Biologicals 60 (2019) 60–67

Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Octanorm [cutaquig®], a new immunoglobulin (human) subcutaneous 16.5% solution for injection (165 mg/mL) – Biochemical characterization, pathogen safety, and stability

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ARTICLE INFO

Keywords:
New SCIG 16.5% liquid
Integrity
Pathogen safety
Stability
Viscosity
Injectability

ABSTRACT

Octanorm (marketed as cutaquig® in Canada and US [2018] and registered in several European countries [2019]) is a new immunoglobulin subcutaneous 16.5% liquid for the treatment of patients with primary immune deficiency (PID) and secondary immune deficiency (SID) depending on country's specific indications.

Octanorm contains ≥96% human IgG and is characterized by especially low concentrations of polymers and aggregates, IgA and IgM, a physiological osmolality along with a low isoagglutinin titer. The Octanorm manufacturing process is based on the well-established IVIG octagam® 5% and 10% process, but yields a higher immunoglobulin concentration of 16.5% in the final product. Octanorm shows a distribution of immunoglobulin G subclasses closely proportional to native human plasma and comprises a broad spectrum of antibodies against infectious agents. Potential procoagulant activity is not detectable. IgG functionality and physico-chemical integrity have been demonstrated by state-of-the-art-methods.

The virus safety of Octanorm is ensured via a combination of three validated independent methods as part of the manufacturing process. Substantial prion depletion during the manufacturing process has also been demonstrated.

Compared with other commercially available subcutaneous immunoglobulin (SCIG) 20% products, Octanorm 16.5% shows a lower viscosity, which is a valuable feature that allows for a more comfortable infusion experience.

1. Introduction

Immunoglobulin concentrates have been successfully used for decades to treat patients with primary or secondary immunodeficiency disorders [1,2]. This treatment has substantially decreased the frequency of life-threatening infections in these patients. Immunoglobulins can be administered via the intravenous, subcutaneous or the intramuscular routes. While immunoglobulins are currently administered as intravenous immunoglobulin (IVIG) products, subcutaneous immunoglobulins (SCIGs) offer several clinical advantages, such as stable and well-balanced serum IgG levels [3,4], high tolerability [5] as well as the possibility of home treatment [6]. Self-administration at home can remarkably improve the patient's quality of life and compliance as it reduces the frequency of hospitalizations and the need for home care. This approach has become even more convenient and popular with the optimization of application devices such as portable pumps.

Octanorm is a ready-to-use, highly purified and concentrated (16.5%) polyvalent immunoglobulin G solution for subcutaneous antibody replacement therapy in patients with primary and secondary humoral immunodeficiency disorders. The product is manufactured from a pool of human fresh-frozen plasma, where single donations are screened using licensed/approved third generation immuno-assays and nucleic acid testing (NAT) for hepatitis B virus (HBV), hepatitis B surface antigen, hepatitis C virus (HCV), HIV and antibodies to HIV-1/2. Furthermore, only donations that are tested negative for HIV, HBV, HCV and HAV and below the acceptance limit for Parvo B19 by Polymerase Chain Reaction (PCR) in mini-pools are accepted for Octanorm production. The large donor pool ensures that Octanorm contains a broad range of antibodies directed against common bacterial

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https://doi.org/10.1016/j.biologicals.2019.05.002
Received 1 February 2019; Received in revised form 15 April 2019; Accepted 2 May 2019
Available online 01 June 2019

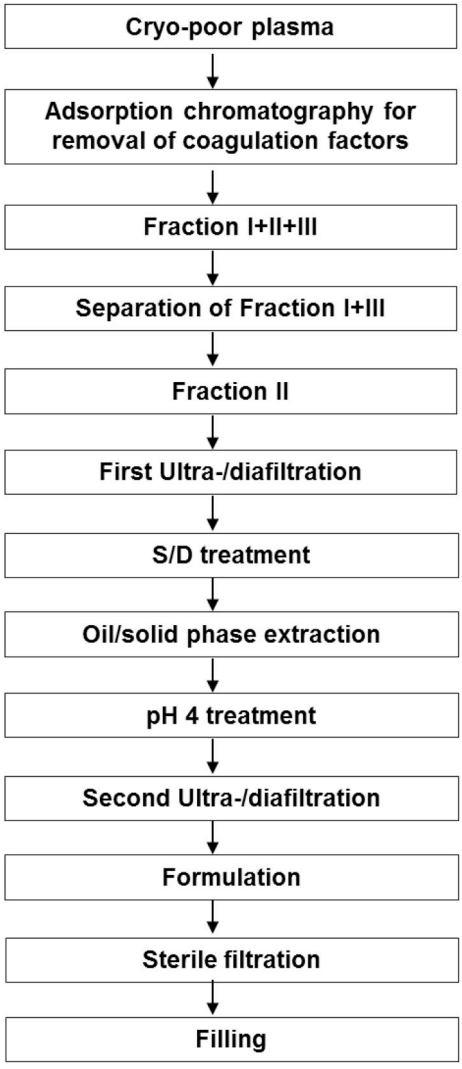

Fig. 1. Manufacturing process of Octanorm/cutaquig®.

and viral pathogens and antigens.

Octanorm was developed based on the manufacturing process of the IVIG octagam®, which includes two dedicated virus reduction steps comprising Solvent/Detergent (S/D) treatment and pH 4 treatment. Another step the cold-ethanol fractionation contributes to the overall pathogen reduction.

In the present study, the biochemical, physico-chemical and functional characteristics of eight consecutive Octanorm batches were assessed. Pathogen safety was validated using downscale models for the dedicated virus and prion inactivation/removal steps.

