5-2014

Comparing Virus Ultrafiltration of Bacteriophages with Filtration of Minute Virus of Mice

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Chemical Engineering

by

Kent Smith
University of Arkansas
Bachelor of Science in Biochemistry, 2009

May 2014
University of Arkansas

This thesis is approved for recommendation to the Graduate Council.

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Abstract

Industrial production of protein therapeutics demand rigorous testing and clearance of viruses. The U.S. Food and Drug Administration dictate the purity of pharmaceuticals with regards to viral contamination. As this testing is time consuming and expensive using mammalian cells and viruses, bacteriophages may provide a faster and cheaper alternative for membrane filtration processes. We used ultrafiltration membranes to filter protein solutions with viruses. Two bacteriophages were tested against membranes with two different pore sizes. These membranes were then tested by inverting the membrane's orientation. Flux measurements and log virus removal data were taken. Flux and log virus removal were seen to be slightly higher than published data for mammalian virus analogue minute virus of mice. Future testing would allow for more precise evaluation, but data suggests bacteriophages provide similar results to mammalian data.

Acknowledgments

I would like to thank Anh Vu and Zizhao Liu for their contributions to the collection of data and SEM images, respectively. Appreciation also goes to Alexandru Avram and Robert Dong for their review of this piece. Finally, this work could not have been completed without the cooperation and camaraderie of the Wickramasinghe research group.

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1 Introduction

Viruses are protein capsules that contain genetic material and have the capability to insert this material into a host's genome. Viruses are critical in the biopharmaceutical industry because of the potential danger of diseases through viral transmission. The general order of processing steps necessary in the creation of a biopharmaceutical begins with cell culture, clarification, recovery, and purification followed by polishing. Additional steps for validation of virus clearance are often required during polishing [1]. A common virus separation technique is membrane filtration. For the past twenty years, membrane filtration for viral rejection and capture has been applied on an industrial scale with obvious success.

Virus clearance is fundamental in the creation of protein based drugs and vaccines for human consumption. Industrial processes concerning biopharmaceuticals fall under federal regulation, and certain precautions are necessary to ensure safety for human usage as medical evidence has supported the need to prevent transmission of blood-borne pathogens [2-5]. The United States Food and Drug Administration (FDA) has strict guidelines on the testing and manufacture of such biopharmaceuticals. One key factor of the FDA's guidelines is validation of viral clearance. Multiple and fundamentally separate or orthogonal steps must also be used in order to achieve greater reduction than what a single process is capable of doing alone [6]. An example of orthogonal steps would be a heat treatment step followed by a filtration step. As each are different in their mechanisms of viral clearance, they can be considered orthogonal. The FDA does not specify number or types of steps necessary for products and allows manufacturers to design their own processes. Instead, final dosage and validation of clearance is mandated. This standard for purity is mandatory and is greatly facilitated through viral filtration.

1.1 Virus Clearance

At least two orthogonal steps are demanded by good manufacturing practice (GMP), though more can be necessary depending on if certain types of viruses are known to be present [7]. Clearance is measured as the $\log_{10}$ removal value (LRV), or the log of the ratio of the feed viral concentration to the permeate viral concentration [8]. Operations are classified as ineffective if LRV is less than one, moderately effective if LRV is between one and four, and effective if LRV is above four. Ineffective steps cannot be considered for total clearance, and orthogonal operations are considered additive to evaluate the total LRV. Most industrial processes desire a single step to at least register as effective to be viable commercially.

Specific categories of viruses must be considered for viral removal: endogenous and adventitious [9]. Endogenous viruses are present due to their use in creating the product. Adventitious viruses are brought in from by external contamination. Given that both may be present in a process, clearance of said viruses is important and must be tested. Two specific viruses are important test markers: parvoviruses and retroviruses. Viruses are sorted by either having a lipid envelope, like many retroviruses, or lacking an envelope, like parvovirus. For example, HIV is an enveloped virus that can easily be inactivated by heat-treatment, as the heat degrades the envelope and reduces its infectivity [10,11]. However, parvoviruses are non-enveloped and are not as easily inactivated by such means. Also given the similar size of the virus, on the range of 20 nm, in comparison to some proteins, around 10 nm for IgG antibody, a process' selectivity with regards to size is very critical [3,12]. Parvoviruses are among the smallest known viruses, and therefore relevant to size exclusion methods of separation as a useful benchmark to challenge a process. Retroviruses are another class of virus that must be taken into consideration for virus clearance [5,9]. Retroviruses use RNA to create proteins that cause insertions into a host's genome.

Many viruses that work in this manner are very efficient in infecting humans. Therefore, extreme care must be taken to reduce the concentration of viruses entering into patients through biopharmaceuticals. Several methods of virus inactivation exist, as **Table 1** demonstrates. Only a few remain viable options considering some virions are more robust than the protein products desired, or the virus itself may be the intended product, as is the case with virus capture. Membrane technology has greatly benefited virus clearance and capture considering that membranes allow for an easily scalable system, can distinguish products based on size, are low in energy cost, and could potentially be cheaper in comparison to additional chromatography steps [13].

Table 1		
Virus Clearance Technologies		
Technology	**Log Removal Value**	**Mechanism**
Chromatography	0-6	Adsorption or exclusion
Filtration	3-6	Size exclusion
Gamma irradiation	>3-6	Inactivation
Heat		Inactivation
Low pH incubation	6+	Inactivation
Membrane adsorber/membrane chromatography	0-6	Adsorption/size exclusion
Solvent/detergent	6+	Inactivation by lipid dissolutiom
Ultraviolet inactivation	>2-6	Inactivation

Table 1: Various methods for viral clearance, LRV values, and mechanism for each [8].

Researchers testing industrial filters and virus removal techniques employ the use of smaller scaled experiments that can be representative of the full scale process. Recent studies often use feeds that contain more impurities than real world processes [14]. This can de deduced considering filtration steps are regularly placed after a single or multiple chromatography steps and most studies do not perform filtrations after performing a chromatography step [8]. The impurities can cause a flux decline through pore blockage, and it could potentially overestimate the LRV properties of a membrane as the additional resistance from the membrane fouling will

cause less viral passage. The study recommended in addition to the regular spiking studies, a protein/virus mixture with a known concentration challenging a filter until a flow decay endpoint, to also use "pre-conditioning" step. This method begins by using only the biologic against the filter until a given volume. Afterwards, a combination of virus and biologic are filtered to a flow decay endpoint. Comparison of the resulting LRV for the two methods provide a more conservative result that would better predict and represent industrial applications.

1.2 Ultrafiltration/Virus Filtration

Given the mechanism of membrane filtration is commonly size exclusion, membrane processes are based on the pore size or the size and type of particles excluded by a given membrane. Proteins and viruses fall under the designation of ultrafiltration (UF), which generally covers pore sizes from 10^{-6} to 10^{-8} m in diameter. This is a pressure driven process, commonly used for separating macromolecules though it is not exclusive for that application. Filtrations are performed in either tangential flow mode (TFF), or dead-end/normal flow mode (NFF). TFF is a method that has the flow of the feed tangential to the membrane surface [6]. This allows for greater surface area due to membrane geometry and reduced fouling due to the sweeping motion of the feed solvent [15]. In operations where the feed contains a high load of foulants, this mode of filtration is valid. The already high purity of the feed reduces this benefit for virus removal considering the downstream placement of most virus filtration (VF) modules in industry. Use of TFF for protein recovery is often applied in other situations, considering the product and the contaminant must be greater than an order of magnitude to each other in size [16,17]. TFF is also predicated on the ability to reuse filters. Though there are benefits to both modes, NFF is the predominant mode of filtration in regards to virus clearance, though it is not exclusive to VF [6,13,16]. In NFF, the entirety of the feed is pushed through the membrane surface. Flux

performance is usually lowered in this mode. The specific restrictions on cleaning and validating virus clearance makes NFF the only practical and economical choice considering the high purity of streams in most operations. This is also taking into account the simplicity of the unit in operations. **Table 2** presents some commercial VF filters.

