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Mechanistic modeling of viral filtration

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ABSTRACT

A simple model based on changes in filter resistance and active area of the membrane has been used to model viral filtration. Viral particles have been modeled as colloidal particles disregarding any specific interaction and considering only passive transport in the system. The model is based on the assumption that purely steric interactions determine the ratio of concentration of viral particles inside the pore to concentration in solution at the pore mouth. Viral particles rejected by the membrane form a layer of high concentration near the membrane and this layer offers additional resistance to filtration. The membrane flux has been calculated by applying Darcy's law. The overall model involves use of six unknown parameters to account for cake formation, nature of virus, interaction between the virus and the membrane, and pore size. The breakthrough of the model virus, bacteriophage ϕX-174, through normal-flow virus filters using commercial process fluids has been chosen as the system used for model validation. The model has been fitted to the time profile of flux and the log reduction value (LRV) of viral particles across the different types of commercially available filters. The model will be useful when performing studies using scale down models for correlating LRV to flux decline. The model also provides us insights into the underlying mechanisms behind viral clearance achieved from the various commercially available viral filters.

1. Introduction

Monoclonal antibody (mAb) products have emerged as an extremely important and valuable class of therapeutic products for treatment of cancer and various other diseases like rheumatoid arthritis, auto-inflammatory disease, allergic asthma and multiple sclerosis [1]. Mammalian cell lines that are most commonly used for expression of this class of product are also known to be prone to contamination with adventitious viruses and endogenous retroviral particles [2–7]. As a result, creating and demonstrating a comprehensive viral clearance strategy is a regulatory requirement for successful commercialization of these products.

To ensure robust viral clearance, a typical downstream process has multiple steps that are capable of either virus removal or inactivation. Examples of steps that provide significant viral clearance include low pH inactivation [8], chromatography [9] and virus filtration [10]. Since a single step is not sufficient to provide adequate clearance to all types of viruses, a combination of orthogonal techniques is required. Endogenous enveloped retroviral particles can be successfully cleared through low pH inactivation and chromatography [11]. Small, non-enveloped adventitious viral particles (e.g. parvoviridae family) are however relatively difficult to remove by these methods. Normal-flow filtration has been successfully used to remove the latter type of viruses [10,12]. Commercially available filters are typically of two kinds: one for the removal of large retroviruses and the other for the removal of both large retroviruses and small proviruses.

Development of a viral filtration step is based on spike/recovery experiments that are performed using scale-down models in the lab and using multiple model viruses [6,13]. Use of the scale down models is cost effective and practically feasible [10]. Once the viral filtration process has been developed, validation is performed by spiking known amounts of model viral particles in process intermediate and quantifying viral LRV across a given membrane filter. Most virus retentive filters provide a LRV of 3–5 [11,14]. Though most commercially available filters provide the required clearance, these filters are invariably expensive due to the precise pore size distribution that is required and are also prone to fouling due to the small pore size (<20 nm). As a result, viral filtration makes a very significant contribution to the overall cost of downstream processing of biotech therapeutics.

In view of variability in the feed stream characteristics as well as filter properties (lot-to-lot), sizing the viral filter for use in a

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manufacturing plant is a complex task. In absence of a mechanistic model, manufacturers tend to oversize the filters as a precautionary measure (by as much as 30%) further adding expenditure to manufacturing. Thus, a model based on transport mechanism that can correlate flux to LRV will be useful in calculating the desired time and the filter area required to provide the require clearance and will improve our accuracy of sizing a viral filter and also reducing the wasteful and expensive oversizing.

With respect to fouling of viral filters, four mechanisms have been proposed: complete pore blocking, intermediate pore blocking, cake filtration and standard blocking [15–17]. The mechanisms differ in whether the particles deposit inside or on top of the membrane pores. Recently, all of these models have been combined and applied towards microfiltration and ultrafiltration of biological fluids [18]. The application involved filtration of virus from a solution containing human plasma IgG and BSA. The combined model was shown to fit well both the modes of filtration – constant pressure filtration and constant flux filtration. More recently, an extension of these models has been suggested to incorporate the effect of flow rate on the deposition of particles for constant pressure filtration [19]. The possibility that a greater flow rate would mean a lower residence time and hence lower deposition of particles inside the pores has been highlighted. The model was shown to provide improved fits for filtration of BSA and human IgG. Another model that combines pore blocking and cake filtration as two stages of filtration to describe the stirred cell filtration through microfiltration membranes has also been proposed [20]. This model accounts for the difference in the thickness of the cake across the membrane and provides good fit for the filtration of BSA. It has, however, not been tested for filtration of viruses.