2. Materials and methods

2.1. Manufacturing process

Octanorm is prepared by cold-ethanol fractionation of donated human fresh frozen plasma based on the well-established octagam® 5% and 10% process. This process includes S/D treatment, subsequent removal of residual S/D reagents by oil extraction and C18 chromatography as well as pH 4 treatment (Fig. 1). The entire manufacturing process is carried out at a low pH to maintain the nativity of the IgG molecules. Octanorm production comprises a final concentration step to the target strength of 16.5% at the end of the manufacturing process and adjustment of the excipients maltose and Polysorbate 80 (Fig. 1). After formulation, the product solution is sterile filtered and filled into 10, 20, 30, and 50 mL vials, respectively.

2.2. Biochemical investigations

For product characterization, final product samples of eight consecutive Octanorm batches manufactured at production scale were biochemically analyzed.

IgG-related parameters: Concentration of total protein was determined using a Biuret method. Protein composition [% IgG] was determined by agarose gel electrophoresis according to European Pharmacopeia (EP) monograph 8.0 [7]. IgG subclass concentrations were quantified using a nephelometric method (Siemens BN Nephelometer Systems; Erlangen, Germany). Molecular size distribution of monomers, dimers, polymers and fragments were determined according to EP 8.0 [7] 0918, general method 2.2.29 and 2.2.46, by size exclusion chromatography (SEC). Hemagglutinins: Anti-A and Anti-B titers (direct method) were tested according to EP 8.0 [7] 2.6.26.

Accompanying plasma proteins: IgA, IgM, albumin, transferrin, and fibrinogen were quantified using in-house enzyme-linked immunosorbent assay (ELISA) methods. Proteolytic activity was assessed using the chromogenic substrate CS-05(88) (Hyphen Biomed, Neuville-sur-Oise, France). Prekallikrein activator (PKA) and PKA blank were measured according to EP [7] 2.6.15 using the chromogenic substrate S-2302™ (Chromogenix; Instrumentation Laboratory, Bedford, MA, USA).

Functional parameters: Fc function was determined according to EP [7] 2.7.9. Binding to cellular Fcγ receptor was determined using fluorescence-activated cell sorting (FACS) (Glycotope GmbH, Berlin, Germany). Opsonophagocytosis capacity was assessed using a neutrophil-based assay investigating the opsonic activity of IgG for the bacterial strain *Streptococcus pneumoniae* (Serotype 6B, DSMZ 11867). The bacterial strain was labeled with FITC and the marked bacteria were opsonized by bacteria specific IgG molecules. The IgG-bacteria-complex interacted with HL60 cells, which were separated by FACS (Glycotope GmbH, Berlin, Germany). The results of both assays were expressed in relation to the Human Immunoglobulin Biological Reference Preparation (BRP) batch 1; supplied by the European Directory for the Quality of Medicines (EDQM), which was set to 100%.

Physicochemical parameters: Osmolality was determined according to EP 8.0 [7] 2.2.35. Viscosity was measured with a fallingsphere viscometer (AMVn; Anton Paar GmbH, Graz, Austria). Density was determined with a DMA 4500 M instrument (Anton Paar GmbH, Graz, Austria). Maltose content was quantified by High Performance Anion Exchange Chromatography and Pulsed Electrochemical Detection (HPAEC-PED). Polysorbate 80 was determined by colorimetric measurement of a blue colored complex that formed after reaction of the polyethoxylated compounds of Polysorbate 80 with ammonium cobalt thiocyanate using spectrophotometer at a wavelength of 620 nm.

Antibody titers: Hepatitis A titers and hepatitis B surface antigen were determined by chemiluminescence immune assay (CLIA) methods (Access; Beckman Coulter Inc., Brea, CA, USA) and Parvovirus B19 titers by ELISA methods (Mikrogen, Neuried, Germany). Polio and measles titers were measured by in vitro neutralization tests and antistreptolysin O titers by nephelometric methods (Labor Enders, Stuttgart, Germany). Diphtheria titers were determined by in vivo neutralization (Medical University Vienna, Department für Biomedizinische Forschung, Abteilung Labortierkunde und Genetik, Himberg, Austria). Titers against Tetanus toxin, *Haemophilus influenzae* type b, Varicella Zoster virus, Rubella virus and *Streptococcus pneumoniae* (serotypes 1–5, 6B, 7F, 8, 9 N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 20, 22F, 23F, 33F) were quantified by ELISA methods (Labor Enders, Stuttgart, Germany).

Residual coagulation factors: Coagulation factors FII, FVII, and FX were quantified based on the prothrombin time (PT) test. Factors IX, FXI and FXII were assessed using a modified activated partial thromboplastin time (aPTT) method. Activated Factor VII (FVIIa) was

determined using the Staclot® VIIa-rTF assay kit (Diagnostica Stago SAS, Asnieres sur Seine, France). Clotting activities were assessed using the ACL System (Werfen, Kirchheim, Germany). Factor IXa activity was determined with the BIOPHEN® Factor IXa Kit (Hyphen Biomed, Neuville-sur-Oise, France) and FXIa with the Rox Factor XIa test kit (Rossix, Mölndal, Sweden).

