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Ultrafiltration of Parvovirus

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Abstract: Ultrafiltration is frequently used in the biotechnology industry for protein purification. The main applications of ultrafiltration are for protein concentration and buffer exchange. This paper focuses on ultrafiltration for purification of parvovirus. The feasibility of using ultrafiltration to remove contaminating host cell proteins from the virus particles has been investigated. Purification of virus particles and virus vectors for clinical applications of gene therapy and in the manufacture of viral vaccines is a major large-scale separations problem. Today, parvoviruses, such as adeno associated virus, are being extensively investigated as gene therapy vectors. Consequently, development of robust purification operations will be essential.

Tangential flow ultrafiltration and high performance tangential flow filtration of Aedes Aegypti densonucleosis virus has been investigated using flat sheet membranes with a nominal molecular weight cut off of 100 and 300 kD. Virus particles were detected in the permeate of the 300 kD membrane for both modes of operation. In tangential flow filtration no virus particles were detected in the permeate from the 100 kD membrane. However, during high performance tangential flow filtration significant passage of virus particles through the membrane was observed. The results obtained here are in general agreement with results obtained in previous studies of high performance tangential flow filtration for protein purification. Optimization in the operating conditions of high performance tangential flow filtration

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may result in a highly selective unit operation for purification of virus particles and virus vectors.

Keywords: High performance tangential flow filtration, membrane, parvovirus, ultrafiltration, virus filtration

INTRODUCTION

Purification of viruses and virus vectors for clinical applications of gene therapy and in the manufacture of viral vaccines will require the development of robust unit operations (1). Gene therapy based treatments of diseases, such as cancer, arteriosclerosis and osteoporosis, involve delivering DNA specifically to affected cells in order to reinitiate cellular production of therapeutic proteins or peptides. Modified virus vectors represent one possible delivery vehicle. Similarly, vaccine vector delivery systems are essential if vaccines are to be developed against viruses such as immunodeficiency virus (HIV) (2). Promising delivery vectors for HIV vaccines include plasmid DNA and replication incompetent adenoviruses (3, 4).

Tangential flow ultrafiltration is frequently used in the biotechnology industry during protein purification for concentration and buffer exchange. Ultrafiltration membranes have pore sizes ranging from 1 to 100 nm. If the protein is larger than the nominal molecular weight cut off of the membrane, ultrafiltration may be used to concentrate the protein product. In diafiltration mode a buffer is added to the feed reservoir while the permeate is removed. If the buffer is added at the same rate at which the permeate is removed, the process is known as constant volume diafiltration. The buffer that is added may be the same or different to the feed buffer. By using a different buffer the protein product will be suspended in a new buffer (buffer exchange) (5).

Large-scale fractionation of proteins by ultrafiltation membranes has been investigated in the past. The required separation factor for a given unit operation depends on the specific product being purified, the toxicity of the contaminant, and the separation factors obtained by other unit operations in the purification train. It has generally been assumed that separation of solutes, which differ in size by less than an order of magnitude, is difficult by tangential flow ultrafiltration. However many recent studies indicate that by carefully controlling the operating conditions efficient separation of solutes, which differ in size by less than an order of magnitude, is possible (6). For example, Pujar and Zydney (7) have demonstrated the importance of electrostatic and electrokinetic interactions in determining selective protein transport through ultrafiltration membranes. Saksena and Zydney (8), showed that the selectivity (defined as the ratio of protein sieving coefficients) for bovine serum albumin (BSA) and immunoglobulin G (IgG) could be increased from 2 at pH 7 to more than 30 by adjusting the pH to 4.7 and lowering the solution ionic strength. Similar improvements in performance

have been reported for laboratory scale filtration of BSA and hemoglobin (van Eijndhoven et al. (9)), BSA and lysozyme (Iritani et al. (10)) and myoglobin and cytochrome C (Yang and Tong (11)).

Van Reis et, al. (12) have investigated the possibility of protein fractionation using high performance tangential flow filtration (HPTFF). In conventional tangential flow ultrafiltration (TFF), the feed pressure varies along the feed flow path from the inlet pressure (P_i) to the outlet pressure (P_o). Usually P_o is close to atmospheric pressure. The average transmembrane pressure (TMP) is given by $TMP = ((P_i + P_o)/2) - P_p$ where P_p is the permeate side pressure. However since the feed side pressure varies from P_i to P_o the TMP will also vary along the feed flow path. This variation in TMP along the feed channel will reduce the resolving power of TFF systems (12). Figure 1 is a schematic representation of the TFF set up. Permeate is collected in a permeate reservoir while the retentate is returned to the feed reservoir. In HPTFF, part of the permeate is returned to the permeate side of the module (see Fig. 2) such that the permeate flows co-current to the feed. By creating an axial pressure drop along the permeate flow channel, the TMP is more nearly constant throughout the module.

Many investigators (12–16) have found that high separation factors for solute species with size differences less than an order of magnitude were obtainable using HPTFF. Their results indicate that maintaining a constant transmembrane pressure across the entire length of the membrane leads to a much finer fractionation of proteins.