Table 2		
Commercial Virus Filtration Products		
Company	**Product**	**Log Removal Value**
Asahi	Planova 15N (TFF/NFF)	>6.2 parvovirus, >6.7 poliovirus
	Planova 20N (TFF/NFF)	>4.3 parvovirus, >5.4 encephalomyocarditis
	Planova 35N (TFF/NFF)	>5.9 bovine viral diarrhea virus, >7.3 HIV
Millipore	NFP (NFF)	>4 PhiX-174 bacteriophage
	NFR (NFF)	>6 retrovirus
	Viresolve 70 (TFF)	>4 polio, >7 retrovirus
	Viresolve 180 (TFF)	>3 polio, >6 retrovirus
Pall	DV20 (NFF)	>3 PP7, >6 PR772 bacteriophage
	DV50 (NFF)	>6 PR772 bacteriophage
Sartorius	Virosart CPV (NFF)	>4 PP7 bacteriophage, >6 retrovirus

Table 2: Filters with mode of filtration and LVR by company [8].

Fouling is a large issue with any filtration process, and virus filtration is no different [18]. One might normally consider fouling to be the result of impurities in the feed, but for protein based separations, the product is often the largest foulant. Normally, UF filtrations are run with the barrier facing the feed and a support structure underneath it for mechanical stability. However, VF experiments have shown improvements in flux if this orientation is reversed [13,18,19,20].

It is generally assumed that the support structure acts as a kind of pre-filter to prevent protein aggregates from irreversibly fouling the membrane as this decreases flux, product recovery, and therefore cost of product [21]. VF has a special caveat that separates it from UF in a specific regard concerning fouling. In most forms of filtration, it is not advantageous to irreversibly foul the membrane since it lowers flux and product recovery. VF can actually be improved by irreversible fouling, assuming what is being irreversibly fouled is a viral contaminant as this fouling increases the rejection of particles [22]. The overall goals of industrial filtration are dictated by product

purity, potency, and quality [23]. In general, virus filters follow no standard in what manufacturers report concerning the performance or properties of the filters themselves [6]. Often researchers must perform specific experiments in order to accurately compare membranes, even with virus filtration filters [13]. The virus size is also substantial considering the larger the virus, the easier the separation will become, and accumulation of virus particles also supports the separation in the same manner [6]. Buffer solutions can play important roles in filtration as the main foulants are proteins, which have pH and ionic sensitive properties [6,27]. Protein passage and yield is extremely important and could vary the cost effectiveness of a process. Sizing membranes is also useful as the membranes demand high throughput before being disposed [3,6]. Balancing process time and volume demand are the factors in play, specifically with regards to protein flux versus volume. Location of viral clearance in the operations is critical as well [3]. These are generally placed after a chromatography step, but as there are several chromatography steps in place, placement can determine the amount and the kind of foulant load on the system. Many factors can be discussed in optimizing virus clearance, including previously mentioned modes and membrane orientation.

While pore size was mentioned previously, manufacturers often describe membranes by their molecular weight cut-off (MWCO) as well. This is the molecular weight of the particle having a rejection of at least 90%, and it represents another adequate membrane separation characteristic [24]. It is a good indicator for characterizing the sieving properties of a membrane, since a molecule's separation depends on many complex parameters, such as three dimensional shape or its ability to form dimers or trimers in different solvents. Thus the solvent properties, such as pH, temperature, ionic strength, etc are extremely significant when designing the filtration process. However, MWCO is not an industry standard and can often be more of an estimation

than a prediction. The aforementioned solvent pH can affect flow rate, product recovery, and run time [25]. Proteins carry different configurations at different pH values. This configuration change can also affect how the biologic interacts with the virus, other biologic molecules, and the filter. A study using antihemophilic factor IX as the biologic had longer run times and significantly reduced recovery rates as the NaOH used to raise the pH would actually inactivate the product. Testing is necessary for each biologic in question as denaturing a protein could allow for higher passage through a membrane's pore, but should the protein not spontaneously refold it would likely be inactivated.

Though this research attempts to investigate filtration through experimental work, numerical modeling of filtration is also used academically. Recent work shows how capsule shaped objects, such as bacteria or viruses, behave in a cylindrical pore [26]. Smaller and more spherical objects have a larger impact from hydrodynamic interactions on rejection than larger species, which have more influence from steric restrictions. This follows some logic as one could expect the fluid to essentially dominate the effects on the particle as small particles will "see" the fluid more in comparison to the walls of the pore or other particles.

1.3 Viruses

The main fear of mammalian virus usage is from zoonotic diseases, diseases found in animals that infect humans. Large evolutionary distance is seen as a barrier for transferring zoonotic diseases. Though this distance is an imperfect barrier, it is relevant concerning the use of bacteria as "safer" alternatives to mammalian hosts. Mammalian cells and viruses demand more resources to test and cultivate in comparison to bacterial ones when considering the doubling time (>10 hours for mammalian cells in comparison to 20 minutes for *E. coli*), biosafety standards, and robustness of the host. Although bacterial validation cannot completely replace the use of

mammalian validation, preliminary testing for viral clearance using bacteriophages is possible to reduce the cost and difficulty of these tests, as the FDA allows specific bacteriophages as models for mammalian viruses [20,28].

Specific bacteriophages mimic the size, envelope, and genome of these particles, which allows for comparison studies. While this is less relevant for steps using chemical interactions such as packed-bed chromatography, it becomes significant for membrane filtration separations as these separations rely on size exclusion. VF membranes, while similar in terms of pore size to UF

Table 3			
Common Viruses for Viral Clearance Studies			
Virus	**Genome**	**Enveloped**	**Size (nm)**
BVDV	RNA	Yes	50-70
Encephalomyocarditis virus	RNA	No	25-30
MVM	DNA	No	18-25
MuLV	RNA	Yes	80-110
Parainfluenza	RNA	Yes	100-200
Parvoviruses	DNA	No	18-24
PhiX-174	DNA	No	25-27
Poliovirus sabin type I	RNA	No	25-30
PR772	DNA	No	53
Pseudorabies virus	DNA	Yes	150-200
Reovirus 3	RNA	No	60-80
Sindbis virus	RNA	Yes	60-70
SV40	DNA	No	40-50

Table 3: Viruses commonly used for clearance studies with genome, envelope presence, and diameter [8,28]

membranes, demand less pore size variability than UF membranes. **Table 3** provides a list of viruses used for clearance studies.