Thrombin generation assay (TGA): Thrombin generation in FXI-deficient plasma was measured using the Technothrombin® assay (Technoclone GmbH, Vienna, Austria) and internal calibration with FXIa (National Institute for Biological Standards and Control (NIBSC) reference reagent 11/236). The Technothrombin® TGA kit is based on monitoring the fluorescence generated by the cleavage of a fluorogenic substrate by thrombin over time upon activation of the coagulation cascade by defined concentrations of tissue factor in the presence of negatively charged phospholipids in plasma. From the changes in fluorescence over time and the calculation of the 1st derivative, the procoagulant activity in the sample was calculated from a calibration curve of FXIa in Hepes/NaCl/BSA-buffer. The response was evaluated as FXIa-like activity. For measurement, all IgG samples were pre-diluted to 5% total protein. Thromboelastography: clotting time in recalcified FXI-deficient plasma (non-activated thromboelastometry; NATEM) was determined using a ROTEG® 05 instrument (TEM Innovations GmbH, Munich, Germany).

Dynamic light scattering (DLS) measurements were performed using a Malvern Zetasizer Nano-ZS (Malvern Instruments Ltd, Malvern, UK). The results were provided as intensity-based size distribution profile and polydispersity index (PDI) and Z-average (intensity-weighted mean diameter derived from cumulants analysis) [8]. The PDI value is a dimensionless measure describing the broadness of the size distribution, ranging from 0 (theoretical) for a perfectly monodispersed sample to 1 for a sample with a very broad size distribution containing multiple particle sizes. The phenomenon multiple scattering occurs in highly concentrated protein solutions [8,9], and consequently, diffusion coefficient cannot be determined unambiguously from the decay rate of the autocorrelation function. Therefore, the values for the Z-average determined in undiluted samples do not reflect the actual radii for IgG [10]. A further effect, which occurs in highly concentrated solutions and limits independent scattering, is particle interaction [8]. The behavior of highly concentrated protein solutions tending to show self-association due to electrostatic interactions is reversible upon dilution [11]. Therefore, the highly concentrated Octanorm final container samples (165 mg/mL) were diluted 1:5 to run DLS measurements of Octanorm samples without the limitations described above. Triplicate measurements were performed and the results represent the respective arithmetic mean. Samples were measured undiluted (data not shown), as well as in a 1:5 dilution using 154 mM NaCl at measurement position 4.65 mm.

2.3. Pathogen safety

To confirm the capacity of the Octanorm manufacturing process to robustly inactivate/remove pathogens, spiking experiments with viruses and prions were conducted. The relevant manufacturing process steps were investigated on a validated laboratory scale. The pathogen safety studies were performed in GLP-certified laboratories. The viruses used represented different families and classes: human immunodeficiency virus type 1 (HIV-1), Pseudorabies Virus (PRV), Sindbis Virus (SBV), Mouse Encephalomyelitis Virus (MEV) and Porcine Parvovirus (PPV). The hamster-adapted scrapie strain 263K (HAS 263 K) represented transmissible spongiform encephalopathies (TSE).

Methods for virus titer calculation: Virus titers were determined using the Tissue Culture Infectious Dose ($TCID_{50}$) assay based on endpoint dilution. The titer was estimated using the Spearman Kaerber Method [12]. In cases were only a few positive cultures were found, the most probable number (MPN) method was used [13–16].

The large volume plating (LVP) assay was used to improve the limit of detection by increasing the tested sample volume. The LVP assay was performed for process samples, which were expected to contain none or only few infectious viruses. The virus titers for samples where no positive cultures were found was determined according the Poisson distribution at 95% confidence limits [17]. If positive cultures were found in the LVP assay, the MPN method was used to estimate the virus titer. The calculations of the $\log_{10}$ reduction factors (LRFs) and 95% confidence intervals were undertaken using the methodology outlined in European regulatory guidelines [18].

Experimental setup of the virus safety studies: A) Cold ethanol fractionation. The process intermediate (fraction I + II + III) was resuspended and cooled to approximately +1.0 °C. After spiking with virus the material was stirred for about 2 h. The pH value was checked and adjusted to approximately pH 4.8. This step was followed by a buffer addition, a pH adjustment and addition of water. Subsequently ethanol was added while the temperature was lowered to −4.0 °C within 4 h. The pH value was checked and adjusted to approximately pH 5.2, followed by a stirring phase. In the next step the precipitated fraction I + III was separated by filtration, followed by a post-wash. Test samples were drawn during the fractionation process to determine the virus reduction factor and to confirm the mode of virus reduction. These samples were investigated by endpoint dilution assay. In addition, the sample of filtrate I + III + W was analyzed by LVP assay. Experiments were carried out with the enveloped viruses HIV-1, PRV and SBV, as well as with the non-enveloped viruses MEV and PPV. Studies were performed in duplicate to show reproducibility.

B) S/D treatment. The process intermediate was cooled to about +6.0 °C. S/D reagents were added in order to reach a final concentration of 0.3% (w/w) tri(n-butyl)phosphate (TNBP) and 1.0% (w/w) Octoxynol-9 (Triton™ X-100). After spiking with virus the material was incubated under stirring for 4 h and a pH value of approximately pH 5.3. To determine the kinetics of virus inactivation, test samples were collected at pre-defined intervals during the process, and investigated by endpoint dilution assay. In addition, the samples were analyzed by LVP assay. Experiments were carried out with the enveloped viruses HIV-1, PRV and SBV. Studies were performed in duplicate to show reproducibility.

C) pH 4 treatment. The process intermediate was cooled to approximately +8 °C. After pH adjustment to pH 3.90–3.92 the process intermediate was passed through a filter (0.22 μm). The filtrate was adjusted to +37 ± 1 °C and spiked with virus. This starting material was incubated under stirring for 24 h at +37 ± 1 °C and a pH value of pH 3.90–4.00. To determine the kinetics of virus inactivation, test samples were collected at pre-defined intervals during the process, and investigated by endpoint dilution assay. In addition, the samples after 8 or 22 h (MEV) and 24 h were analyzed by LVP assay. Experiments were carried out with the enveloped viruses HIV-1, PRV and SBV, as well as with the non-enveloped virus MEV. Studies were performed in duplicate to show reproducibility.