Here, we have investigated the use of ultrafiltration for purification of Aedes aegypti densonucleosisvirus (AeDNV). The virus particles are 18–26 nm in diameter. Removal of host cell proteins is likely to be challenging as the virus particles are very small. In this work we compare the passage of virus particle and host cell proteins through the pores of 100 and 300 kD membranes. Ideally, we aim to retain virus particles in the retentate while removing host cell proteins in the permeate. TFF and HPTFF modes of

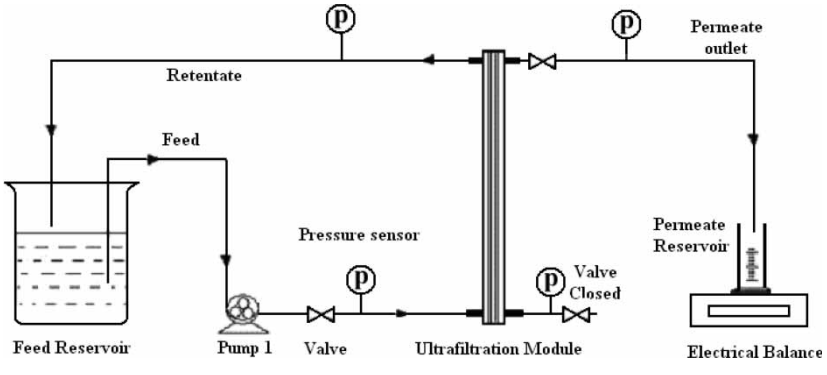

Figure 1. Tangential flow filtration setup.

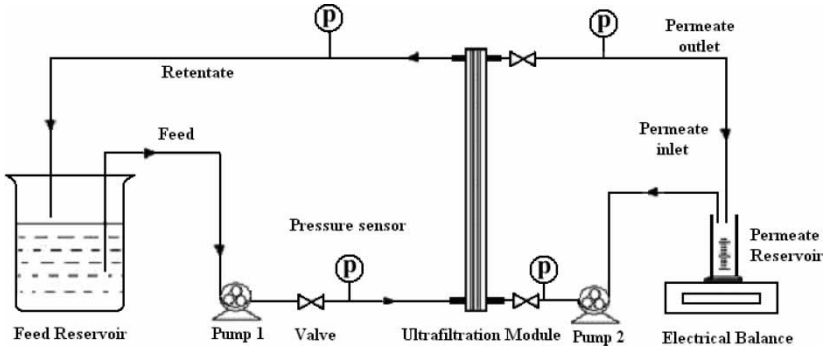

Figure 2. High performance tangential flow filtration setup.

operation are compared. We focus on ultrafiltration of AeDNV for a number of reasons. AeDNV is highly pathogenic to Aedes aegypti and a number of other Culicine mosquito species. The Aedes aegypti mosquito is a vector of the viruses that cause dengue and yellow fever, and AeDNV shows promise as a biological control agent with important applications in integrated vector control programs against mosquito-born diseases.

AeDNV, like AAV, is a parvovirus. Both viruses are similar in size (18–26 nm). AeDNV particles are easy to grow using a cell culture based system. Further, a PCR based assay is available, which allows easy measurement of the virus titer (17, 18). Consequently, AeDNV could serve as a model for studying tangential flow filtration of parvoviruses. AAV are being extensively investigated as gene therapy vectors since they do not induce an immune response toward viral components; they can integrate into human chromosome 19 and they do not require active dividing cells for transduction and are non pathogenic (1). Currently, purification AAV from cell lysates is generally conducted using density gradient centrifugation. However, these vector preparations are contaminated with impurities that cause local inflammation in vivo (19–21). Thus there is a need to develop robust and scalable purification operations for AAV.

EXPERIMENTAL

Production of AeDNV Particles in Serum Free Medium

AeDNV particles were produced using the A. albopictus cell line C6/36 in a serum and protein free medium (SFPFM) (SF-900 II SFM, Invitrogen Corporation, Grand Island, NY). The C6/36 cell line was grown at 28°C in T-75 flasks containing 14 ml SFPFM medium supplemented with 1% penicillin-streptomycin (Invitrogen Co., Carlsbad, CA). The pH of the medium was 6.2–6.4. When the cells reached 40–80% confluency, the A. albopictus cell

line C6/36 was transfected with pUCA, an infectious clone containing the AeDNV genome, by using Qiagen effectine kit (Qiagen, Valencia, CA) (22). The media was changed 8 to 18 hours post-transfection to remove the toxic Qiagen transfection solution. Four days post-transfection, after a clear cell monolayer was visible, transfected C6/36 cells were transferred from the T-75 flask to a 125 mL spin flask (stirred bioreactor) (Wheaton Science Products, Millville, NJ) at a cell concentration of 5.5×10^5 cell/mL. The total medium volume was 100 mL. The bioreactor was stirred at 300 rpm at 28°C. Five to six days later, when the cell concentration reached around $5-6 \times 10^6$ cells/mL, cells were transferred to a second 500 mL bioreactor at a cell concentration of 5.5×10^5 cell/mL and a medium volume of 300 mL. About a week later, the cells were collected and frozen, and thawed three times, then centrifuged at 3,750 rpm for 15 min at 4°C to remove cell debris. The supernatant containing AeDNV particles was filtered using a 0.45 μm filter and stored at −80°C for future use as the feed stream for the ultrafiltration experiments. Consequently, the feed solution in our ultrafiltration experiments contains virus particles, host cell proteins and DNA, as well as, growth medium. In addition, cell fragments that were not removed by the centrifugation and prefiltration steps may also be present. Based on the results of SDS PAGE we find the molecular weight of host cell proteins vary from 14 kD to well over 100 kD.