There are various considerations for clearance studies. These include the health hazard to those workers performing the tests, the ability to create a high titer with that virus, and the ability to test with reliable sensitivity at every step of the manufacturing process [8]. Murine leukemia virus (MuLV) and minute virus of mice (MVM) are commonly used since they serve as appropriate

retrovirus and parvovirus models. Using bacteriophages to mimic these virus models would be the goal for such studies. For the purposes of this study, UF membranes will be challenged using bacteriophages in order to compare MVM clearance studies. Testing commercially available UF membranes has the benefit of reducing the cost in such filtrations in comparison to VF membranes, as well as ease of membrane running experiments.

2 Methods

2.1 Virus Stock Creation

Host cells and viruses were purchased from ATCC (Manassas, Virginia). The two bacterial hosts were *Escherichia coli* (Migula) Castellani and Chalmers (ATCC 13706) and *Escherichia coli* (Migula) Castellani and Chalmers (ATCC BAA-769). The two virus types were purchased in conjunction with the host: phi X174 (ATCC 13706-B1), strain Phi X174 and *Escherichia coli* phage (ATCC BAA-769-B1), strain PR772 (HER 221). The specific host was used to propagate its corresponding virus. Phi X174 is reported as 25 nm in diameter and PR772 is reported as 53 nm in diameter [29].

Bacteria were grown in 2XYT medium broth (AMRESCO; Salon, Ohio, VWR Cat. #97063-442) until the media reached an optical density of 0.300 to 0.500 at a wavelength of 600 nm. All bacteria were grown at 37 degrees Celsius in aerobic conditions while shaking at approximately 200 rpms. When it reached the appropriate optical density, the media was spiked with 1 mL of virus stock and allowed to shake overnight. This culture was then centrifuged at 9500 g for 30 minutes, and the supernatant was filtered with a 0.2 µm polyethersulfone filter membrane. This resulted in a solution that was free from microbes while having a relatively high concentration of virus particles (generally around 4 to 6 $\log_{10}$ virus particles per mL). This stock was used to create new stocks as well as run filtration experiments. This procedure was performed

on both virus types with no deviation. When not in use, the stocks were kept at 4 degree Celsius or stored long-term frozen.

2.2 Filtration

Filtration experiments used two polyethersulfone membranes of MWCO, 10 kDa MWCO Ultrafiltration membrane from Sartorius Stedim Biotech (Göttingen, Germany) and 300 kDa MWCO Omega Ultrafiltration membrane from Pall Life Sciences (Port Washington, New York). Both are asymmetric membranes, with a barrier or skin-side that faces the feed and an open, support-side. These were selected for comparison to previous studies using similarly sized membranes. Membranes were either rinsed or soaked in DI water according to manufacturer's instructions. All experiments using 10 kDa membranes were performed in an 8200 Amicon stirred cell without any stirring mechanism in dead-end filtration mode at a constant pressure of 300 kPa. All experiments using the 300 kDa membranes were performed in an 8050 Amicon stirred cell without any stirring mechanism in dead-end filtration mode at a constant pressure of 70 kPa. The 8200 cell has a reported filter diameter of 63.5 mm and the 8050 has a reported filter diameter of 44.5 mm. A total volume of 60 mL virus stock was run through both stirred cells in all experiments. Fractions were taken at every 5 mL in permeate volume. Water or media was generally run through the membrane before any virus stock was used. Each membrane was tested in two different orientations, barrier side facing the feed and support side facing the feed. Each membrane was tested with each virus strain and each orientation. The 300 kDa membranes also were tested with 0% weight by volume [w/v] BSA virus stock and 1% w/v BSA virus stock. After adding the solid BSA to the stock, the solution was stirred for thirty minutes and then filtered with a 0.2 µm polyethersulfone filter membrane. A single experiment with the 10 kDa membrane utilized 1% BSA while the rest used 0% BSA. A water flux and a media flux control were

performed for each membrane, and an additional control using 1% BSA in media was performed for the 300 kDa membrane. This produced five experiments for the 10 kDa membrane and eight for the 300 kDa membrane. Controls were performed using a mass balance as fractions were not necessitated for those runs.

2.3 Titer Analysis

Each fraction was tested for its concentration of virus particles using a culture plate and plaque count method. Plate media was made of 2xYT medium broth (31g/L) and agar (15g/L). Host bacteria were grown to an optical density of 0.300-0.600 at a wavelength of 600 nm. 200 µL aliquots of the bacteria were spiked with 10 µL of either a diluted or an undiluted fraction. Several sample plates were made for each filtration to determine what level of dilution, if any, was necessary for that result. Dilutions were either 10-fold or 100-fold dilutions using 2xYT medium broth. The 210 µL of bacteria and virus solution were vortexed and allowed to sit for five minutes to allow for infection. An agar-media solution (31g/L 2xYT medium broth and 7g/L agar) was heated to melting. 3 mL of this solution was combined with the 210 µL of bacteria/virus solution and vortexed. This was poured over a prepared culture plate and allowed to solidify. This plate was placed in a 37 degree Celsius incubator overnight. Each plate was made in triplicate. Plaque counting was then performed on each plate. Values below 10 plaques per plate or above 350 plaques per plate were not considered, and the fraction was repeated at a different dilution. This would produce a minimum of thirty six plates for each filtration. If any plates failed to cultivate any growth or if the results were not discernable, the plates were repeated. General methods for the titer analysis were derived from *Benson's Microbiological Applications* [30]. Each data point is the average LRV of three titer plates.

2.4 Scanning Electron Microscope

Scanning Electron Microscope (SEM) images were taken of both membranes. Images were taken of the barrier layer, support layer, and a cross-section of the membrane. The SEM is an FEI Novalab 200 Duo-Beam Workstation. It was operated with an accelerating voltage of 15 kV. 5 cm by 5 cm pieces of membrane were cut and coated in gold. Membranes used for cross section images were immersed in liquid nitrogen and then fractured. Images were taken at 500X and 10,000X magnification for both membranes and all orientations.

3 Results

3.1 SEM Images

Fig. 1 shows the SEM images of the two membranes taken to show the barrier layer (B, G, H), the support layer (C, D, F), and the cross section (A, E). Barrier layers for each membrane are nondescript at 500 times magnification. A 10,000 times magnification for the 300 kDa membrane provided visibility to the pores (H). Increasing the magnification 10,000 times for the 10 kDa membrane damaged the membrane while failing to provide any information about the pores, which is expected given the low molecular weight cut off. Smoothness of the surface also did not allow for much visualization. Cross-sectional views of the 10 kDa membrane show the widening of the pores from the barrier layer to the support layer, as well as some channel interconnection. Visualization for the 300 kDa cross-section is more difficult since the membrane was difficult to cleanly crack after being submerged in liquid nitrogen. The various layers of the membrane inhibit much else from being seen. Support structures can be seen for the 300 kDa membrane at 500 times magnification, but the 10 kDa membrane requires 10,000 times magnification to see the openness of the support layer. Several of the cross section images of the 300 kDa membrane appear with a ring or halo like appearance in the background, such as **Fig. 1 E**. These are not significant to the

image as a portion of the membrane was directed perpendicular to the resting surface of the rest of the membrane, and the background was likely the mount in the SEM.