Experimental setup of the prion safety studies: To test for TSE removal, preparations derived from the hamster-adapted scrapie strain 263K (microsomal/cytosolic fraction) were used. This fraction was prepared from crude brain homogenate by differential centrifugation to remove larger aggregates, leaving only the smaller microsomal membrane fragments in the supernatant. This spike preparation was used for the cold ethanol fractionation and for the total process post-ethanol fractionation. Process intermediate was spiked with hamster-adapted scrapie strain 263K (HAS 263K) and the samples collected following cold ethanol fractionation or the total process post-ethanol fractionation were assessed for PrP^{Sc} levels using Western blot analysis [19]. Studies were performed in duplicate to show reproducibility.

2.4. Stability assessment

The stability study for Octanorm was performed according to the current guidelines of the International Conference on Harmonisation of stability testing [20,21]. For evaluation of long term stability, six

Octanorm final container batches were stored at +5 °C ± 3 °C as well as at +25 ± 2 °C with 60% ± 5% relative humidity for 24 months (each inverted in the dark).

The stability parameters, tested at each time point (0, 3, 6, 9, 12, 18, and 24 months), included the following: molecular size distribution (polymers, monomers and dimers, fragments), anti-hepatitis A virus antibody, anti-hepatitis B virus antibody, Diphtheria antitoxin, and Parvovirus B19 antibodies. Mean values and standard deviations (SD) were calculated.

2.5. Injectability

Injectability was tested using Material Testing Machine zwickiLine 1 kN (Zwick Roell GmbH & Co.KG, Germany) equipped with 500 N load cell in compression mode. Octanorm and two marketed 20% SCIG products equilibrated to laboratory temperature were tested in combination with commonly used subcutaneous syringe/needles (10 mL Omnifix® luer lock syringe (B. Braun, Germany) and 25G and, 23G x 3/4" Butterfly winged infusion sets (Hospira, USA)). Glide force (mean force required to sustain the movement of the syringe plunger) was recorded at the crosshead speeds of 10 mm/min and 7.5 mm/min (corresponding to 2 mL/min and 1.5 mL/min injection speeds). All measurements (Setting 1: 25 G, 2 mL/min; Setting 2: 25 G, 1.5 mL/min and Setting 3: 23 G, 2 mL/min) were conducted in triplicate, and the results are presented as mean values ± standard deviations.

3. Results

3.1. Biochemical data

Results of the biochemical characterization, physicochemical and functional parameters, antibody titers and procoagulant activity from eight consecutive Octanorm batches are summarized in Tables 1–5. The results of all parameters are presented as mean values ± standard deviations.

Table 1
Product characteristics of Octanorm (n = 8 batches).

Parameter	Units	Mean ± SD
Total protein	mg/mL	163.0 ± 5.1
Protein composition [% IgG]	%	98.0 ± 0.9
IgG molecular size distribution[a]		
Monomer + dimer	%	99.5 ± 0.4
Polymer	%	≤1
Fragments	%	< 0.5
IgG subclass distribution[b]		
IgG1	%	70.6 ± 0.8
IgG2	%	25.1 ± 0.8
IgG3	%	2.8 ± 0.2
IgG4	%	1.6 ± 0.1
Accompanying plasma proteins		
Anti-A-hemagglutinin	Titer	4 to 8
Anti-B-hemagglutinin	Titer	2 to 4
IgA	mg/mL	0.17 ± 0.04
IgM	mg/mL	0.005 ± 0.003
PKA	IU/mL	< 2
PKA blank	IU/mL	< 2
Proteolytic activity	U/L	< 3
Albumin	mg/mL	1.17 ± 0.69
Transferrin	mg/mL	0.28 ± 0.03
Fibrinogen	µg/mL	< 0.02 to 1.2
Physicochemical parameters		
pH		5.3 ± 0.04
Osmolality	mOsmol/kg	332.0 ± 15.2
Maltose	mg/mL	77.0 ± 2.4
Polysorbate	µg/mL	31.0 ± 3.7
Relative density (20 °C)	g/mL	1.072 ± 0.002

PKA prekallikrein activator.
[a] Expressed as a percentage of the total chromatogram area.
[b] Expressed as a percentage of total IgG.

Table 2
Viscosity of Octanorm in comparison to 20% SCIG products.

Parameter	Immunoglobulin product	Mean ± SD
Viscosity (20 °C/40° angle), [mPa*s]	Octanorm 16.5% (n = 6)	11.6 ± 0.6
	20% SCIG A (n = 3)	15.5 ± 0.9
	20% SCIG B (n = 1)	16.0

SCIG immunoglobulin product for subcutaneous administration.

Table 3
Functional characteristics of Octanorm (n = 8 batches).

Parameter	Units	Mean ± SD
Fc function	%	97.0 ± 5.8
Fcγ receptor binding	% relative to BRP	123.0 ± 8.5*
Opsonophagocytosis	% relative to BRP	123.0 ± 5.3*

* 7 batches tested.
BRP Human immune globulin biological reference preparation Y0001512 batch 1.

Table 4
Antibody titers for pathogens of Octanorm (n = 8 batches).