TFF and HPTFF Experiments

Experiments were conducted using flat sheet Sartocon® Slice 200 cassettes (Sartorius AG, Germany). The Sartocon® Slice 200 cassettes are 15 cm in length. The nominal molecular weight cutoffs of 100 and 300 kD (Sartorius polyethersulfone Cat No.: 100 kD, 3081465002E-SG; 300 kD, 3081467902E-SG) were used. The filtration area is 200 cm^2 (0.02 m^2). The membranes were gifted by Sartorius. Figure 1 shows the experimental setup during TFF, where only a feed pump is used. Figure 2 shows the experimental set up during HPTFF, where both a feed and permeate pump are used.

Prior to conducting virus filtration experiments deionized (DI) water fluxes were measured at the operating conditions using the TFF mode of operation. At a flow rate of 150 mL min^{-1} and an average transmembrane pressure of 0.14 bar, the water fluxes were 55 and 104 L m^{-2} hr^{-1} for the 100 and 300 kD membranes respectively. TFF and HPTFF experiments were conducted using the thawed supernatant containing virus particles (see preparation of AeDNV particles in serum free medium) as the feed stream. The initial feed volume was 500 ml. The feed was concentrated about 10 times resulting in a retentate volume of 50 mL.

In all the experiments, the feed flow rate was set to 150 mL min^{-1} using a peristaltic pump. In the HPTFF mode, retentate was returned to the feed reservoir. The permeate was weighed on a balance (Mettler Toledo,

Columbus, OH), see Fig. 2. Permeate, from the permeate reservoir, was pumped using a second peristaltic pump to the permeate inlet of the membrane module. The permeate recycle flow rate was set to 10 mL min^{-1}.

At the commencement of an experiment, the feed pump was started and the permeate outlet closed. For TFF, after a 10–15 minutes, the permeate outlet was opened. For HPTFF, after 10–15 minutes, the permeate outlet was opened and the permeate pump started. For both methods of operation, the mass of permeate collected was measured by a balance and recorded by an online personal computer.

For TFF, the feed, retentate, and permeate pressures were measured by three pressure sensors (Honeywell International Inc., Morristown, NJ). For HPTFF, the feed, retentate, permeate inlet, and permeate outlet pressures were measured using four pressure sensors. The data were automatically recorded on a personal computer. The transmembrane pressure was calculated, using the following equation. For TFF operation:

$$TMP = \frac{P_{Feed} + P_{Retentate}}{2} - P_{permeate}$$

For the HPTFF:

$$TMP = \frac{P_{Feed} + P_{Retentate}}{2} - \frac{P_{permeate\ inlet} + P_{permeate\ outlet}}{2}$$

The average transmembrane pressure for the single pump mode of operation was around 0.372 bar with a variation from 0.379–0.345 bar. The average transmembrane pressure for the HPTFF mode of operation was around 0.155 bar with a variation from 0.138-0.172 bar. Some variation in transmembrane pressure, for both modes of operation, was caused by the action of the peristaltic pump. Pressure readings were accurate to $\pm 5\%$ of the average value.

In both modes of operation, 1 mL samples were taken at regular intervals, from the feed, the retentate, and permeate streams for analysis of virus titer and protein concentration. Filtration was continued until 400–450 mL of permeate had been collected i.e. the contents of the feed reservoir were concentrated about 10 times.

At the end of the virus filtration experiment, the membrane was flushed using DI water followed by 1 mol/L NaOH solution at 50°C for 1 hour. Then the DI water flux was measured. After cleaning it was ensured that the water flux was at least 95% of the initial water flux. The membrane was then stored in a 0.1 mol/L NaOH solution supplemented with 20% ethanol by volume.

PCR Assay

A quantitative real time PCR (QPCR) based assay was used to determine the virus titer in the infective solutions as AeDNV does not show cytopathic effects. The primers and probe were designed within a conserved region of

the viral NS1 gene. Primer Express® oligo design software (Applied Biosystems, Foster City, CA) was used to design forward primer: CAT ACT ACA CAT TCG TCC ACA A, reverse primer: CTT GCT GAT TCT GGT TCT GAC TCT T, and TaqMan Probe: FAMCCA GGG CCA AGC AAG CGC CTAMRA. The reaction was performed in 96-well format skirted v-bottomed polypropylene microplates (MJ Research, Inc., Waltham, MA) with optical caps (Applied Biosystems, Foster City, CA).