3.2 Flux Data

Flux control experiments for the 10 kDa membrane are similar to the reported water flux by Sartorius, as seen in **Fig. 2** [31]. Though the flux matches the reported flux, the reported flux has a pressure at 400 kPa. The control experiment was performed at 300 kPa which should cause the flux to be roughly seventy five percent of the reported flux as flux should be linearly proportional to pressure. A solution of 2xYT media shows a decline in the flux without the presence of virus or protein. There is significant flux decline with media alone, but the addition of 1% BSA causes the flux to drop below 10 LMH. The 300 kDa membrane control, **Fig. 3**, demonstrated a drop in flux with media in solution compared to water with a strong drop in flux with BSA also in solution. Pall's product information is unclear about the concentration of BSA used, but the 300 kDa membrane solute flux was within the range of the reported flux [32]. Flux values for 10 kDa membrane experiments, **Fig. 4**, show a significant decrease in the flux when protein is used. What was accomplished suggests little difference in the flux with or without virions. For each virus type, inverting the membrane saw an improvement in the flux. It is difficult to establish significant differences in flux between the virus types for this data set. Considering the propensity for the membrane to be fouled by the protein, this is expected. **Fig. 5** provides the data for the 300 kDa membrane, and provides a more dramatic change in flux with the addition of 1% BSA. All inverted flux experiments had higher fluxes than their non-inverted counterparts. The large difference between the fluxes for the two different membranes either with water or the solute fluxes is clearly related to the pore openness and size. The SEM images show that the 300 kDa have more open pores and its support structure is more open than the 10 kDa membrane even in design.

The 300 kDa membrane's support is not composed of discrete pores like the 10 kDa membrane. Rather, the support of the 300 kDa membrane shows individual fibers which are layered and overlapping. The solution will flow around these fibers and allow for a higher flux. As proteins will foul membranes of these sizes, all solutions containing media or BSA will experience flux decline. **Table 4** provides the design of experiments.

Table 4	
Design of Experiments	
10 kDa Pore Size	**300 kDa Pore Size**
Inverted Membrane, Phi X174	Inverted Membrane, Phi X174
Non-inverted Membrane, Phi X174	Non-inverted Membrane, Phi X174
Inverted Membrane, PR 772	Inverted Membrane, PR 772
Non-inverted Membrane, PR 772	Non-inverted Membrane, PR 772

Table 4: Design of experiments. Eight total experiments consisting of two different viruses, two different membranes, and two different membrane orientations.

3.3 Viral Rejection

Virus rejection data is presented in **Fig. 6** and **Fig. 7**. Except for the initial data points for non-inverted 10 kDa membrane with 0% BSA and the PR772 virus, all experiments showed a rejection beyond our methods to detect viable virions. The aforementioned data set rises in LRV with each data point until it reaches the limit of detection likely due to an increase in membrane resistance. The 10 kDa membrane showed it was proficient at rejecting both virus types. The 300 kDa membrane gave high viral rejection except in the presence of BSA. **Fig. 7** shows that all runs with BSA had reduced levels of rejection compared to those without BSA. The major variations between all runs is the initial titer of each stock. It also appears that for the inverted and non-inverted Phi-X 174 with 1% BSA runs that the rejection increased with time as membrane resistance increased.

4 Discussion

Although one parameter tested was the inclusion of 1% BSA, it is obvious that protein was

present in every filtration regardless of the addition of BSA as 2xYT medium broth was used to grow the *E. coli* hosts for virus production [33]. Thus all filtrations contained the broth in solution. The broth was a product of AMRESCO and their product information gives the formula for the broth as containing per liter 16.0 g of tryptone and 10.0 g of yeast extract. While perhaps it is possible to discern the common amount tryptone present in these solutions, each batch of broth produced would contain inconsistent amounts in part to the inclusion of yeast extract. As such, it is not feasible to be able to accurately calculate the protein concentration of any filtrations using this broth. This also complicates quantifying protein recovery. One possible solution around this issue would be to use a bacterial host in combination with specific minimal media that could allow bacteria to thrive without containing a significant portion of protein already present in solution. It would not, however, be able to remove the host proteins produced during the virus creation process. Considering the comparison study does not attempt to remove these proteins and describes the solution as "low protein," these are not considered significant contributions to the foulant load [13]. Another avenue would involve using BSA in phosphate buffer then adding virus to conduct control experiments since the feed would have much less protein.

Comparing flux data with Wickramasinghe et al. shows the MVM feeds to have mostly higher fluxes for the Omega 300 membrane and fluxes between the Omega 10 and the Sartorius 10kDa membrane [13]. Fluxes ranged from about 400 liters per meter squared per hour (LMH) to below 10 LMH in **Fig. 5**. The comparison study maintained a flux of above 1000 LMH for Omega 300, even with 1% BSA, and at a lower pressure. Obviously the fluxes from these experiments are expected to be lower simply due to the presence of protein in every run. The Omega 10 and the Sartorius 10kDa membranes faired more comparably with protein fluxes falling below 10 LMH for both studies, regardless of viral presence. The largest deviation between the

two studies' data concerns LRV. The MVM study showed two membranes as having substantial virus removal properties, which were DV20 and Omega 10. DV20 is specifically designed for virus removal, unlike the ultrafiltration membranes used, and one of Omega 10's suggested usage is for vaccine concentration. What was significant in those experiments was the membrane orientation, as they showed a higher LRV when the separation surface was facing the feed. **Fig. 6** and **Fig. 7** show no effect when the orientation of the membrane is changed. Only with the addition of BSA for the Omega 300 membrane is there any reduction in LRV. Data for the 10 kDa membrane is insufficient to determine if the addition of BSA would have significant effects on LRV. For the Omega 300 membrane, it follows that the increased protein concentration fouled enough of the smaller pores such that the larger ones were open enough to allow virus particles to reach the permeate. This illustrates why having a narrow range of pore sizes is crucial for successful virus capture. Without BSA, both ultrafiltration membranes were adequate at providing some level of virus removal for the two model viruses, which suggests the membranes are acceptable for future testing. However, specific concentrations of protein would need to be quantifiable for future experiments. Initial virus concentrations would need to be more consistent for greater analysis on specific membrane performance for each virus.

5 Future Recommendations

Additional research concerning these experiments would benefit from several adjustments to the methodology discussed previously. Duplication of fluxes should be made for all runs, and averages could be made for the runs. Doubling or tripling the number of measurements taken during the run would provide protection against errant values from disrupting any analysis performed on the flux data. More data concerning the 10 kDa membrane flux with 1% BSA is necessary, however care must be taken when using that combination. The UF membranes used

are designed for non-inverted orientation. Placing the support to face the feed could cause leakage as the solution is directed not through the membrane but rather around or over it. The stirred cells used in these experiments are unlike the virus filtration cells used in industry, which are designed around not being susceptible to such leaking issues.

Protein concentration and quantification are useful parameters to control. The viral stocks contained protein that was difficult to quantify. Assays that could determine protein concentration before, during, and after runs would provide BSA rejection data and allow for standardization between runs. This would allow for examination of fouling should different effects be seen for the various parameters. Another method to reduce extra protein from the media could be obtained through centrifugation. The methods previously used centrifugation to remove cellular debris. If a second centrifugation was used to make the virus particles into a pellet and resuspend it in phosphate buffer solution. Though it may not remove all of it, this technique would greatly reduce non-BSA protein in the feed solution.