Antibody specificity	Units	Mean ± SD
Hepatitis A virus	IU/mL	26.7 ± 6.6
Hepatitis B virus surface antigen	IU/mg of IgG	70.9 ± 17.2
Parvovirus B19	IU/mL	547 ± 35.1
Poliovirus	relative to NIH176	1.1 ± 0.6
Measles virus	relative to NIH176	0.8 ± 0.2
Diptheria toxin	U/mL	16.5 ± 4.8
Haemophilus influenzae type b	µg/mL	42.2 ± 13.2
Rubella virus	IU/mL	694 ± 131
Streptococcus pneumoniae	µg/mL	953 ± 351
Streptolysin O	IU/mL	2913 ± 321
Tetanus toxin	IU/mL	48.5 ± 14.5
Varicella zoster virus	mIU/mL	19,100 ± 8955

Table 5
Coagulation parameters of Octanorm (n = 8 batches).

Parameter	Units	Value
TGA: FXIa-like activity	mIU/mL	< 1
NATEM: clotting time	s	> 1500
FII	IU/mL	< 0.016
FVII	IU/mL	< 0.016
FVIIa	mIU/mL	< 9.1
FIX	IU/mL	< 0.016
FIXa	mIU/mL	< 1.73
FX	IU/mL	< 0.016
FXI	IU/mL	< 0.015
FXIa	mIU/mL	< 1
FXII	U/mL	< 0.015

TGA thrombin generation assay.
NATEM non-activated thromboelastometry.

In terms of physicochemical parameters, Octanorm showed a pH of 5.3 and an osmolality of 332 mOsmol/kg (Table 1). Octanorm revealed IgG monomer and dimer contents of 99.5% on average, as measured by size exclusion chromatography and a corresponding polymer and aggregate concentration of ≤1% aggregates. Fragments were not detectable. In Fig. 2 a SEC chromatogram showing the molecular size distribution of the IgG molecules of an Octanorm final container batch is displayed. The product contained 0.17 mg/mL of IgA and even lower amounts of IgM, thereby reflecting the low presence of accompanying plasma proteins (Table 1). Hemagglutinins were present at low titers: anti-A ranged from 4 to 8, and anti-B from 2 to 4. All IgG subclasses are present in Octanorm at the following percentages: IgG1 (71%), IgG2 (25%), IgG3 (2.8%) and IgG4 (1.6%), on average (Table 1), which is

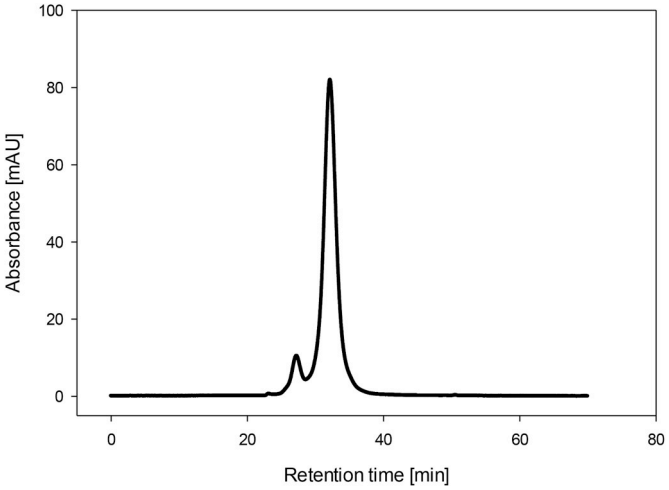

Fig. 2. SEC chromatogram of an Octanorm final container batch.

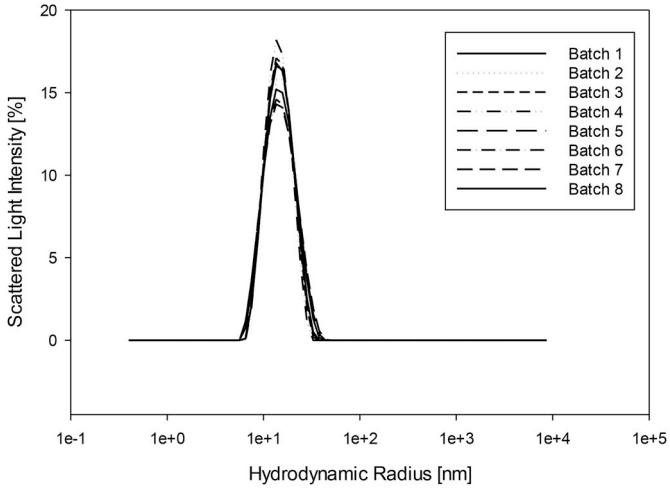

Fig. 3. Dynamic light scattering of Octanorm (overlay of eight batches); samples diluted 1:5 in 0.9% sodium chloride. Triplicate measurements of each Octanorm batch were performed and the results represent the respective arithmetic mean.

closely proportional to the subclass distribution typical for human plasma [22]. Relative to subcutaneous immunoglobulin (SCIG) 20% products, Octanorm 16.5% shows a low viscosity of 11.6 mPa*s at 20 °C (Table 2).

The manufacturing process of Octanorm provides an IgG concentrate with fully intact Fc portion of 97% on average in the final product (Table 3). Fcγ receptor-mediated binding and opsonophagocytosis both determined by cell-based flow cytometric assays demonstrated the integrity of the Fc and the Fab parts of the IgG molecules in Octanorm (relative to that of the BRP standard) (Table 3).

Octanorm contains a broad spectrum of IgG antibodies against bacterial and viral pathogens that are capable of opsonization and neutralization of microbes and toxins, which is required for successful replacement therapies in immune-deficient patients. Octanorm batches showed comparable results for the following specific antibody titers: Hepatitis A, Hepatitis B surface antigen, Parvo B19, Measles, Diphtheria, Polio as well as the presence of additional antibody titers: Streptolysin, Tetanus toxin, *Haemophilus influenzae* type b, *Varicella zoster*, *Rubella*, and *Streptococcus pneumoniae* providing an extended view on the antibody panel present (Table 4).