The Brilliant® Quantitative polymerase chain reaction core reagent kit (Stratagene, La Jolla, CA) was used as the QPCR master mix. Each well consisted of 4 μL of unknown sample or standard control DNA pUCA plasmid, 10 μL master mix, 2 μL of 0.05 mmol/L forward primer, 2 μL of 0.05 mmol/L reverse primer, and 2 μL of 5×10^{-3} mmol/L probe. The thermal cycling conditions were: stage 1 50°C for 2 min, stage 2 95°C for 10 min, stage 3 95°C for 15 sec, stage 4 60°C for 1 min, and then the stages 3 and 4 were repeated 39 times. All reactions were performed in the Opticon 2 DNA Engine (MJ Research, Inc.). All samples were analyzed three times and average results are reported. The accuracy of the PCR assay was determined by analyzing 12 samples of the same infective solution and found to be within $\pm$ 0.5 log units. Further details of the PCR assay are given by Afanasiev et al. 1999 (23).

A QPCR based method was used for the quantification of AeDNV virus since more conventional biological assays are not straightforward (23). The quantitative QPCR assay is a rapid, sensitive, and efficient way to compare samples. Though similar results could be obtained with naked viral genomic DNA, when batches of AeDNV prepared from cell culture or mosquito larvae, as described in the manuscript, are exposed to pancreatic DNase prior to QPCR, there is little or no reduction in signal. Also QPCR on pellet fractions after ultracentrifugation under conditions that should pellet virus particles indicates that most of the DNA is pelleted. These results give us confidence that we are measuring DNA from virus particles in these preparations rather than DNA from plasmid transfections or replicative forms. Further, the PCR assay will not detect host cell DNA.

Protein Assay

Protein concentration was measured using a BCA Protein Assay Kit (Pierce, Rockford, IL) following the manufacturer's instruction. Using a 96 well microplate (Nalge Nunc International) 25 μL of unknown sample or standard albumin were added to the wells. Next 200 μL of working reagent were added to each well. The plate was covered and incubated at 37°C for 30 min. After cooling to room temperature, the absorbance of each sample at 562 nm was measured using a microplate spectrophometer (Benchmark Plus Microplate Spectrophometer, Bio-Rad Laboratories, Hercules, CA). As described by the manufacturer, the protein concentration is determined and

reported with reference to a standard albumin solution provided by the manufacturer. All samples were analyzed in triplicate and average values reported. The accuracy of the protein assay is better than 6% of the mean. Since the working range of the assay is 20–2,000 μg/mL, samples were diluted 10 fold.

RESULTS

The variation of the permeate flux with permeate volume is given in Fig. 3. Results are shown for the 100 and 300 kD membranes in TFF mode as well as 100 kD membrane in HPTFF mode. The permeate flux for the 100 kD membrane is less than the permeate flux for the 300 kD membrane. In general, the permeate flux decreases with decreasing nominal molecular weight cutoff, providing the porosity of the membrane is approximately constant (25). As can be seen, the permeate flux for HPTFF is much lower than the flux for TFF using the 100 kD membrane. HPTFF using the 300 kD membrane (not shown) also gave a lower permeate flux than TFF using the 300 kD membrane. This result is not surprising as the transmembrane pressure is lower during HPTFF operation. Van Reis and Zydney (14) indicate that at the same transmembrane pressure, HPTFF has an equivalent throughput to TFF measured in mass of product per unit membrane area and time. Importantly as indicated by van Reis (12, 16), by maintaining a more constant transmembrane pressure along the feed flow channel, the variation of permeate flux with time is much less than for conventional TFF.

Figure 4 gives the variation of the relative permeate flux versus the permeate volume. The relative permeate flux is defined as the permeate flux divided by the DI water flux at the same TMP for TFF. As can be seen, the decrease in relative permeate flux with time for the 300 kD membrane is

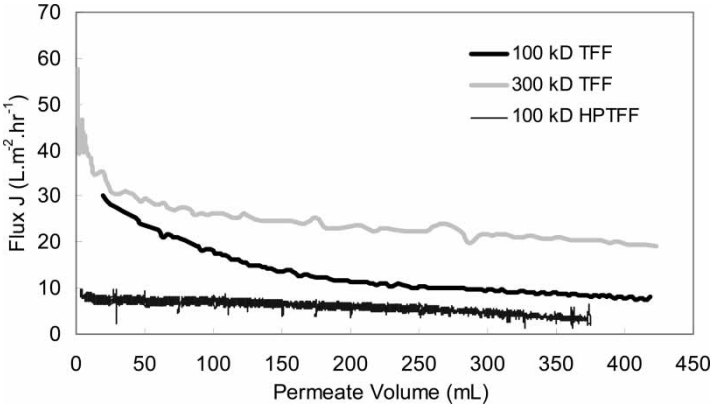

Figure 3. Variation of permeate flux with permeate volume for TFF and HPTFF mode.