In this paper, we evaluate previously published data [21] and propose a model that explains the fouling mechanism during viral filtration, wherein viral removal is based entirely on size exclusion as opposed to adsorption [22]. The proposed model accurately fits change in LRV and flux as a function of time for the various different kinds of viral filters that are commercially available. The model has been validated using data on filtration of parvovirus (ϕX-174) by direct-flow small-virus retentive filters.

2. Materials and methods

2.1. Model protein

The model TP (therapeutic protein), a human IgG, is an in-process intermediate from a proprietary protein product from Eli Lilly and Co. Monomer TP was generated by filtration sequentially through two 10.16 cm (4 in.) Viresolve NFP cartridges containing V180 membrane. The detailed procedure for preparing stock protein solution has been described elsewhere [22].

2.2. Phage procedures

ΦX-174 and *Escherichia coli* C (A.T.C.C. 13706) were obtained from the Felix d'Herelle Reference Center for Bacterial Viruses (Quebec, Canada). The detail procedure for preparing stock viral particle has been described elsewhere [22].

2.3. Phage-retention experiments

The filtration methodology included collection and assay of fractions taken over the course of filtration ('grab samples'). The test filters were small-scale disc or hollow fiber devices supplied by the four filter manufacturers for use in scale-down validation studies. Filters from Millipore and Sartorius Stedim were commercially available devices (25 mm syringe filter units). Filters from Pall were coupons in stainless steel housings. In all cases, the filter is composed of multiple layers "fused" together. These were Optiscale-25 devices with Viresolve NFP membrane (3.5 cm^2 surface area Millipore), Virosart CPV filter discs (5.3 cm^2 surface area; Sartorius), Ultipor VF grade DV20 filter discs in a reusable stainless-steel housing unit (Pall housing part number FTK200; 11.1 cm^2 surface area Pall Corp.), and Planova 20N small-scale hollow-fiber devices (10.0 cm^2 surface area Asahi Kasei). Filtration experiments were performed at ambient temperature and at vendor-recommended pressures of 207 kPa (30 lbf/in.^{2}) for Viresolve NFP, Virosart CPV, and Ultipor DV20 and of 98 kPa (14.2 lbf/in.^{2}) for Planova 20N. Devices were first pre-wetted with test buffer (without protein). The initial two hold-up volumes (containing mostly buffer, not model protein/phage solution) were discarded and not counted towards throughput in l/m^2. Challenge solutions were processed through a 0.22-μm-pore-size filter before being added to the pressure vessel. The system was then pressurized and the volume of filtrate measured every 1–5 min for the duration of the test using precision timers and balances (process fluid density is assumed to be 1 g/ml). The initial flow rate of buffer was used to calculate flow rates at specific time or passage volume points. The degree of decay from the initial flow varied with the particular filter and protein/phage combination. Samples were collected directly from the filter devices at the start of the run, and at the target flow reductions or passage volume points. These samples measure the phage-retention capability of the filter at the time of sample collection, as opposed to pooled filtrate samples typically collected in validation studies, which measure virus retention of the filter over the course of an entire run. LRVs were calculated as follows:

$$\mathrm{LRV} = \log(C_b/C_p) \tag{1}$$

where, C_b is the phage titer in the challenge solution and C_p is phage titer in the filtrate grab samples (permeate).

3. Model

A simple model based on changes in filter resistance and active filter area has been used to model viral filtration. This model has been used to fit the previously published data on viral filtration [21]. Viral particles are modeled as colloidal particles disregarding any specific interactions and considering only passive transport in the system. The model is based on the following arguments:

- Purely steric interaction is assumed to determine the distribution coefficient (Φ), i.e., ratio of concentration of viral particles inside the pore to concentration in solution just at the pore mouth. Using either geometrical [23] or statistical arguments [24], Φ can be related to the ratio of size of viral particles to size of pore of membrane ($\lambda = r_v/r_p$) as given below

$$\Phi = (1-\lambda)^2 \tag{2}$$

- Viral particles rejected by the membrane form a layer of high concentration called the concentration polarization (CP) layer at the membrane surface which offers additional resistance to filtration.
- The CP layer grows with time as an unsteady-state penetration layer where the growth rate is independent of concentration and depends on diffusion coefficient. Concentration and rejection determine the range of concentrations encountered in diffusion boundary layer. The time dependent thickness of the CP layer (δ) can be related to the time of filtration as given