octaplex® state-of-the-art: implementation of a new nanofilter

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Background and Objectives

octaplex® 500 is a state-of-the-art prothrombin complex concentrate (PCC) for the substitution of clotting factors II, VII, IX and X and is used for the treatment of hereditary or acquired coagulation factor disorders. octaplex® contains also the vitamin K dependent proteins C and S in therapeutically relevant concentrations.

The manufacturing process of octaplex® comprises two chromatographic steps for product purification and two dedicated orthogonal pathogen reduction steps, i.e. solvent detergent (S/D) treatment and nanofiltration to inactivate/remove, respectively, any potentially present lipid enveloped viruses (LEV), non-enveloped viruses (NEV) and infectious prion protein. In order to maintain and further increase the overall pathogen safety, a new nanofilter (20 nm) was implemented replacing the former pathogen filtration step while the product specifications and additional biochemical characteristics had to be maintained.

The aim of this study was to demonstrate the contribution of the recently implemented new nanofilter to the overall pathogen safety of octaplex® 500 while preserving reproducible composition of the product with only low levels of activation markers.

octaplex® Manufacturing Process

Cryo-poor Plasma

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1st Ion Exchange Chromatography (IEC)

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S/D Treatment

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2nd Ion Exchange Chromatography (IEC)

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Nanofiltration 20 nm (NF20)

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Formulation / Sterile Filtration / Filling

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Lyophilisation

Materials and Methods

Pathogen Safety Studies

All pathogen safety studies are according to the international pathogen safety guidelines and were performed with appropriate process intermediates from production batches. All fractions were analyzed by infectivity assays with susceptible cells to determine the virus loads before and after the steps. Prion (263K strain of hamster-adapted scrapie, PrP^{Sc}) removal was quantified by the Western blot assay.

Virus and Cells

Human immunodeficiency virus type 1 (HIV-1) was propagated and titrated on MT-2 cells or C8166 cells. Pseudorabies virus (PRV) and Sindbis virus (SBV) were propagated and titrated on Vero cells. Bovine viral diarrhea virus (BVDV) was propagated and titrated on MDBK cells. Hepatitis A virus (HAV) was propagated and titrated on FRhK-4 cells. Porcine parvovirus (PPV) was propagated and titrated on PK13 cells. The cell cultures were maintained in a CO_2 incubator at + 37°C.

Determination of Virus Titres and Virus Reduction Factors

Virus titres were determined as 50% tissue culture infective doses ($TCID_{50}$/ml) using cells in 96-well microtitre plates (100 µl/well; serial 1:3 dilutions, 8 replicates per dilution). After 7 days (PRV, SBV, BVDV HAV and BVDV), after 15 days (HIV-1/MT-2) and after 10 days (HIV-1/C8166) post inoculation, respectively, cultures were evaluated for virus-induced cytopathic effects. Virus titres were calculated according to the method of Spearman and Kaerber and the relevant CPMP guidelines (CPMP/BWP/268/95).

1st IEC: Batch adsorption with QAE-Sephadex A-50

Due to the fact that the S/D treatment is highly effective against enveloped viruses, this chromatographic step was exclusively examined for its capacity to remove NEVs and prions. The capture step has therefore been validated for HAV, PPV and PrP^{Sc}, supporting the high removal capacity of the nanofiltration step.

An in-process sample was spiked with a defined amount of virus or rather prion (spike ratio: 1:13.6 for HAV, 1:20 for PrP^{Sc} and 1:34 for PPV) and incubated with QAE-Sephadex A-50 in a batch procedure.

S/D treatment with TNBP and Polysorbate 80

The S/D treatment is based on the lipid-membrane destroying properties of TNBP (solvent) and Polysorbate 80 (detergent) towards LEVs. The total capacity of the S/D treatment to inactivate a panel of model viruses with different physicochemical properties was evaluated under process and worst-case conditions. The process intermediates were spiked 1 in 10 with virus and treated with 0.3% TNBP and 1.0% Polysorbate 80 at 27°C for 6 hours. The laboratory process was stopped by a 1:100 dilution with cell culture medium at different kinetic points. Additionally, robustness studies were performed at a lowered S/D concentration and/or a lowered process temperature.

2nd IEC: DEAE-Sepharose FF

An in-process sample was spiked 1:21 with PrP^{Sc} and applied to a DEAE-Sepharose FF radial column.

Nanofiltration with Planova 20N

Virus and prion safety studies using the Planova 20 N filter (Asahi Kasei Co., Ltd., Japan) were performed with the LEVs HIV-1, PRV, SBV, BVDV and NEVs HAV, PPV and PrP^{Sc}, respectively.