6 Conclusions

Virus filtrations were performed using commercially obtained asymmetric, ultrafiltration membranes with bacteriophages. 10 kDa and 300 kDa MWCO PES membranes exhibited virus removal capabilities for bacterial parvovirus and retrovirus, Phi X174 and PR772 respectively, in the presence of protein. These viruses are approved by the FDA as mammalian virus analogues. Results confirm that using the support structure as a pre-filter allows for better performance by reducing fouling on the separating surface of the membrane. High protein concentration, greater than 1% w/v, negatively impacted the membrane's flux, while virus size did not appear to affect either flux or LRV.

7 Figures

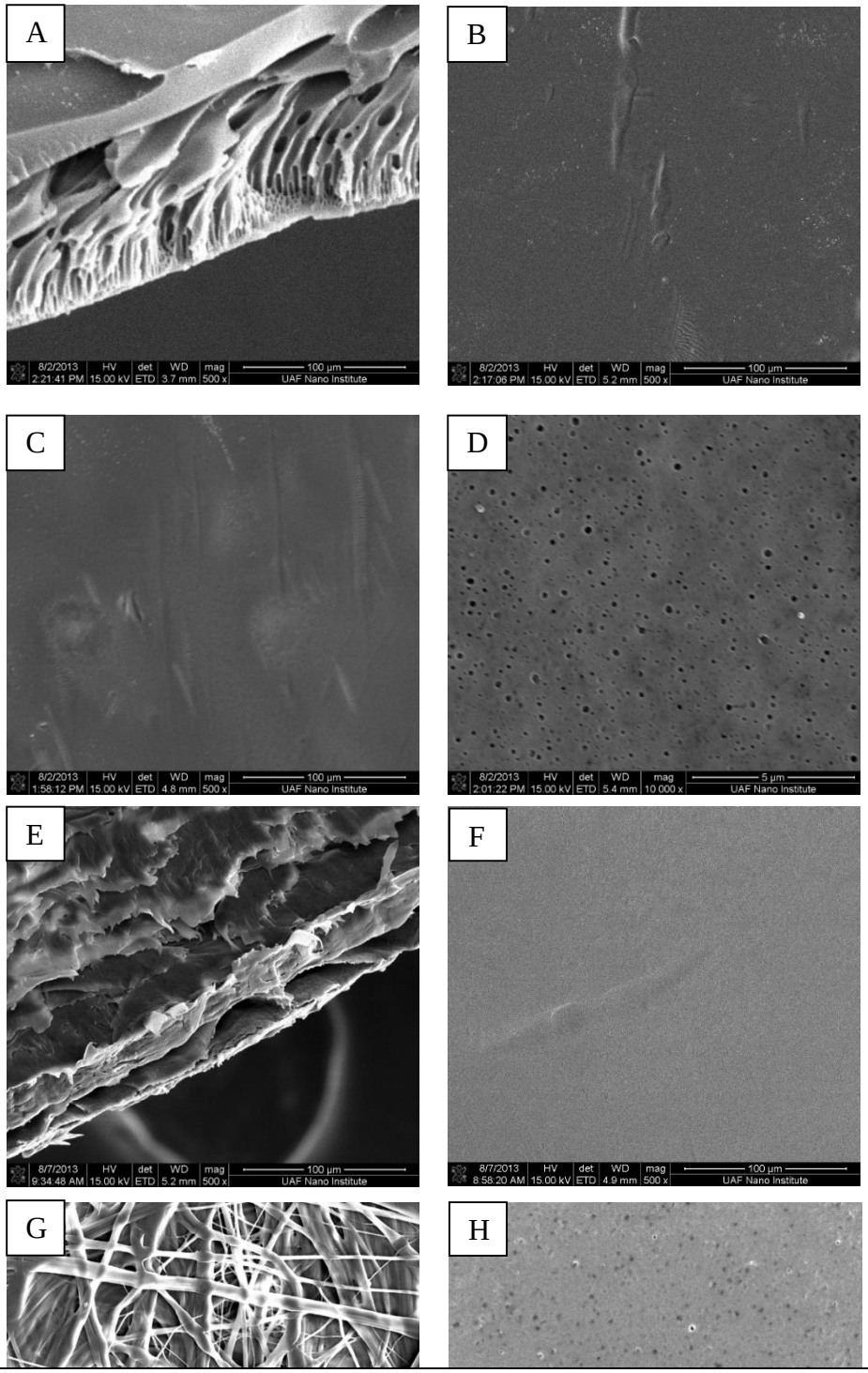


Fig. 1 SEM images of 10 kDa Sartorius PES membrane A) cross-section B) barrier C) support at 500 times magnification and D) support at 10,000 times magnification. Remainder at 300 kDa Pall PES membrane E) cross-section F) barrier G) support at 500 times magnification and H) barrier at 10,000 times magnification.