The upstream manufacture of Octanorm includes an adsorption step for the removal of coagulation factor XI/XIa (Fig. 1). Accordingly, in all Octanorm final container samples, FXIa-like activity, as determined by the TGA system, was < 1 mIU/mL (Table 5). Measurement of clotting potential using NATEM in FXI-deficient plasma showed that no clotting activity was detected within 1500 s (Table 5). Proteolytic activity (Table 1), PKA/PKA blank (Table 1), as well as all measured coagulation factor activities, were found below the limits of quantification (Table 5), indicating a robust removal of procoagulant activities during the Octanorm production process.

DLS is a non-invasive technique for measuring the size distribution of particles and molecules either in suspension or in solution. For Octanorm a Z-average range from 6.77 to 7.09 r.nm and a PDI between 0.070 and 0.129 were detected in samples diluted 1:5 with 0.9% NaCl. Low PDI values and symmetric unimodal size distribution of the DLS pattern confirmed a very reproducible Octanorm manufacturing process, resulting in consistent molecular size distributions of the IgG molecules (Fig. 3).

3.2. Pathogen safety

Following the requirements of the “guidelines on plasma-derived medicinal products” [23–25], the production process of Octanorm comprises orthogonal and effective steps for the inactivation/removal of pathogens. Corresponding log reduction factors and global reduction factors are summarized in Table 6.

The virus safety study portfolio included standard process and robustness conditions of the Octanorm cold ethanol fractionation process step (precipitation and separation of Fraction I + III), solvent detergent (S/D) treatment and pH 4 treatment (Table 6). Prior to these virus safety studies, cytotoxicity/interference studies were completed to ensure the integrity of the test systems. For precipitation and separation of fraction I + III, all virus safety studies showed reduction factors below the limit of detection (LOD) under standard conditions when using the $TCID_{50}$ assay or large volume plating (LVP) (Table 6). Robustness of the cold ethanol precipitation was thoroughly confirmed. The S/D treatment demonstrated its efficacy to inactivate lipid enveloped viruses rapidly and irreversibly under standard treatment conditions (Table 6). The robustness of this inactivation step was shown for all tested viruses.

Furthermore, the pH 4 treatment confirmed efficient inactivation of enveloped viruses and the non-enveloped virus MEV (Table 6). While no significant inactivation of PPV was found upon pH 4 treatment, it has to be considered that this model virus is known to be extremely resistant to physicochemical conditions. The human B19V has been reported by another working group to be sensitive to pH 4 treatment [26]. Therefore, efficient inactivation of B19V was observed in an IgG concentrate solution at a temperature of +37 °C, which is comparable to the conditions used in the respective step of Octanorm production.

In conclusion, the validated very high and robust removal capacity of PPV by cold ethanol fraction, the known sensitivity of B19V to low pH exposure and the specified anti-B19V titers ensure a high safety margin regarding a potential parvovirus contamination.

Prion safety was demonstrated for the cold ethanol fractionation and the downstream manufacturing process after ethanol precipitation as a whole. Both investigations demonstrated a removal to below the limit of detection (Table 6).

3.3. Stability data

Stability was tested according to ICH guidelines. Assessment of long-term stability revealed that Octanorm was stable during storage at +5 °C for at least 24 months. As demonstrated in Fig. 4a, no statistically relevant decrease in IgG content was observed. The polymer content only minimally increased (Fig. 4b), and accordingly, monomer and dimer concentrations remained constant at > 98% within this period of time (Fig. 4c). Antibody levels of antibodies against hepatitis A virus

Table 6
In vitro reduction factor during Octanorm liquid manufacturing.

Production step		In vitro reduction factor [log_{10}]					
		Enveloped viruses			Non-enveloped viruses		Prions
	Pathogen	HIV	HBV	HCV	HAV	B19V	PrP^{Sc}
	Model Pathogen	HIV-1	PRV	SBV	MEV	PPV	HAS 263 K
Ethanol fractionation		≥4.81	≥6.28	≥7.13	≥7.13	≥6.53	≥3.16
S/D treatment		≥4.93	5.23	≥6.77	n.a.	n.a.	
							≥3.0 (Total process post-ethanol fractionation)
pH 4 treatment		≥4.33	≥6.71	6.71	5.07	< 1*	n.d.
Global reduction factor		**≥14.07**	**≥18.22**	**≥20.61**	**≥12.20**	**≥6.53**	**≥6.16**

n.a. not applicable.
n.d. not done.
B19V Human Parvovirus B19.
HAV Hepatitis A virus.
HBV Hepatitis B virus.
HCV Hepatitis C virus.
HIV-1 Human immunodeficiency virus – type 1.
MEV Mouse encephalomyelitis virus.
PPV Porcine Parvovirus.
PrP^{Sc} Protease resistant prion protein.
PRV Pseudorabies virus.
SBV Sindbis virus.
*not calculated for global reduction factor.

