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Determination of pore size gradients of virus filtration membranes using gold nanoparticles and their relation to fouling with protein containing feed streams

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ABSTRACT

Virus filtration membranes contribute to the virus safety of biopharmaceutical drugs due to their capability to retain virus particles mainly based on size-exclusion mechanisms. Typical product molecules like monoclonal antibodies with 9–12 nm in hydrodynamic diameter have to be transmitted by > 95% while small viruses, e.g. *parvoviridae* (B19, MVM, PPV) with a diameter of 18–26 nm, have to be retained by at least 99.99%. Therefore, membrane fouling caused by product aggregates, which are similar in size compared to the viruses that have to be retained, is a common observation. Minimal membrane fouling is a requirement for economical processes and is influenced by both the membrane surface chemistry and the membrane structure, particularly with regard to the pore size gradient (PSG). In this work, virus filtration membranes were challenged with gold nanoparticles (GNPs) in order to determine PSGs for a wide range of different commercial and non-commercial parvovirus retentive membranes differing in structure, material and surface chemistry. GNP adsorption to the membrane material was suppressed by the use of an anionic surfactant, allowing to gain insights into size-exclusion properties of the membranes. Membrane performance with regard to fouling was further investigated by determination of protein mass throughputs up-to a defined membrane flux decay using solutions containing intravenous immunoglobulin (IVIG) as model protein. Additionally, the fouling mechanism of IVIG was investigated and confirmed to be caused by trace amounts of species larger than IVIG monomers and dimers, which were already present in the feed. The fouling results are discussed in relationship to the determined PSGs, since the porous support structure of virus filtration membranes can act as a depth pre-filter protecting the separation-active layer from particulate foulants.

1. Introduction

Parvovirus retentive virus filters (VFs), designed to remove even small viruses like parvoviruses with 18–24 nm in diameter, have become a widely applied and mature industry standard [1,2]. These filters are expected to provide robust parvovirus reduction of ≥99.99% by size-exclusion and thereby significantly contribute to pathogen safety of biopharmaceutical drugs derived from mammalian cell culture or from human blood plasma. IgG-type antibodies, a major class of biopharmaceutical molecules to which most of the commercial monoclonal antibodies belong and which are the main component of intravenous immunoglobulin (IVIG) [3], exhibit hydrodynamic diameters of 9–12 nm for aggregate free solutions [4]. VFs, which are expected to transmit such product molecules by > 95%, are reported to have only slightly larger mean pore diameters of 12–17 nm [5,6]. By having narrow pore size distributions, VFs achieve high selectivities which enable the mandatory high virus retention while allowing quantitative product transmission.

Typical product related membrane foulants, e.g. protein aggregates, often are in the size range of parvoviruses and effectively foul the membrane due to pore blocking [7,8]. Such membrane fouling was also found to reduce virus retention performance of VFs [9]. Studies of Barnard et al. using monoclonal antibodies revealed that trace amounts of protein aggregates (1×10^{-4}% of the total mass of protein in solution) in the size range of 20–40 nm are sufficient to cause significant flux decay during virus filtration [7]. Furthermore, Brown et al. determined the size range of dimers up to pentamers of IgG-type monoclonal antibodies to be 16–23 nm with respect to their hydrodynamic diameter, which is very similar to the reported size range for parvoviruses [8]. Other smaller impurities like product fragments, host cell

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List of symbols

A	Membrane area [m^2]
$A_{220\ nm}$	Absorbance at 220 nm [a.u.]
$A_{280\ nm}$	Absorbance at 280 nm [a.u.]
$d_{particle}$	Particle diameter [nm]
d_{pore}	Effective pore diameter [nm]
d_{TEM}	Particle diameter measured with TEM [nm]
J_{IVIG}/J_{H2O}	Flux ratio of IVIG solution to pure water [dimensionless]
m/A	Protein mass throughput per membrane area [kg/m^2]
x	Membrane depth [μm]
x_{max}	Membrane depth at intensity maximum of GNP band [μm]
$\delta_{hydrodyn}$	Hydrodynamic correction term [nm]
δ_{SDS}	Correction term for pore narrowing by SDS adsorption [nm]
∇d_{pore}	Pore size gradient [nm/μm]

proteins and DNA can also cause fouling due to adsorption to the membrane. One strategy to maximize the throughput of the virus filtration step is to locate the unit operation of virus filtration close to the end of the downstream purification process, where the feed typically has already a high purity [10]. Another strategy involves the specific optimization of the unit operations upstream of the virus filtration to further reduce impurities [11].

Fouling resistance can also be achieved by developing membranes with low interacting surfaces. Therefore, membrane manufacturers use low interacting base materials like regenerated cellulose (RC) or render high interacting base materials like polyethersulfone (PES) or polyvinylidene fluoride (PVDF) low interacting by a separate surface modification step.