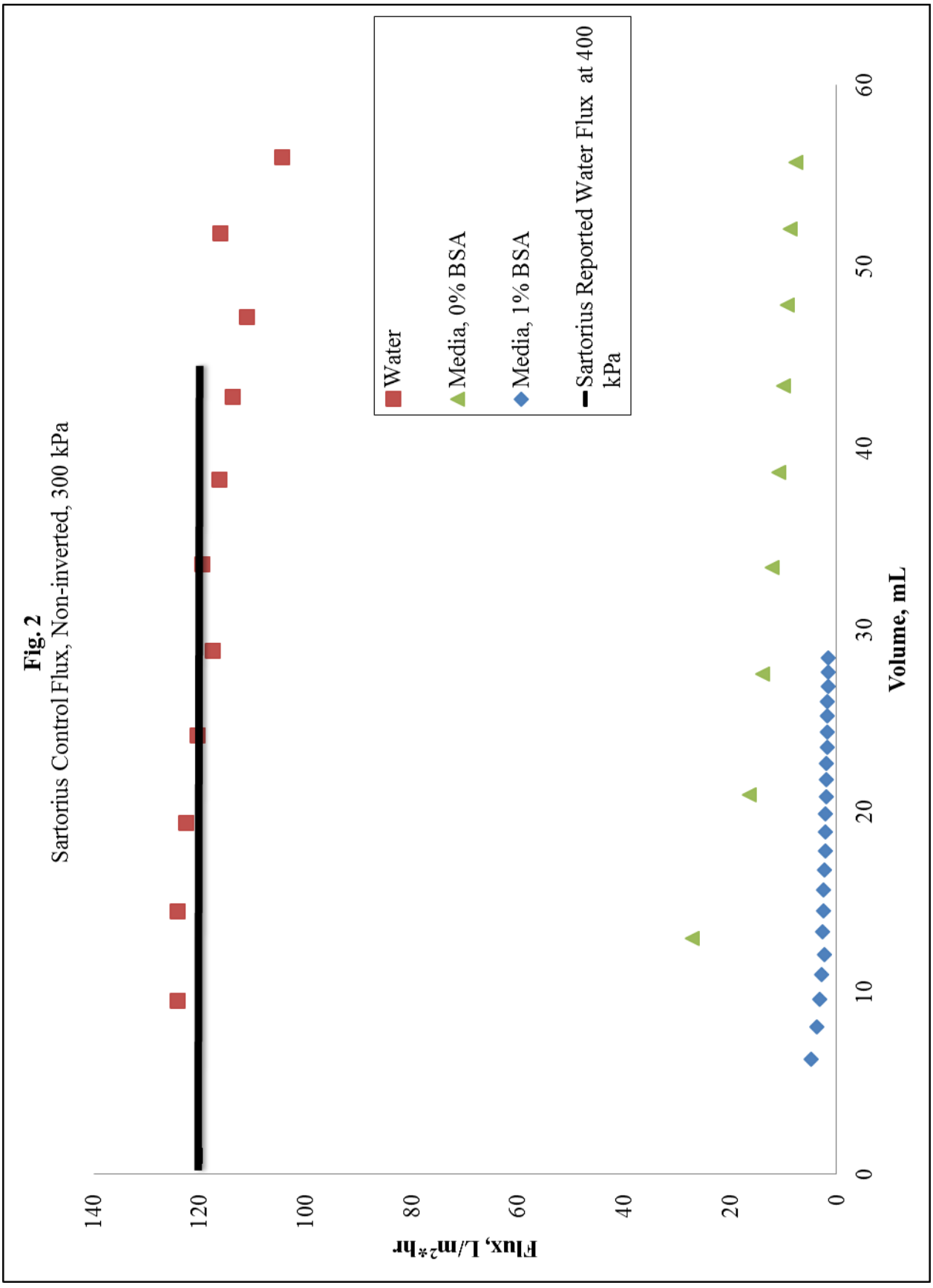


Fig. 2
Sartorius Control Flux, Non-inverted, 300 kPa
Water
Media, 0% BSA
Media, 1% BSA
Sartorius Reported Water Flux at 400 kPa
Flux, L/m²*hr
Volume, mL
0
20
40
60
80
100
120
140
0
10
20
30
40
50
60

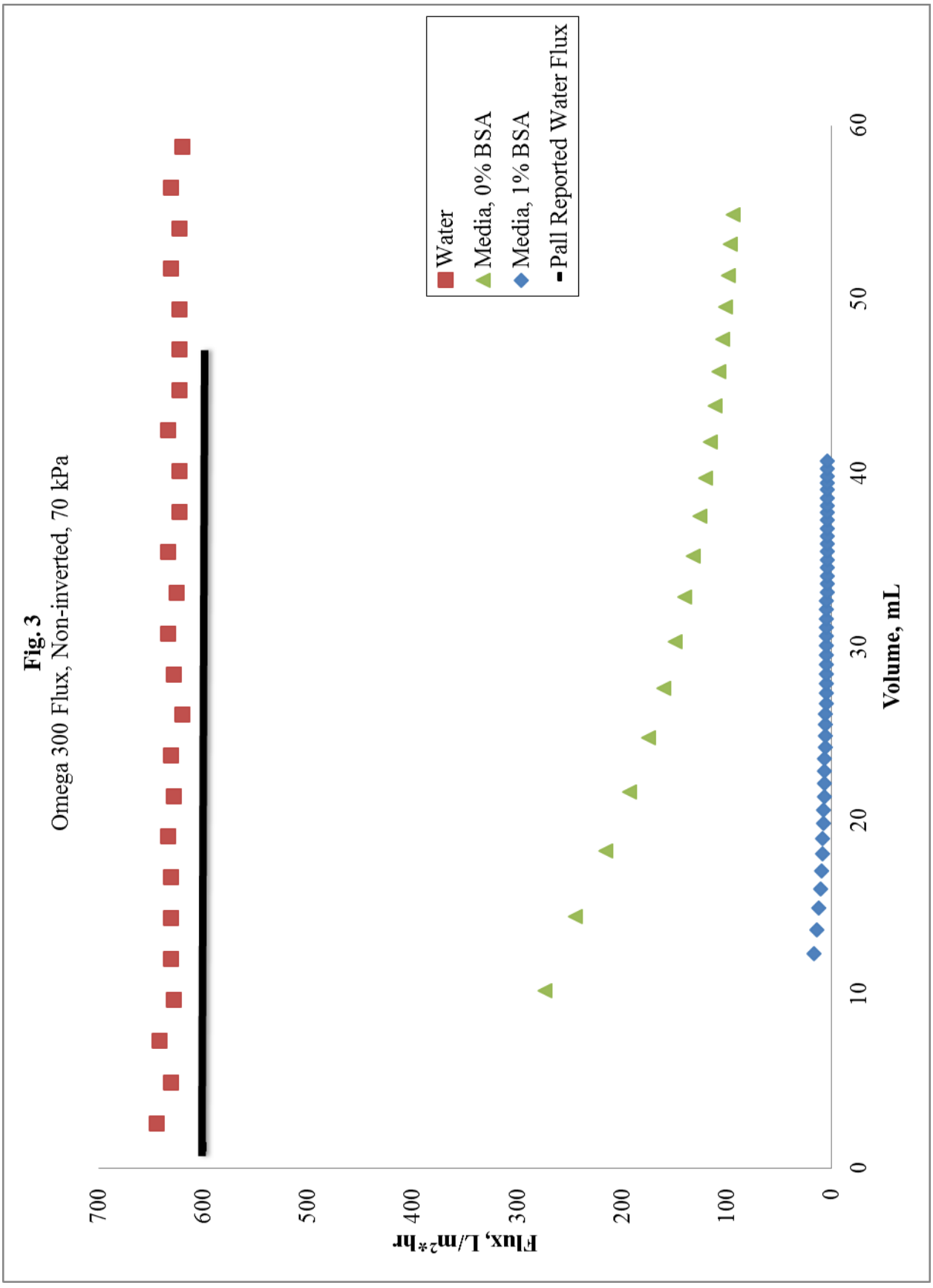


Fig. 3
Omega 300 Flux, Non-inverted, 70 kPa
Water
Media, 0% BSA
Media, 1% BSA
Pall Reported Water Flux
Flux, L/m²*hr
Volume, mL
0
100
200
300
400
500
600
700
0
10
20
30
40
50
60

