingly high protein wall concentrations or protein aggregation. The data established that operating at the plateau of the curve provided optimal conditions for crossflow, TMP pressure and reduced product passes, thus reducing processing time and product stress thereby resulting in improved drug substance stability. To further reduce protein shear during the UF/DF operation, a rotary lobe pump was replaced with a diaphragm Quattro flow pump. The Quattro flow pump's method of operation allows it to gently, safely and securely convey the shear sensitive IVIG product. The four-piston design does not include a mechanical shaft seal or wetted rotating parts, ensuring total product containment without abrasion or particulate generation.

Process control of the downstream process comprising C-18 chromatography, Q-Sepharose chromatography, nanofiltration, ultrafiltration/diafiltration, formulation and filling is equally as critical in the production of IVIG. During C-18 column chromatography, flow rate must be optimized to ensure removal of Triton X-100 and TnBP from the drug substance. Additionally, buffer addition quantity and rate as well as pH titration rate are critical for conditioning of the eluate. Q-Sepharose chromatography uses a strong anion-exchange resin with quaternary ammonium as its functional groups to remove impurities with a net negative charge. At pH 7.5, IgG has a net positive charge and passes through the column. Overall, this step is effective in reducing aggregates and hydrophobic proteins at the process defined conductivity. Critical operating parameters include loading flow rate and the amount and rate of acetic acid addition. Nanofiltration is critical for reducing viral burden; the critical parameter for this step is filtration pressure. The second ultrafiltration/diafiltration exchanges proteins into the formulation buffer used in the final drug product and concentrates the product for the final formulation steps. The diavolume of formulation buffer is the critical parameter that is controlled to attain final concentration weight and protein concentration. For final filtration and formulation, filtration pressure and the amount of Polysorbate 80 are integral to formulating bulk drug substance.

Table 3. Process improvements and enhanced controls resulting from procedural and engineering changes.

Process step	Improvements
Pooling and thawing of plasma(Step 1)	The temperature gradient in the pooling tank is minimized by allowing the Temperature Control Module to cycle longer (reduced hysteresis). As a result, the plasma thawing process is more consistent and excessive foaming due to ice formation is eliminated. Reducing the temperature gradient in the tank also allows better separation of fibrinogen from the cryoprecipitate. Minimizing the differences in piping diameter and flow meter from the pooling tank reduces the inherent change in pressure that can contribute to protein shearing and denaturation.
Throughout the fractionation process(Steps 2, 3, 4 and 5)	Use of a peristaltic pump for acetate/acetic acid pH 4.0 buffer addition provides for a more controlled and consistent flowrate. Reducing flowrates avoids localized areas of lower pH and potential protein denaturation. Adequate aging time allows proteins to undergo the stages of precipitation (nucleation, flocculation and maximum particle growth), increasing the protein separation and subsequently reducing the potential for protein carryover at each fraction. Incrementally increasing the alcohol addition rate based on both the temperature and alcohol concentration of the product reduces localized heating, minimizing the potential for protein denaturation and coprecipitation of undesirable proteins and optimizing the nucleation of protein precipitates.
Resuspension of Fr II+III/Fr II + IIIw(Steps 4 and 5)	Incremental addition of paste to the resuspension tank (with ~2–3 min between additions) reduces the likelihood for accumulation of paste at the bottom of the tank and the overall time required to achieve a uniform suspension paste. Reducing the time to achieve a uniform suspension reduces exposure of the protein to shearing and denaturation due to the high speeds of the mixers on the resuspension tank. Switch to concave base mixing tank and single pitch-blade mixer from flat-base mixing tank with two propeller-type mixers allows smoother, more uniform mixing (less shearing force); reducing the mixing speed from 731 to 1192 rpm or 872 to 1044 rpm to 200–300 rpm allows for a gentler mixing of the resuspension and reduces the potential activation of unwanted coagulation factors.
Fr II+IIIw precipitation/separation(Step 4)	Establishment of a maximum addition rate for sodium phosphate buffer increases control and reproducibility of the manufacturing process. Reducing the mixing speed during sodium phosphate buffer addition allows for a gentler mixing of the resuspension and reduces the potential activation of unwanted coagulation factors.
Fraction III precipitation/separation(Step 5)	Increasing pH from 5.20 ± 0.05 to pH 5.40 ± 0.10 allows more effective removal of impurities such as fibrinogen, Factor XI (Xla), Factor XII, prekallikrein activator, immunoglobulin M, plasmin/plasminogen, proteolytic activity, and complement protein C3; the higher pH has no impact on the immunoglobulin G yield.
pH adjustment of Fraction III filtrate(Step 6)	Reducing the addition rate of 2.0 N acetic acid and adding at two points reduces the potential for localized areas of low pH due to the introduction of strong acid, reducing the potential for protein denaturation while remaining within the overall addition rate specification. Holding the vessel containing the acid at a lower temperature reduces the difference between the temperature of the solution and the suspension to which it is being added.
Ultrafiltration/diafiltration-1(Step 7)	UF1 product tank cooling prevents the potential for freezing of the product as it enters the tank and is exposed to the precooled tank wall.
Viral inactivation(Step 8)	Utilization of a pipette provides for a more constant flowrate of the addition of Tri-n-Butyl Phosphate. Use of a peristaltic pump provides for a more controlled and consistent flowrate for addition of 20% Triton X-100, reducing the localized areas of buffer and potential protein denaturation. Reducing the heating ramp up time reduces product exposure to solvent-detergent and total process time, thus reduces potential protein aggregation. This is achieved by switching from an electrically coiled tank with a heating ramp up time ≥ 7 h to a jacketed tank with heating ramp up time ≤ 1.0 h.
C-18 chromatography and Q-sepharose chromatography(Steps 9 and 10)	Use of a longer dip-tube in the collection tank configuration reduces potential foaming due to turbulence at the product surface.
Ultrafiltration/diafiltration2(Step 12)	Use of a smaller concentration tank that is currently used in the downstream processing step will ensure the complete submersion of the agitator throughout the concentration step reducing the potential for foaming due to product and agitator levels being approximately equal (toward the end of concentration). Changes were also implemented in piping at the pressure gauge connections. These changes eliminated different piping diameters throughout the UF-2 system, thus minimizing the pressure difference which can contribute to protein shearing and denaturation. Switch to diaphragm pump with ultrafiltration/diafiltration membrane area 20–30 l/m² from a rotary lobe pump with ultrafiltration/diafiltration membrane area 45 l/m² reduces the shear and thermal stress to protein products observed in rotary lobe pump; reducing the sample load to ultrafiltration/diafiltration membrane area ratio decreases potential shear stress to the product by shortening the time that product passes through the pump and membrane
Bulk drug substance filtration(Step 13)	Utilization of a pipette provides for a more constant flowrate of the addition of 20% Polysorbate 80. Pre-filtration mixing ensures solution homogeneity.