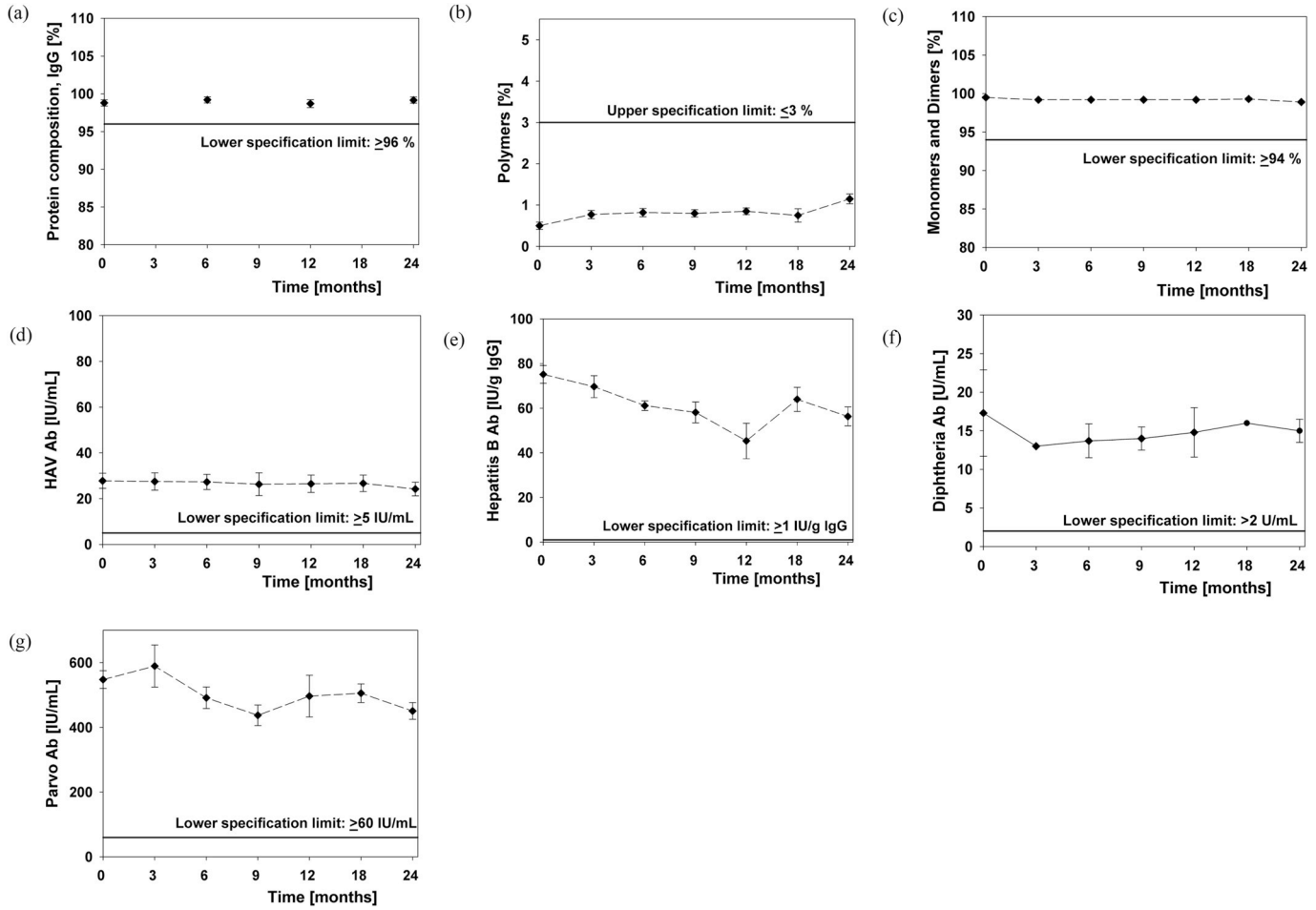

Fig. 4. Graphical representation of selected stability indicating parameters of six Octanorm batches stored over 24 months at +5 °C ± 3 °C. a) Protein composition [IgG%], b) polymers [%*], c) monomers and dimers [%*], d) anti-hepatitis A antibodies [IU/mL], e) anti-hepatitis B antibodies [IU/g IgG], f) Diphtheria antitoxin [U/mL], and g) Parvovirus B19 antibodies [IU/mL]. Mean values and standard deviations (SD) were calculated. *percent of total chromatography area.

Table 7
Injectability test results.

Sample	Viscosity (mPa*s)	Mean Glide Force (N)		
	20 °C	Setting 1	Setting 2	Setting 3
		(25G 2 mL/min)	(25G 1.5 mL/min)	(23G 2 mL/min)
Octanorm	11.3	11.1 ± 0.6	10.0 ± 1.4	6.6 ± 0.8
20% SCIG 1	15.2	13.4 ± 0.6	11.8 ± 0.4	7.7 ± 0.6
20% SCIG 2	16.1	14.9 ± 1.1	13.0 ± 0.0	8.2 ± 0.6

(Fig. 4d), hepatitis B virus (Fig. 4e), Diphtheria antitoxin (Fig. 4f), and Parvovirus B19 (Fig. 4g) remained above the respective specification limits throughout 24 months.

At +25 °C, Octanorm remained within the predefined specifications – as defined by EP, US Pharmacopeia (USP), and internal limits – throughout a storage period of six months (data not shown).

3.4. Injectability

Injectability (i.e., force required for injection) is a major performance parameter of parenteral products and affects the ease of administration by patients or health care professionals. As patients have to take care for multiple steps in the application, the ease of infusion is important.

Glide force results showed that for Octanorm infusion, up to 11 N force is needed using either 23G or 25G needles at given infusion rates (Table 7). For comparison, marketed 20% SCIG products were investigated in the same settings (Table 7). Considering that the flow of Newtonian liquids through a needle is characterized by the Poiseuille law [27], the final force is dependent on needle diameter, injection rate and drug product viscosity. In all three investigated settings, the use of Octanorm was associated with less injection force than the 20% SCIG products, with differences up to 21% (SCIG 1) and 34% (SCIG 2). With tissue back-pressure involved, which is known to be linearly dependent on product viscosity [28], the difference would be most likely even more discernible. These data indicate an easier administration of Octanorm relative to SCIG products with higher concentrations. Such a difference can be particularly relevant for elderly patients or those suffering from hand dexterity symptoms [29].