Fig. 4

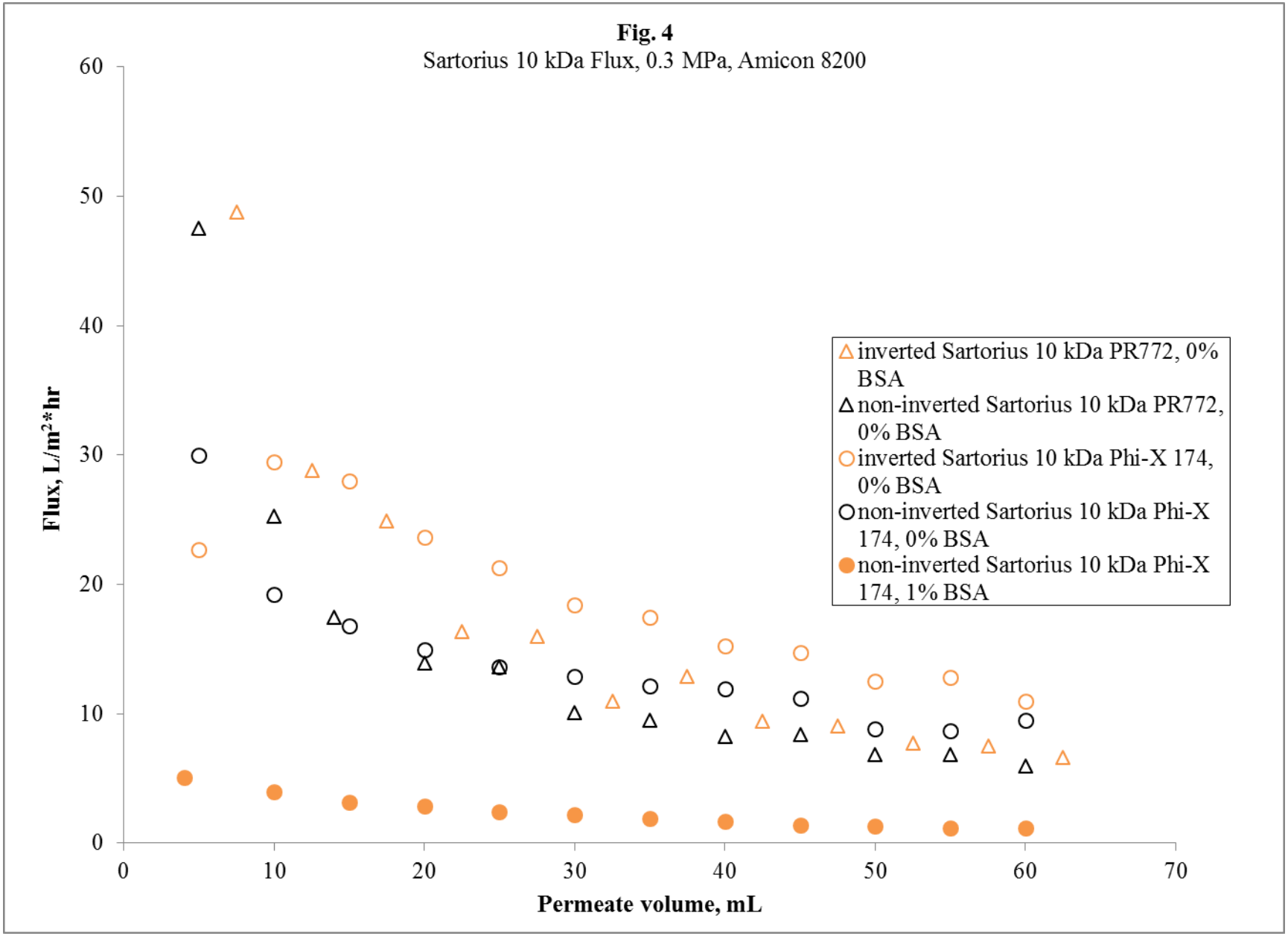

Sartorius 10 kDa Flux, 0.3 MPa, Amicon 8200
Flux, L/m²*hr
Permeate volume, mL
0
10
20
30
40
50
60
70
inverted Sartorius 10 kDa PR772, 0% BSA
non-inverted Sartorius 10 kDa PR772, 0% BSA
inverted Sartorius 10 kDa Phi-X 174, 0% BSA
non-inverted Sartorius 10 kDa Phi-X 174, 0% BSA
non-inverted Sartorius 10 kDa Phi-X 174, 1% BSA