Outcome: results of ADMA's robust IVIG process optimization

The optimization project identified several parameters with the potential to increase process consistency and robustness, decrease product variability and enhance removal of impurities that lead to product aggregation and particle formation. Overall, process improvements included reductions in thermal, physical and chemical product stressors, engineering modifications, and enhancements to procedural controls. Adopted modifications to the original process are outlined in [Table 3](#) and include: a reduction in mixing speed and the use of a concave tank for process step 4 and 5 resuspensions; pH adjustment to 5.4 during fraction III precipitation and resuspension in order to reduce protein impurities; use of a water jacketed tank with faster heat ramping time and uniform heat transfer distribution to minimize protein aggregation and prolonged incubation at elevated temperatures

Figure 1. Changes to physical stressors in the IVIG manufacturing process reduce impurities. (A) Average (SD) PKA levels (IU/ml) in Fraction II+IIIw resuspension at mixing speeds of 1200 rpm (pre-optimization, black) and 300 rpm (post-optimization, white). (B) Short temperature ramp up time (post-optimization, white bar) during viral inactivation achieved by utilization of a jacketed tank impacts mean (SD) concentration of subvisible particles $>2\ \mu\text{m}$. (C) Submicron particles by nanoparticles tracking analysis in UF bulk during diafiltration process in clogged lot (black) and nonclogged lot (white). (D) Effect of pump type on quantity of subvisible particles of sizes ranging from 10 to 25 μm to $\geq 25\ \mu\text{m}$ (white) at the end of diafiltration using a quattro flow diaphragm (post-optimization, black) versus rotary lobe (pre-optimization, white).

DF: Diafiltration; IVIG: Intravenous immunoglobulin; PKA: Prekallikrein activator; UF: Ultrafiltration.

during viral inactivation, establishing optimum process control of flux rate and TMP parameters to minimize protein shear; use of a diaphragm pump versus a rotary lobe pump reducing shearing effect during the second ultrafiltration/diafiltration step to further reduce physical and thermal stresses on the IgG protein. A variety of other engineering and procedure changes were also implemented to increase consistency based on the results of the criticality assessment (Table 3). Further, batch records were consolidated and additional controls were included to further increase process consistency.