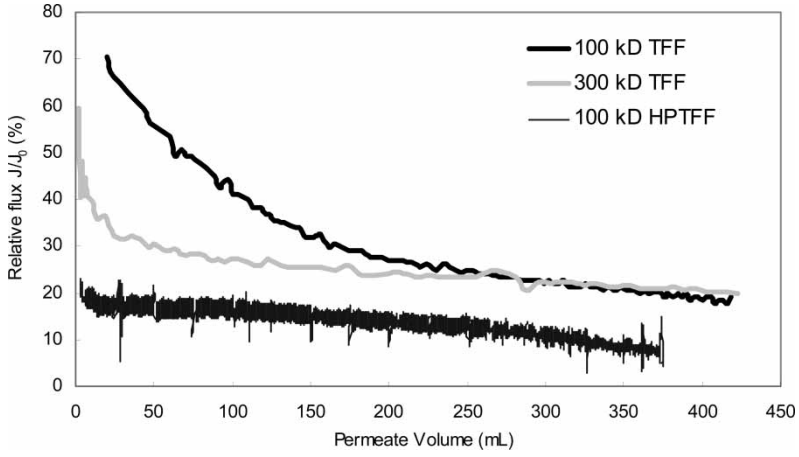

Figure 4. Variation of relative permeate flux with permeate volume for TFF and HPTFF mode. Relative Flux is defined as the measured flux divided by the water flux.

much more rapid than the 100 kD membrane in TFF mode. Grzenia et al. (25), point out that this result is most likely due to entrapment of virus particles in the membrane pores. Since the 100 kD membranes, has a smaller pore size, virus particles do not enter the pores as easily; thus, the decrease in permeate flux with time is much slower. While the permeate flux for HPTFF mode using the 100 kD membrane is much lower than for TFF

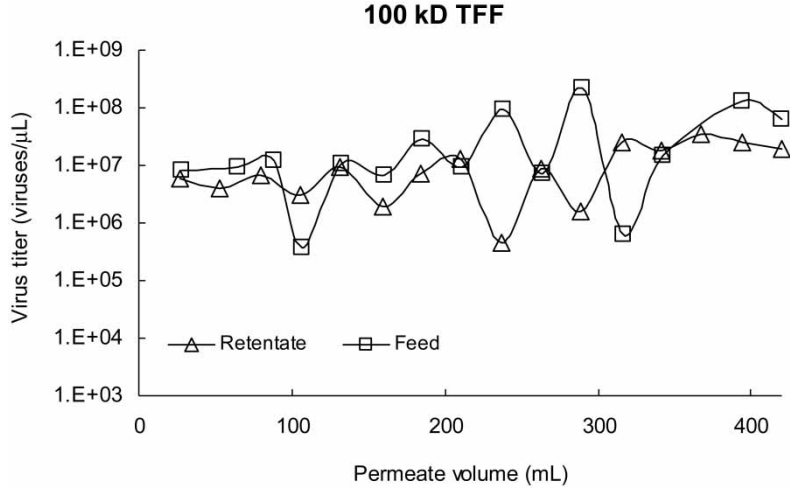

Figure 5. Variation of the virus titer with permeate volume for the 100 kD membrane during TFF. No virus particles were detected in the permeate.

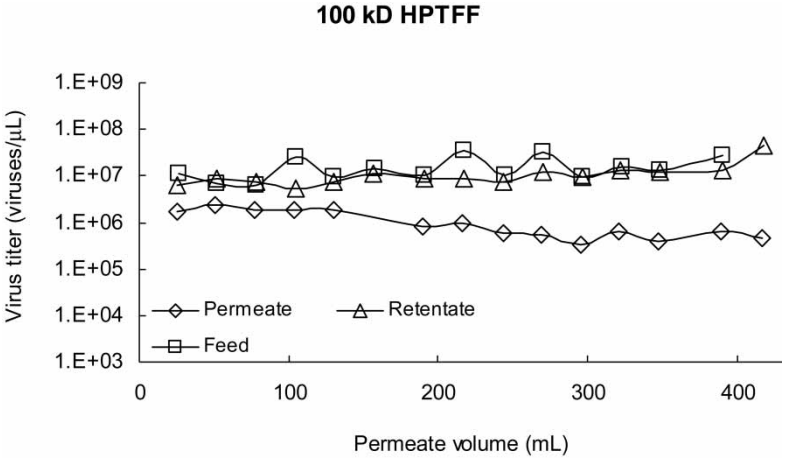

Figure 6. Variation of the virus titer with permeate volume for the 100 kD membrane during HPTFF.

mode, the permeate flux is much more stable. Similar results were obtained for the 300 kD membrane (not shown).