Fig. 5

Omega 300 Flux, 70 kPa, Amicon 8050

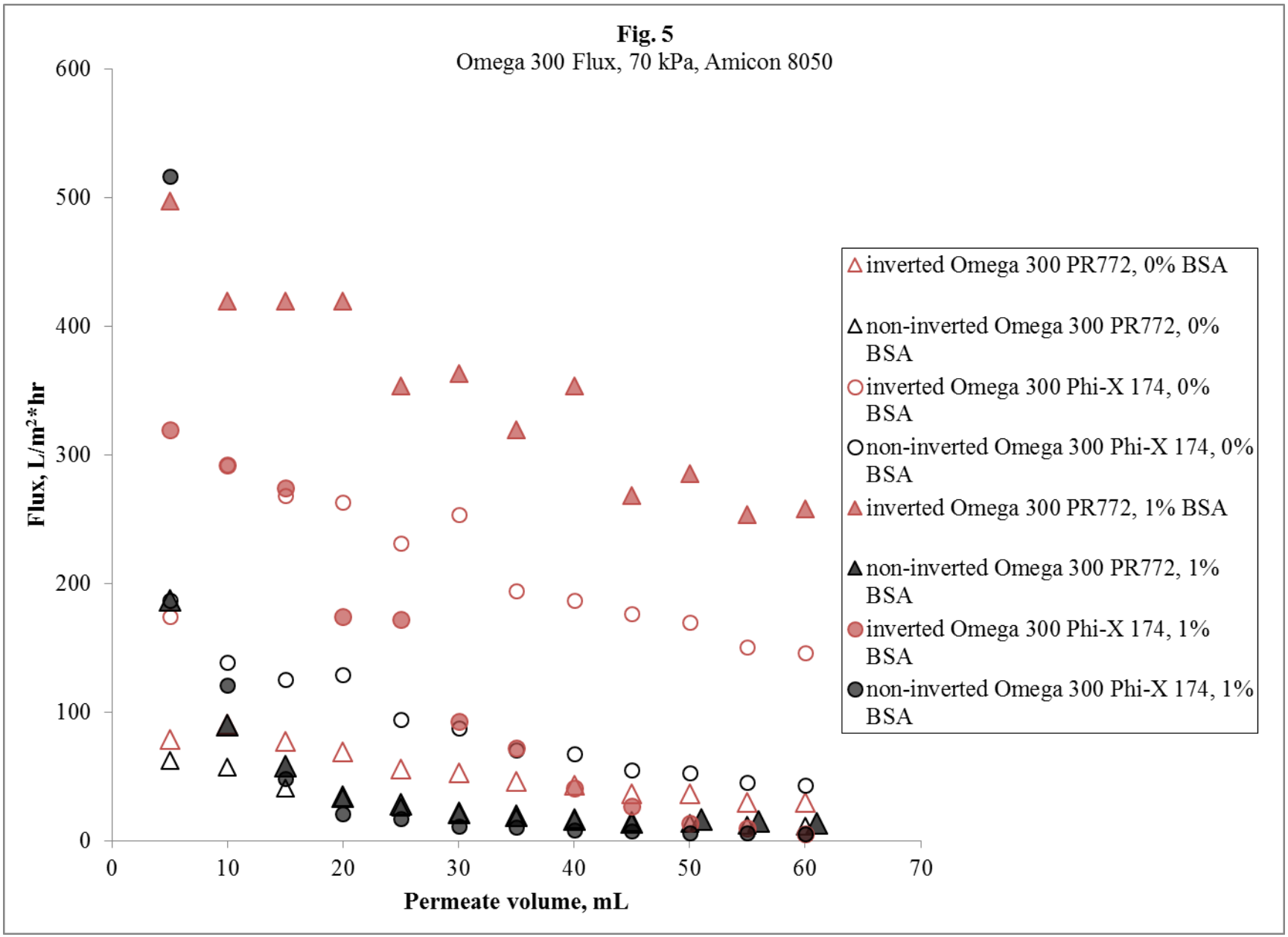


Fig. 6

Sartorius 10 kDa LRV, 0.3 MPa, Amicon 8200

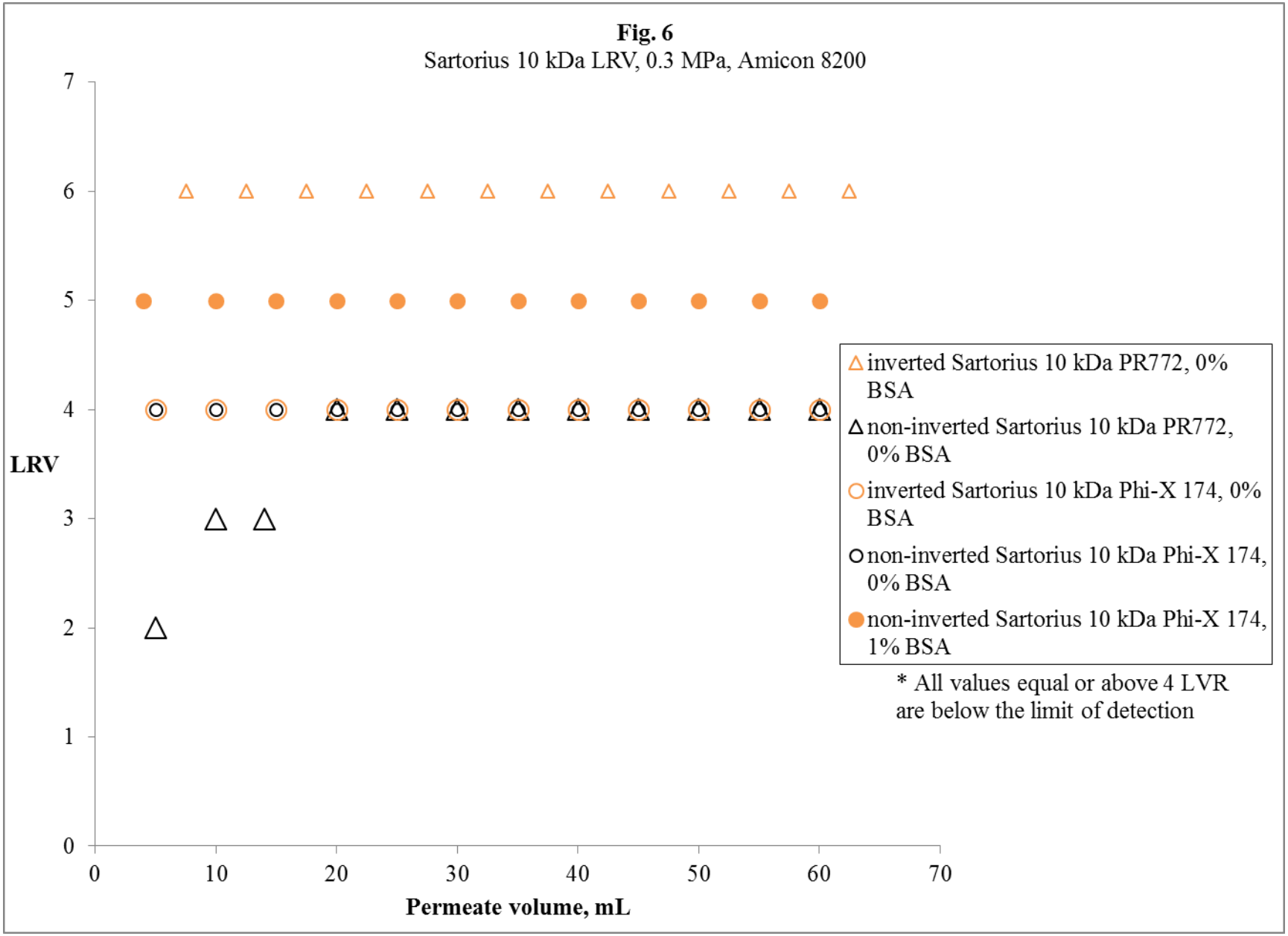
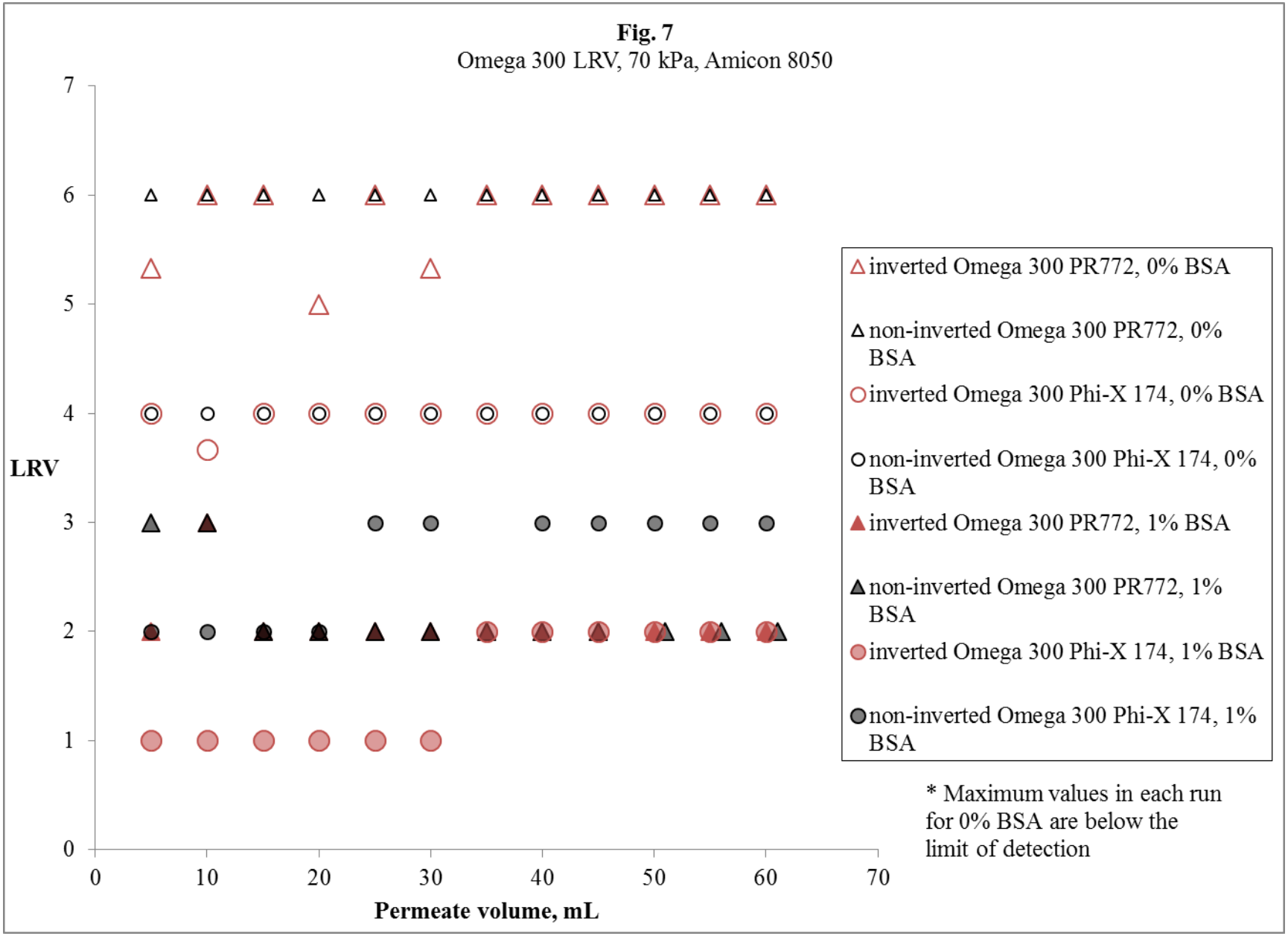


Fig. 7

Omega 300 LRV, 70 kPa, Amicon 8050



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