Successful minimization of thermal, physical and chemical stressors was further achieved through modification of the tanks during different process steps including improved tank geometry during Fraction III paste resuspension and the use of a jacketed tank during the inactivation process step to allow faster heat ramp up via optimized heat exchange. Further, gentler mixing and a new blade design and diameter for more consistent mixing and pressures resulted in minimization of the activation of coagulation factors during the Fraction III resuspension step. Figure 1A demonstrates a substantial reduction in average PKA levels following Fraction II paste resuspension at 300 versus

Table 4. Reduction of clinically relevant safety related impurities in the drug substance using the ADMA optimized manufacturing process as compared with the pre-optimization manufacturing process

Analytical test	Acceptance criteria	Pre-optimization mean (n = 3)	Post-optimization mean (n = 3)
Fibrinogen	≤600 µg/ml	99 µg/ml	3 µg/ml
Complement protein C3	<10 µg/ml	2 µg/ml	1 µg/ml
Factor XIa	<80 mIU/ml	11 mIU/ml	0 mIU/ml
Factor XI	≤900 ng/ml	185 ng/ml	36 ng/ml
Factor XII	≤1200 ng/ml	200 ng/ml	65 ng/ml
FlowCam	Particles/ml	50,501 particles/ml	7705 particles/ml

1200 rpm. Tank improvements during the viral inactivation step resulted in six to seven fewer hours of product exposure to heat, solvent and detergent yielding a five- to sixfold reduction in subvisible particles greater than 2 µm at the end of the process step (Figure 1B). Filter clogging and particle formation were addressed by optimization of the UF/DF 2 process including changing the pump from a rotary lobe to a diaphragm pump and control of critical process parameters such as product to membrane area, cross flow and TMPs. These interventions resulted in large reductions in submicron particles across the process step (Figure 1C). The effect of the change in pump type on the quantity of subvisible particles at the end of diafiltration is presented for representative lots in Figure 1D.

Analytical comparability testing of intermediates, drug substance and drug product was performed between the optimized ADMA process and original IVIG process in separate studies designed to assess comparability of intermediates and drug substance, comparability of drug product [ADMA BIOLOGICS, DATA ON FILE]. Overall, impurities including fibrinogen (Figure 2A) and Factor XI (Figure 2B) were reduced more than two- to threefold across the optimized process. The reduction in impurities and aggregation provided a substantial improvement in bulk filterability (Figure 2C). Increased purity of drug substance and drug product was partly attained through optimization of the Fraction III precipitation step by increasing target pH from 5.2 to 5.4 and eliminating post wash of the paste during centrifugation to reduce the potential of introducing impurities into the Fraction III supernatant. The effect of changing pH from 5.20 to 5.40 during the first ultrafiltration/diafiltration process step on impurities including fibrinogen, Factor XI, and Factor XII is presented in Figures 2D–F. Fibrinogen, Factor XI, Factor XII and PKA are effectively reduced with the increase in Fraction III precipitation pH with a minimal product loss. Based on these findings, it was concluded that shifting Fraction III precipitation pH from 5.20 ± 0.05 to 5.40 ± 0.10 was a critical parameter that resulted in improved filterability of the final bulk product.

In all comparability and stability studies, the evaluated IVIG processes met predetermined acceptance criteria for all time points tested to date [ADMA BIOLOGICS, DATA ON FILE]. Overall, these process improvements resulted in significant decreases in aggregate formation, particulates in intermediates prior to filtration, improved bulk filterability, decreased protein impurities including fibrinogen, Factor XI, Factor XIa, Factor XII and PKA. Pre- and post-optimization mean values of key CQAs in drug substance that are relevant to the safety profile of IVIG are presented in Table 4.

All process changes were implemented for both ASCENIV and BIVIGAM. The ADMA optimized IVIG manufacturing process yields significant improvements in purity that continue to be reproducible in currently available commercial product(s). The optimized manufacturing process also incorporated increased controls and robustness for each unit operation including standardization of total processing time and improvements in format and structure of production batch records to allow evaluation of performance for all unit operations.

Future perspective

Manufacturing processes for biologic products, and specifically plasma protein products such as IVIG, are subject to physical, chemical and thermal stressors that can result in product impurities and decrease product yield. A stepwise, small-scale, quality by design approach can optimize this process and guide manufacturing improvements to achieve a consistent and higher purity product, improving product safety, quality and yield. This risk-based approach was focused on establishing CQAs, CPPs and key process parameters based on a sound scientific and chemical engineering data driven foundation. As a result, both BIVIGAM and ASCENIV are fully characterized, meet all acceptance criteria and can be manufactured consistently and reproducibly in ADMA's wholly owned and operated manufacturing facility. Both products are manufactured through an identical manufacturing process with the only exception being the differences in plasma pool requirements and patented methods used for ASCENIV.