Figures 5–8 give the variation of virus titer with permeate volume. All four figures indicate that the retentate and feed virus titers are the same. This result is expected as the permeate flux is much lower than the feed flow rate. Consequently, the permeate removed per pass through the

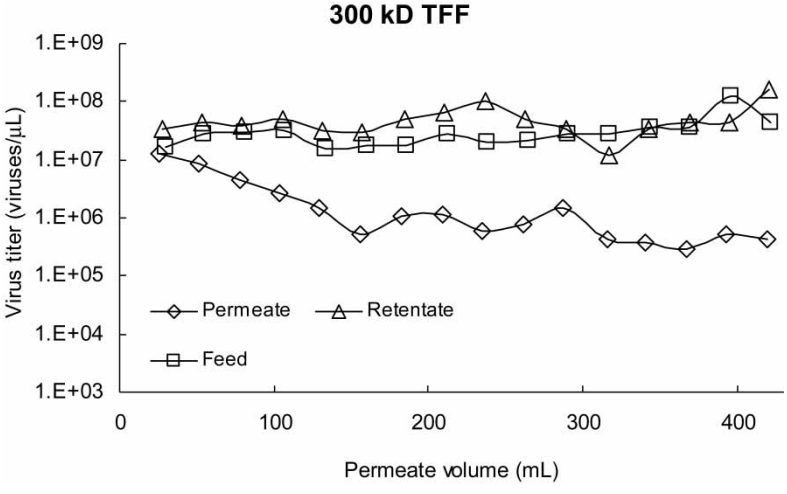

Figure 7. Variation of the virus titer with permeate volume for the 300 kD membrane during TFF.

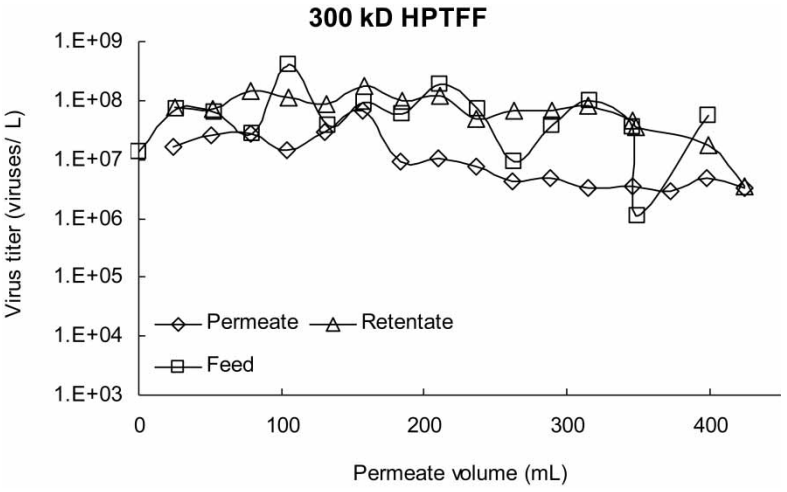

Figure 8. Variation of the virus titer with permeate volume for the 300 kD membrane during HPTFF.

module, is low relative to the feed flow rate. Figures 5 and 6 give results for the 100 kD membrane for TFF and HPTFF. In Fig. 5 the virus titer in the permeate was below the detection limit of the PCR assay. Since the PCR assay is sensitive to much less than 1 log virus/μL there was less than 1 log virus particles per μL in the permeate from the 100 kD membrane. Comparing Figs. 5 and 6, it can be seen that the permeate virus titer is much higher for HPTFF. The results suggest that in HPTFF mode, there is much better transmission of virus particles through the membrane pores.

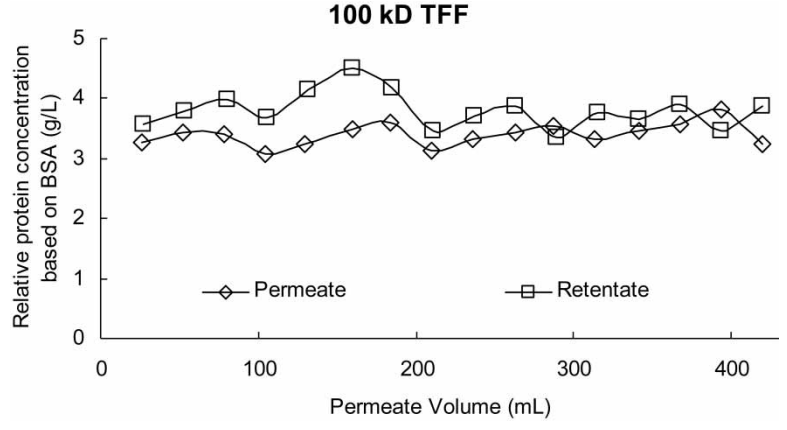

Figure 9. Variation of the protein concentration with permeate volume for the 100 kD membrane during TFF.

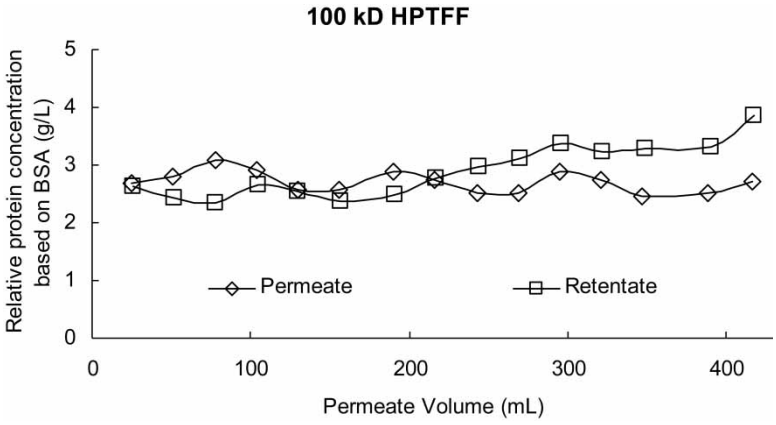

Figure 10. Variation of the protein concentration with permeate volume for the 100 kD membrane during HPTFF.

