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Short paper

Virus removal capacity at varying ionic strength during nanofiltration of AlphaNine® SD

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ABSTRACT

Nanofiltration is incorporated into the manufacturing processes of many protein biopharmaceuticals to enhance safety by providing the capacity to retain pathogens while allowing protein drugs to pass through the filter. Retention is mainly a function of size; however, the shape of the pathogen may also influence retention. The ability of the Viresolve® Pro nanofilter to remove different sized viruses during the manufacture of a Coagulation Factor IX (Alphanine® SD) was studied at varying ionic strength, a process condition with the potential to affect virus shape and, hence, virus retention. Eight viruses were tested in a scale-down of the nanofiltration process. Five of the viruses (EMCV, Reo, BVDV, HIV, PRV) were nanofiltered at normal sodium processing conditions and three (PPV, HAV and WNV) were nanofiltered at higher and lower sodium. Representative Reduction Factors for all viruses were ≥4.50 logs and removal was consistent over a wide range of ionic strength.

1. Introduction

The ability of nanofiltration to enhance the safety margin of human plasma-derived protein biopharmaceuticals has been demonstrated in studies showing removal of viruses and the agents associated with transmissible spongiform encephalopathies (TSEs) when they are spiked into laboratory models that mimic the industrial scale nanofiltration conditions [1–4]. The Coagulation Factor IX (FIX) product, AlphaNine® SD, is a nanofiltered, plasma-derived product that is used extensively in the treatment of Hemophilia B [5]. It is currently nanofiltered through a Viresolve® 70 (V70) nanofilter (Millipore Corporation) which has a mean pore size <20 nm. In order to identify an alternate to the V70 nanofilter, we evaluated several other nanofilters also with mean pore sizes ≤20 nm and identified one, the Viresolve® Pro (Millipore Corporation), with protein sieving characteristics similar to the V70 [6]. This is a recently marketed nanofilter and laboratory studies demonstrating its robustness in removing multiple viruses with different physico-chemical properties have not been extensively reported. Therefore, we report here the results of virus validation studies performed with the VPro nanofilter and AlphaNine® SD that show that this filter is capable of efficiently removing both enveloped and non-enveloped viruses spiked into a plasma protein solution over a wide range of process conditions. These studies were performed according to international guidelines [7–10].

2. Materials and methods

2.1. Nanofiltration samples

The intermediate used in these studies was manufactured at Grifols and represented the purified elution pool from the final chromatography column in the AlphaNine® SD manufacturing process [3]. The elution pool contained sodium citrate and sodium chloride at pH 6.8. The sodium concentration of the pools used in this study ranged from 0.63 to 0.66 Eq/L which is representative of the normal process sodium at this stage and within the range evaluated in earlier studies [6]. For some experiments, an elution pool with a higher sodium concentration (0.70 Eq/L) was prepared by the addition of NaCl. For other experiments, an elution pool with a lower sodium concentration (0.40 Eq/L) was prepared by diluting a normal elution pool sample with a low sodium buffer. If needed, the salt adjusted samples were ultrafiltered to reach the protein concentration of the typical elution pool.

2.2. Viruses and infectivity assays

Infectivity was quantified by the 50% Tissue Culture Infectious Dose ($TCID_{50}$) infectivity assay. Five-fold serial dilutions of samples