Figure 2. The optimized IGIV manufacturing process results in increased bulk filterability and reductions in the concentration of impurities including fibrinogen, Factor XI and Factor XII. **(A)** The optimized IGIV process reduced fibrinogen, a contributor to filter clogging, in multiple unit operations. **(B)** The optimized IGIV process reduced Factor XI, a cause of thromboembolic events in patients, in multiple unit operations. **(C)** Mean (SD) bulk filterability was increased post-optimization. **(D–F)** Mean (SD) fibrinogen, Factor XI and Factor XII post-Fraction III ultrafiltration/diafiltration step at pH 5.2 (pre-optimization) and pH 5.4 (post-optimization).
Fr: Fraction; IgG: Immunoglobulin G.

Improvements directly guided by small-scale production studies and current Good Manufacturing Practices (cGMP) have led to implementation of exacting in-process controls including regulated processing time requirements, acceptance criteria, critical process step changes, engineering changes, procedural changes and a consolidated, data driven batch record. These changes have resulted in a production process that is validated, reliable, reproducible and efficient with demonstrated lot-to-lot consistency. At this time, both BIVIGAM and ASCENIV use this optimized process as FDA has approved both product submissions in early 2019. A data driven approach to identify critical process steps, quality parameters and quality attributes that impact product characteristics is recommended to improve future therapeutics.

Executive summary

- ADMA Biologics' IVIG products, BIVIGAM[®] and ASCENIV[™] for the treatment of primary immunodeficiency disease patients are produced in ADMA's manufacturing facility using similar methodologies. These two products have undergone manufacturing process optimization to increase consistency, purity and stability.
- ASCENIV[™] is manufactured from plasma donors using a patented method for collection and plasma pooling.

The original intravenous immunoglobulin process for BIVIGAM & ASCENIV (pre-optimization)

- ADMA's intravenous immunoglobulin (IVIG) manufacturing process is based on a modified Cohn-Oncley cold fractionation method followed by chromatographic purification procedures consisting of 13 steps.
- The process incorporates a minimum of four validated viral inactivation steps to remove or inactivate any blood-borne pathogens.
- The original process for production of BIVIGAM was associated with several manufacturing problems including filter clogging, excessive foaming, impurities, protein aggregation and particulates that were found in process intermediates as well as in the final drug product.

Process optimization: a methodical & systematic approach

- IVIG manufacturing process optimization for BIVIGAM and ASCENIV was performed in small-scale production studies to reduce protein impurities and aggregation in drug substance and drug product.
- Critical process parameters and attributes were defined and evaluated with a focus on increasing manufacturing process control, minimizing thermal, physical and chemical stressors, minimizing filter clogging and particle formation problems and increasing the overall purity of drug substance and drug product.

Outcome: results of ADMA's robust IVIG process optimization

- Implementation of procedural, engineering and process-related changes resulted in significant improvements in product quality and consistency for ASCENIV and BIVIGAM.
- Data show the optimized process is robust with thermal, chemical and physical stresses being significantly reduced throughout the manufacturing process. Critical process parameters and critical quality attributes developed and implemented provide a scientific basis for process improvement and qualification. The critical process parameter and critical quality attribute as well as key product attributes are fully validated and support critical production equipment documentation packages. Established processing times during qualification runs were achieved within specification for each of the conformance batches produced.
- The optimized manufacturing process is fully characterized with evidence that the process is capable of removing relevant impurities such as coagulation factor Factor XIa and other high molecular weight proteins from drug substance and drug product.

Financial & competing interests disclosure

RL Wasserman is a consultant for Korean Green Cross, Prometic, Takeda, Therapure; an investigator for Korean Green Cross, Octapharma, Takeda, Therapure; a speaker for CSL Behring, Takeda. J Mond is the chief medical and chief scientific officer of ADMA Biologics, and holds United States patent US 9,107,906, 9,815,886 and 10,259,865. D Garcia is the vice president of IgG product management at ADMA Biologics. K Kestenberg is the vice president of compliance, project management and clinical operations at ADMA Biologics. A Pinkert is the vice president of quality operations and regulatory affairs management at ADMA Biologics. A Grossman is the president and chief executive officer of ADMA Biologics. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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