Figures 7 and 8 give analogous results for the 300 kD membrane. Though there is significant passage of virus particles during TFF, in HPTFF mode the virus titer in the permeate is closer to the feed and retentate titers. This result again indicates better passage of virus particles during HPTFF.

Figures 9–12 give the variation of protein concentration with permeate volume. Figures 9 and 10 give results for the 100 kD membrane for TFF and HPTFF. Figures 11 and 12 give analogous results for the 300 kD membrane. The protein concentration reported is the concentration with reference to a standard albumin solution. As was the case for the virus titer,

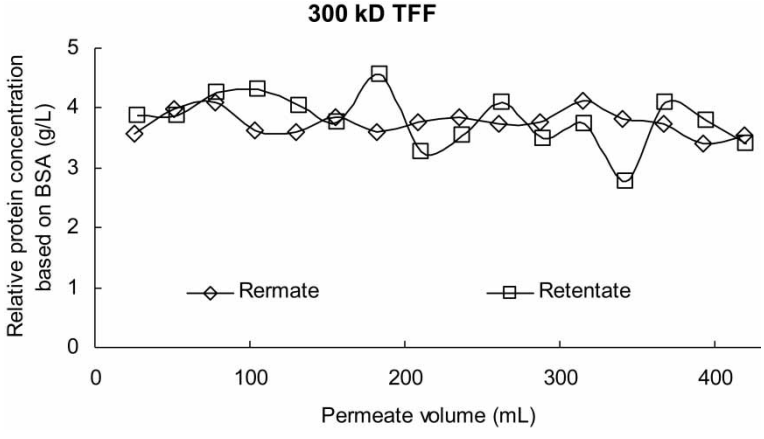

Figure 11. Variation of the protein concentration with permeate volume for the 300 kD membrane during TFF.

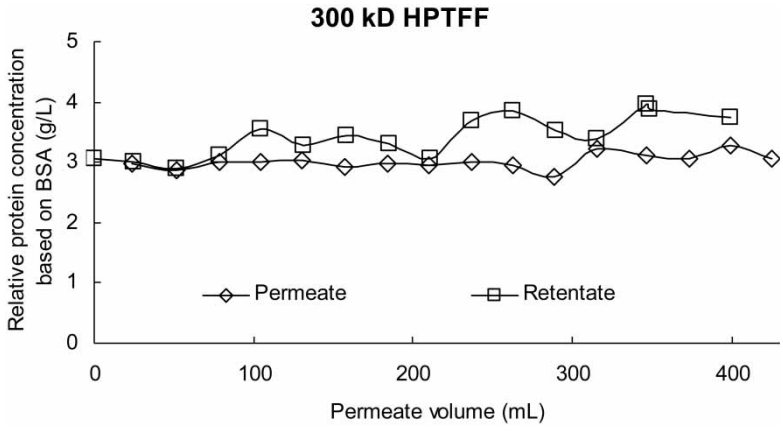

Figure 12. Variation of the protein concentration with permeate volume for the 300 kD membrane during HPTFF.

the protein concentration in the feed and retentate was the same as at a given instant. Consequently, the results for the feed were not shown. The growth medium used here was serum and protein free. Therefore, the protein assay detects the presence of polypeptides and amino acids which are referred to as "proteins". In all four figures, the protein concentration in the retentate and permeate is similar indicating little rejection of the proteins.

DISCUSSION

Figures 3 and 4 indicate that for TFF the permeate flux for the 300 kD membrane decreases much more rapidly than for the 100 kD membrane. In fact Fig. 4 indicates the relative flux for the 300 kD membrane is less than the relative flux for the 100 kD membrane for the first 250 ml of operation. Comparing Figs. 5 and 7, it can be seen that while virus particles were found in the permeate from the 300 kD membrane none were detected in the permeate from the 100 kD membrane. Thus, it is likely that virus particles are being trapped in the pores of the 300 kD membrane, which leads to the rapid decrease in permeate flux. Grzenia et al. (25) report a similar result.

Comparing Figs. 5 and 6, it can be seen that there is a significant passage of virus particles for the 100 kD membrane in HPTFF. As has been observed by others (12–16), better protein passage is obtained during HPTFF for proteins that are similar in size to the nominal molecular weight cut off of the membrane. The ability of the membrane to more precisely reject proteins that are bigger than the nominal molecular weight cut off and, yet, to allow more complete passage proteins that are slightly smaller than the

nominal molecular weight cut off, results in higher separation factors for proteins with size differences less than an order of magnitude.