The efficiency of the Planova 20N nanofiltration to remove these viruses and prions was evaluated under standard (only PPV) and robustness conditions (significant increase of throughput). The process intermediates were spiked 1 in 100 with virus/prion. In order to minimise the presence of protein aggregates that could clog the filter membrane non-specifically, a pre-filtration step with a 0.22-µm filter was applied before the nanofiltration step.

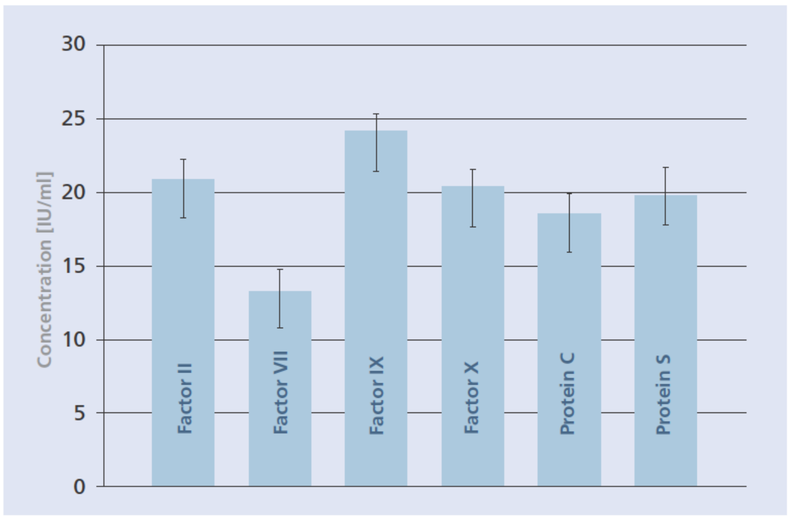
Biochemical Characterisation

Beyond the product release criteria, extended investigations were performed on representative batches octaplex® 500: Prothrombin fragment 1+2 (Enzygnost®, Siemens, Marburg, Germany), Activated factor VII (Staclot VIIa-rTF, Stago, Asnieres, France), FIIa-like activity (Substrate S-2238, Fisher Diagnostics, Vienna, Austria), proteolytic activity (Substrate for Serine proteases CS-05(88), Hyphen Biomed, Neuville-sur-Oise, France), FXa-like activity (Substrate CS-11(32), Hyphen Biomed, Neuville-sur-Oise, France).

Evaluation of a potential thrombogenic risk of octaplex® was performed in the Wessler stasis model based on Wessler et al (J. Appl. Physiol. 14, 943 – 956 (1959).

Results

A balanced content of coagulation factors and inhibitors is maintained in octaplex® 500



The activites of the active ingredients were quantified in octaplex® 500 batches produced with the new nanofilter (n=32, mean ± SD)

Coagulation activation markers are present in low amounts only in octaplex® 500 which is also reflected by the absence of a thrombogenic effect in the Wessler test

Parameter	Mean value ± SD
ratio FVIIa / FVII	0.21 ± 0.03 IU/IU (n=8)
proteolytic activity / FIX	0.22 ± 0.02 mU/IU (n=8)
FIIa – like activity	< 0.17 IU/ml (n=5)
FXa – like activity	< 3.4 nkat/ml (n=5)
Thrombus formation in the Wessler Test	score 0, i.e. no clot (n=3)

Pathogen safety studies show efficient inactivation and reduction of pathogens

Results of the pathogen safety studies are summarized in the table including the reduction factors achieved with the new nanofilter (NF20). Log reduction factors (LRF) and global reduction factors (GRF) of the octaplex® manufacturing process are presented as log_{10}.

Step/ Pathogen	HIV-1	PRV	SBV	BVDV	HAV	PPV	PrP^{Sc}
1st IEC	n.d.	n.d.	n.d.	n.d.	2.63	2.16	2.38*
S/D	≥ 6.37	≥ 7.19	≥ 7.34	n.d.	n.a.	n.a.	n.a.
2nd IEC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.80
NF20	≥ 6.55	≥ 7.09	≥ 6.61	≥ 5.91	≥ 6.08	5.40	≥ 3.58
GRF	≥ 12.92	≥ 14.28	≥ 13.95	≥ 5.91	≥ 8.71	7.56	≥ 7.76

IEC: ion-exchange chromatography; n.d.: not done; n.a.; not applicable;
*combined reduction factor for cryo-precipitation and 1st IEC

Conclusion

The pathogen safety steps implemented in the octaplex® manufacturing process helped to safeguard patients from infectious transmission for more than a decade, while the implementation of a new nanofilter (20 nm) maintained or even further increased pathogen reduction capacities of the process.

The product characteristics were maintained, which was confirmed by extended biochemical investigations. The specified parameters of octaplex® remained unchanged and extended investigations demonstrated that the biochemical characteristics were fully maintained.

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