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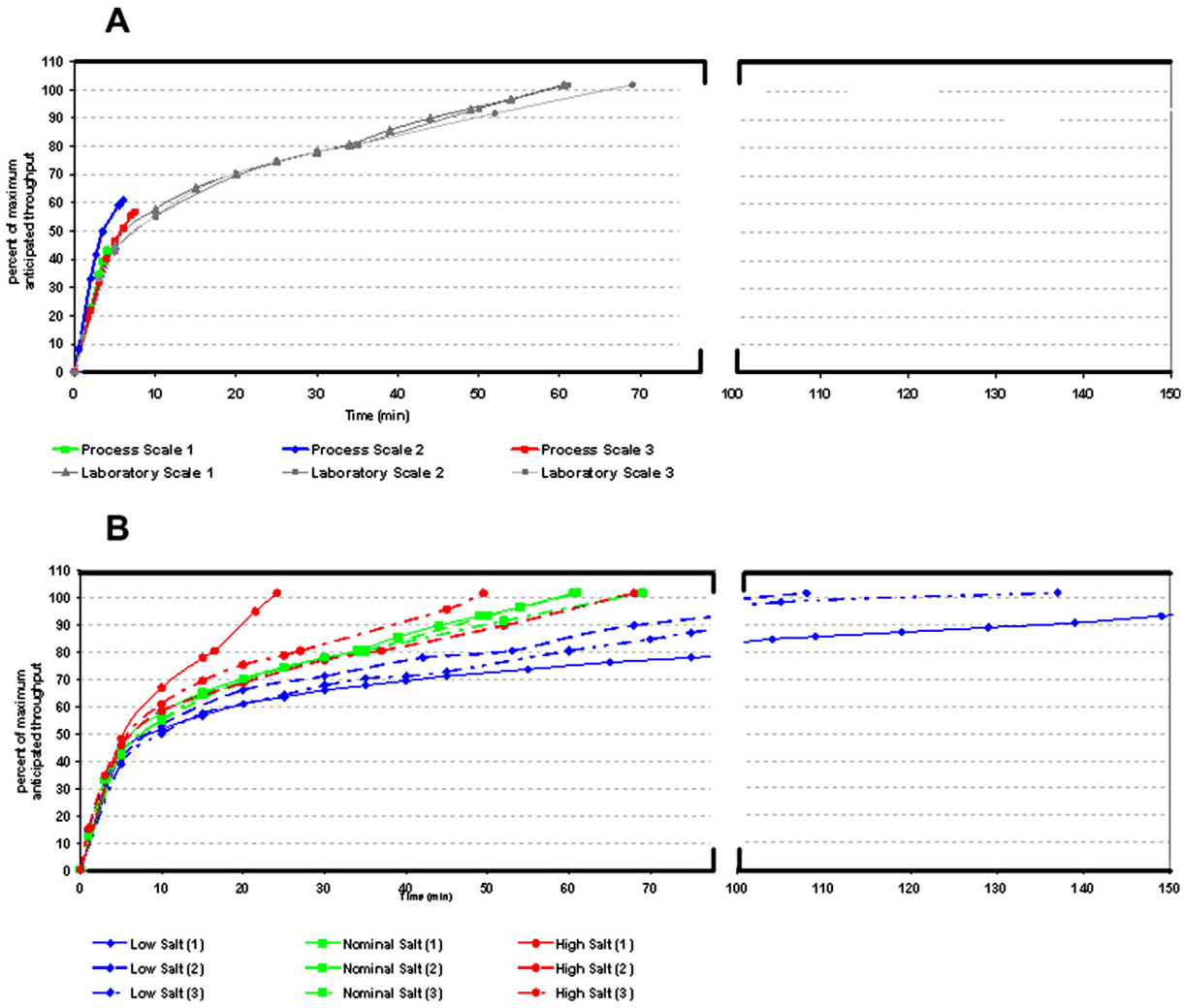

Fig. 1. Viresolve Pro filtration profile. (A) Process scale filtration profile from three industrial lots and three experimental lots at normal salt. (B) Laboratory scale filtration profile from three different experiments at high, normal and low salt conditions.

were prepared in media and each dilution was inoculated at 50 μl (250 μl for HAV) per well into eight replicate wells of a 96-well plate seeded with the indicator cell line. The non-enveloped viruses, porcine parvo (PPV), encephalomyocarditis (EMCV), hepatitis A (HAV) and reo-3 (Reo) were assayed in PK13, L-929, FRhL-4 and L-929 indicator cells, respectively. The enveloped viruses, west nile (WNV), bovine viral diarrhea (BVDV), human immunodeficiency (HIV-1) and pseudorabies (PRV) were assayed in Vero, EBTr, C8166 and Vero indicator cells, respectively.

Virus titers were calculated per Spearman–Kärber [11,12] and expressed as $\log_{10}$ $TCID_{50}$/ml. Before testing, the minimum dilution of product needed to ensure that the product had no toxic effect on indicator cells or virus, was determined. In samples where a low titer was expected, an additional 800 wells were plated with the minimally diluted sample to enhance the limit of detection. When no viruses were detected, the titer was expressed as the limit of detection of the technique with a confidence limit of 95%, and calculated according the Poisson distribution of probabilities [9,10].

2.3. Virus spiking tests

Elution pool samples were spiked at 1% (v/v) with viruses and prefiltered using filters with a pore size slightly larger than the virus (0.1 μm for PPV, EMCV, HAV, Reo, WNV and BVDV; 0.22 μm for HIV; 0.45 μm for PRV). The VPro Micro Devices used in the laboratory studies contained 3.1 cm^2 of filtration area, a 5000-fold scale-down of the nanofiltration manufacturing process. To ensure the robustness of results, multiple lots of filters and product were used in the studies.

Nanofiltration was performed in a dead-end mode at 5 ± 3 °C and a feed pressure of 45 ± 5 psi. Filters were pre-rinsed with water and citrate buffer. After nanofiltration, the nanofilter was post-washed with buffer equal to 20% of the filtrate volume. Filter integrity was confirmed via a post-use air leakage test.

Reduction factors (RFs) for the nanofiltration step were determined as total virus detected in the spiked material before nanofiltration divided by total recovered virus after nanofiltration (including postwash), expressed in $\log_{10}$. When no virus was detected in the nanofiltered product, the amount of virus remaining after nanofiltration was reported as ≤ the limit of detection for the virus infectivity assay. When these values were used to calculate RFs, the RF result is necessarily reported as a ≥ value.

3. Results

The efficiency of virus removal during VPro nanofiltration of AlphaNine® SD was evaluated in spiking studies using eight clinically relevant or model viruses. Studies were performed with