The AeDNV particles range in size from 18–26 nm. Further, smaller particles may be present that represent damaged viral fragments. While the 100–kD membrane appears to reject all virus particles in TFF, it is likely that in HPTFF smaller viral fragments pass through the membrane. Similarly, fractionation of virus particles may be possible using the 300 kD membrane in TFF. Fractionation of virus particles may be of practical value. If, for a given viral purification, only viral particles within a certain size range represent intact virus particles, removing unwanted particles during the filtration step will reduce the requirements on subsequent purification steps such as ion exchange or size exclusion chromatography.

Wickramasinghe et al. (26) have studied TFF of human influenza virus. These enveloped virus particles are 80–120 nm in size. Use of a 0.1 μm pore size microfiltrtion membrane also resulted in a rapid decline in permeate flux again suggesting entrapment of the virus particles in the membrane pores. Further, Wickramasinghe et al. were able to fractionate human influenza virus particles using a 0.1 μm pore size microfiltration membrane. Virus particles produced using a cell culture based method often display a distribution of particle sizes. Particles smaller than the range of sizes of the intact virus particles are due to viral fragments. Larger particles could be due to aggregated viral fragments, as well as virus particles attached to host cell fragments.

In this work, the feed was centrifuged prior to ultrafiltration. In an actual manufacturing process, it is likely that depth filtration will be used to remove host cells debris prior to ultrafiltration (26). Consequently, larger particles representing viral fragment attached to the host cell membrane may also be present in the feed. Thus, particle fractionation to remove these larger particles may be beneficial. Production of adeno associated virus often require the presence of adenovirus. Though adenovirus is also nonenveloped, it is much larger than adeno associated virus being 70–90 nm in size (25). Purification of adeno associate virus requires the removal of contaminating adenovirus particles. Fractionation by ultrafiltration may provide a method to remove adenovirus particles.

Figures 3 and 4 indicate that the permeate flux was much more stable for HPTFF than TFF. Comparing Figures 5–8 it can also be seen that the instantaneous fluctuation in virus titer for the feed and retentate streams is always less for HPTFF compared to TFF. These results are in agreement with earlier protein filtration studies (25) that indicate that maintaining a constant transmembrane pressure along the flow channel leads to a more stable operation.

Figure 5 indicates that the virus concentration in the retentate increases by about 1 log unit. Since the feed is concentrated ten-fold, and no virus particles were detected in the permeate, this result is to be expected. Figure 7 indicates a slight increase in the virus titer in the retentate as the feed is concentrated for

the 300–kD membrane. Since the virus titer in the permeate is much lower than the feed during TFF the 300–kD membrane partially rejects virus particles. Figures 6 and 8 indicate that there is very good passage of virus particles through the 100 and 300–kD membranes during HPTFF operation. Figures 9–12 indicate that there is no rejection of proteins for the 100 and 300–kD membranes.

Comparing Figures 5 and 9 we see that host cell proteins may be removed from the virus particles. It is, however, important to remember that the nominal molecular weigh cut-off of an ultrafiltration membranes refers to the rejection of a specified protein under specified conditions. In the case of AeDNV the viral genome alone consists of 400 bases each about 300 Dalton giving a molecular weight for the genome of 120 kD.

In this study, removal of host cell DNA was not investigated. Here, AeDNV vectors are produced for infection of Aedes aegypti mosquitoes. The overall aim of this work is to infect mosquitoes by AeDNV vectors in order to develop mosquito subpopulations that are resistant to human pathogens such as yellow fever and dengue. This, in turn, could control the spread of mosquito borne pathogens. Removal of host cell DNA from purified AeDNV particles was not required. The results, however, do indicate that removal of host cell proteins and DNA during the purification of other parvovirus particles, such as AAV for gene therapy applications, will be challenging.

Previous experimental studies have indicated the potential of HPTFF for protein fractionation (12–16). The results of the work described here indicate that HPTFF may be very useful in the purification of small virus particles where contaminating host cell proteins and DNA may be of similar size. TFF is limited in its ability to fractionate species with sizes within an order of magnitude of each other. A wide membrane pore size distribution can limit the resolving power of the membrane (27, 28). Further concentration polarization can reduce selectivity and membrane fouling can affect the separation. By carefully optimizing the feed flow rate (wall shear rate), the transmembrane pressure, the pH, and the ionic strength of the buffer, separation of species that are similar in size can be achieved.

The experiments conducted here with AeDNV indicate that there is a much better virus passage for HPTFF compared to TFF. No attempt was made to optimize the buffer pH, and ionic strength. The results appear to indicate that if a constant transmembrane pressure is maintained throughout the length of the feed channel, plugging of the membrane pore can be minimized.

CONCLUSIONS

TFF and HPTFF of feed streams containing AeDNV has been conducted using 100 kD and 300 kD nominal molecular weight cutoff membranes. The

permeate flux is much more stable over time for HPTFF. For the 100 kD membrane, no virus particles were detected in the permeate during TFF. However, for HPTFF significant passage of virus particles through the 100 kD membrane was observed. In both modes of operation, virus particles were detected in the permeate for the 300 kD membrane. Little rejection of polypeptides and amino acids was observed for both membranes in both modes of operation.

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