Besides a low interacting membrane surface, the pore size gradient (PSG) of a VF can have a considerable impact on its fouling resistance, especially with feeds containing impurities larger than the monomeric protein product. In contrast to ultrafilters (UFs), which have thin separation-active layers (SALs) of single digit microns thickness and support structures having pore sizes that are magnitudes larger compared to the ones within the SALs, VFs can have thick SALs with up to 30 μm thickness, consisting of a multitude of pore layers, contributing to the high selectivity [12–14]. While UFs have very steep PSGs, commercially available VFs exhibit a wide range of PSGs from UF-like steep to very shallow [15]. Some early VFs on the market were derived from UFs exhibiting similar membrane structures. For UFs and UF-like VFs operated in cross-flow mode with the SAL facing towards the feed, the support structure acts mainly as mechanical support for the thin SAL and does not contribute to the fouling resistance. Some studies using these early commercial, UF-like structured VFs, intended for application in cross-flow mode, investigated the impact of membrane orientation on fouling behavior [16–18]. The authors of these studies found significantly increased performance with respect to fouling resistance when the membranes were oriented with the SAL facing away from the feed. In such orientation the mode of operation, either dead-end or cross-flow, had no impact on anti-fouling performance. The performance increase was attributed to the porous membrane support structure, acting as a depth pre-filter. This pre-filtration effect protects the SAL from fouling by “trace levels of submicron-sized particles” [17]. Nowadays all commercially available VFs are used in the orientation with the SAL facing away from the feed. Those VFs are also designed for dead-end use. Wide application in dead-end mode is also due to higher simplicity of use and lower capital requirements for filtration equipment.

The effectivity of the support structure acting as a pre-filter is highly dependent on the actual PSG. Commercial VFs with an UF-like steep PSG offer high water permeability. Their thin SAL is the main contributor to the overall hydraulic resistance of the membrane. Due to the steep PSG only a small section of the membrane in close proximity to the SAL has pore sizes in the range of protein aggregates, which are probably < 40 nm [7], resulting in low depth pre-filter capacities and higher susceptibility towards fouling. Accumulation of foulants being retained by size exclusion closely to the SAL of microfiltration membranes having steep PSGs was visualized by other groups using confocal scanning laser microscopy [19,20]. VFs with shallow PSGs have typically lower water permeability as a larger part of the membrane structure contributes to the hydraulic resistance. This can increase processing times which negatively impacts the productivity of such VF. On the other hand, such membranes often show higher robustness against fouling due to the fact, that a larger part of the membrane structure is capable of retaining the fouling species. However, membranes having very shallow PSGs with constant mean pore diameters across the membrane cross-section, in the typical range for parvovirus retentive VFs, could be very susceptible to fouling. As larger foulants would be mostly retained close to or directly on the outer membrane surface facing towards the feed, such a membrane would exhibit low depth pre-filter capacities and low fouling robustness similar to a membrane with a very steep PSG. Track-etched membranes typically have cylindrical pores and therefore no PSG. Such membranes are very rarely used in biopharmaceutical processes due to their high susceptibility towards fouling with aggregate containing protein feed streams [21]. An overview of commercial VFs including a very qualitative classification regarding their PSGs was given by Miesegaes et al. [22].

PSGs that exhibit pore sizes reflecting the particle size of foulants could help to utilize a large extent of the total membrane structure to retain fouling species in order to maximize the depth pre-filter capacity while keeping the hydraulic resistance as low as possible achieving highest possible permeability. However, we are not aware of a technique that is capable to quantitatively determine the PSG in normal direction to the outer surface of a parvovirus retentive membrane. Determining the PSG is an especially challenging task with view on method's applicability towards the high diversity of membrane materials and surface chemistries that commercial VFs exhibit [22].

Utilizing high resolution imaging techniques such as scanning or transmission electron microscopy (SEM/TEM) to investigate membrane cross-sections already provides good qualitative impressions of membrane structures and related PSG. Quantitative analysis using SEM/TEM is significantly more challenging [23] and often limited to determination of pore sizes on the outermost membrane surface [24] rather than within the inner membrane structure. Samples of membrane cross-sections need to be prepared carefully by freeze fracturing or microtomy avoiding artifacts that alter the pore structure (e.g. by compaction or smearing). In contrast to track-etched membranes with cylindrical pores, VFs have a complex sponge-like pore structure with highly interconnected pores. Quantitative image analysis of such structures requires binarization of grayscale images which is a very subjective procedure with resulting pore sizes highly dependent on the individual operator [23]. For the evaluation of two-dimensional images, also simplifications with respect to the definition of the term “pore size” are required. Ziel et al. proposed a computer-aided method to determine PSGs of 0.2 μm rated microfilters measuring the distances of the pore voids using “equidistant lines parallel to the (outer) membrane surface and perpendicular to the flow direction in the membrane” (= mean free path length) [25]. While the PSG throughout the whole cross-section was determined quite well, the magnitude of the mean free path lengths was significantly larger than 0.2 μm, even in the SAL. This is related to the simplified definition of the mean free path length, which can be determined from a single two-dimensional image of a membrane cross-section. For a proper quantitative description of the