4. Discussion

The demand for purified plasma-derived IgG products for the treatment of a number of diseases, including immune-deficiencies, neurological diseases and autoimmune conditions, is increasing. Currently, intravenous immunoglobulin (IVIG), manufactured from pooled human plasma, is the most widely used plasma component in the world [30]. As an alternative to IVIG, SCIG, showing similar efficacy and safety, is increasingly used as replacement therapy in patients with PID [6,31]. In the situation of poor venous access, systemic adverse reactions and the need for frequent hospital visits, SCIGs offer an alternative way of administration. No severe adverse effects of subcutaneous IgGs have been reported and the medication can be self-administered at home, which is associated with a better health-related quality of life for patients [6,32]. The development of highly concentrated IgG products, improved delivery devices, and alternate methods of delivery will further increase the use of SCIG for the treatment of PID with antibody deficiency [33]. In 1991, Gardulf et al. reported the use of infusion pumps to administer SCIG as a rapid infusion [34].

The aim of this work was to develop a ready-to-use, highly purified, concentrated, safe and stable liquid preparation of polyvalent human IgG for subcutaneous administration.

Octanorm (Immunoglobulin (human) subcutaneous, 16.5%) is a sterile liquid preparation of highly purified immunoglobulin G (IgG). The IgG is purified using a combination of cold ethanol fractionation, ultrafiltration and chromatography. The manufacture of Octanorm is based on the production process of octagam®. The tolerability and safety of this IVIG product has been summarized in a recent publication [35].

Precautions against viral transmission include the following: selection of plasma donors, screening of donations and plasma pool, and quality control measurements of the final product.

Effective viral reduction obtained via a combination of cold ethanol fractionation, S/D treatment and pH 4 treatment during the Octanorm production process was validated according to current international guidelines. An effective reduction in prions was demonstrated for the cold ethanol fractionation and the downstream manufacturing process after ethanol precipitation as a whole. Furthermore, the efficacy of S/D treatment within the octagam®/Octanorm (cutaquig®) production process has previously been shown also against novel viruses, such as ZIKA virus, which has been inactivated to below the limit of detection [36].

Octanorm was developed as an IgG product providing osmolality and viscosity suitable for safe and tolerable use in patients. Injectability data indicated an easier administration of Octanorm and such a difference can be especially relevant in rapid push administration for adolescents or elderly patients who frequently suffering from hand dexterity symptoms.

Maltose was defined as excipient for Octanorm at a target concentration of 7.4% to achieve osmolality and assure IgG stability. The pH value of an immunoglobulin concentrate is an important parameter because it influences - dependent on the stabilizer or excipient(s) added - the tolerability, performance and stability of the product, in particular the content of IgG monomers/dimers/polymers in the released product and during shelf-life. For Octanorm, a mean pH value of 5.3 was determined in this study.

Octanorm is characterized by a low and comparable content of polymers, a low level of accompanying plasma protein and a low isoagglutinin titer. The limit for antibodies against blood cell antigens that can cause hemolytic transfusion reactions, is defined in the EP as a titer of 1:64. Octanorm final container batches revealed an anti-A-hemagglutinin titer of not higher than 1:8 and an anti-B-hemagglutinin titer of not higher than 1:4. The Octanorm manufacturing process is based on cold-ethanol fractionation of frozen plasma. The isoagglutinin depletion during Cohn-like purification processes (up to a five-titer step reduction) was confirmed in recent publications [37,38].

Furthermore, Octanorm showed excellent physico-chemical characteristics as demonstrated by molecular size distribution and dynamic light scattering.

Measurement of Fc function is used to ensure that the biological activity of the Fc portion of the IgG molecule is not damaged during the manufacturing process. The result of the Rubella antigen-based test showed a high level of Fc function for Octanorm (97% on average), which exceeded the requirements of the Ph. Eur. to reach more than 60% of the potency of the reference standard.

Beyond Fc-function, opsonophagocytosis of pathogens is an important part of host defence mediated by the immune system (including IgG). The corresponding assay requires functional integrity of both the antigen-binding site for the bacteria and the Fc portion enabling binding to the phagocyting cell. This complex interaction ensures phagocytosis and inactivation of the intruder, thereby contributing to the prevention of infection and its consequences. Compared with the BRP standard, Octanorm revealed efficient opsonophagocytosis. Additionally, in a cell-based Fc binding assay, Octanorm showed effective binding to cellular Fc-receptors, which comprises an important part of immune defence and regulation.

The polyclonality of immunoglobulin concentrates provides a variety of antibody specificities, including important titers against pathogens. A prerequisite for binding to the specific antigen is structural integrity. In Octanorm, antibodies against pathogens or compounds/toxins thereof were shown to be present in expected and clinically relevant titers or concentrations in accordance with the guidelines.

As demonstrated by the assessment of biochemical, functional, physicochemical and safety related parameters, the intended shelf life of Octanorm is 24 months, stored at +2 °C to +8 °C and protected from light; it can also be stored at room temperature for up to six months within its total shelf-life.

In conclusion, the set target product profile was met, providing Octanorm as a SCIG of high purity, nativity, functional integrity and stability. Octanorm was demonstrated to be safe, tolerable and efficacious in pharmacology/toxicology studies as well as in a subsequent clinical phase III study performed in 18 centers in North America and Europe [39].

Acknowledgements

The authors would like to thank Manfred Karlovits and Roman Gruber for excellent technical assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biologicals.2019.05.002.

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