Photo-crosslinked PVA/PEI electrospun nanofiber membranes:
Preparation and preliminary evaluation in virus clearance tests

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Abstract

We report on the preparation of electropositive nanofiber membranes by electrospinning with in situ photo-crosslinking and their preliminary evaluation in virus adsorption and removal tests. Poly(vinyl alcohol) (PVA) and polyethyleneimine (PEI) were modified with glycidyl methacrylate, to form an acrylated crosslinked polymer (a-PVA/a-PEI) upon UV exposure during the electrospinning process. The a-PVA/a-PEI nanofibers were electrospun on a non-woven polyester support to form an electropositive (\(\zeta = 7\) mV at pH 7.4) and hydrophilic (\(\theta_w \approx 53^\circ\)) membrane with the mean pore size of 0.48 \(\mu\)m. The microfilter had the specific permeate flux of \(\sim 6.9 \times 10^4\) L/(m\(^2\)·h·bar), comparable with that of commercially available membranes of similar nominal pore sizes. Adsorption of the negatively charged and hydrophilic bacteriophage MS2 (\(d \approx 27\) nm) onto the membrane followed Freundlich isotherm and could be classified as favorable with the average adsorption intensity \(n^{-1} \approx 0.91\). The 99% retention of MS2 in flow-through virus clearance tests was attributed to adsorption and was likely controlled by the limited detention time within the membrane.

Keywords: electrospinning; nanofiber membranes; photo-crosslinking; microfiltration; bacteriophage MS2
1. Introduction

Electrospinning is commonly used to make nanofibers ranging from ~10 nm to several hundred nanometers in diameter [1]. Comparatively simple and inexpensive [2, 3], the electrospinning method offers an alternative to other nanofiber manufacturing techniques such as drawing, template synthesis, phase separation and self-assembly [4]. Electrospinning relies on electrostatic forces to draw ultrafine solid threads from solutions of polymers of sufficiently high molecular weight and does not require coagulation chemistry or high temperatures [1, 5]. Fiber mats produced by electrospinning are lightweight and characterized by high porosity, small inter-fiber pore size and large surface area [1, 6]. Electrospun nanofiber membranes (ENMs) have found a wide range of applications including drug delivery [7], scaffolding in tissue engineering [8], clothing protection [9], sensing [10], as well as adsorption [11, 12] and filtration [13]. In membrane filtration applications, ENMs have been used as microfilters [14, 15] and as supporting layers for both ultrafilters and salt-rejecting membranes [16]. The high porosity and interconnected pore structure of ENMs brings about increased permeability and higher separation throughput.

Many polymers processable by electrospinning are water-soluble and, when unmodified, are not suitable as materials for water treatment membranes [17, 18]. Physical or chemical crosslinking of polymer structures is a common method of rendering the nanofibers insoluble and increasing their thermal and chemical stability. Most ENMs have been produced by post-spinning crosslinking, as well as pre-crosslinking. Each of these approaches, however, adds another step to the fabrication process. Furthermore, pre-crosslinking, which involves mixing the polymer solution and chemical crosslinker, could lead to gel formation making continuous or large scale production difficult [19]. In our previous study, in situ UV radiation was implemented during electrospinning to achieve crosslinking [12, 20, 21]. In addition to being a very fast one-step process, this novel approach can ensure high crosslinking efficiency to minimize the amount of unreacted and leachable cytotoxic agents.
Key metrics of ENMs are fiber morphology and surface chemistry. The former define the nominal pore size and the permeability of the fiber mat while the latter govern adsorptive characteristics of the material. ENMs can be used to remove microorganisms from water either in purification applications or for pathogen detection. For example, VIRADEL (virus adsorption and elution) method employs charged microporous filters to concentrate viruses from water samples for further detection by downstream assays [22]. Viruses, with their size typically in the 20 to 200 nm range are too small for size exclusion by microfilters to be effective [15]. Instead, electrostatic interactions between the filter surface and viruses are employed to ensure sufficient removal. Because the charge of most viruses at pH typical for natural aqueous media is negative, electropositive filters are a common choice. The attractive feature of electropositive filtration is that virus removal can be achieved even though the pore size is much larger than the virus; the large pore size and porosity enable high permeate fluxes making, high throughput separations possible [23].

Significant amount of work has been done on virus adsorption by free nanofibers (i.e. nanofibers not cast as a membrane) – both electrospun and otherwise. For example, Mi et al. demonstrated that crosslinked N-[(2-hydroxyl-3-trimethylammonium) propyl] chitosan (HTCC)– polyvinyl alcohol (PVA) nanofibers adsorbed enveloped and nonenveloped viruses [24]; although ENMs were produced in this study, their separation properties (e.g. water permeability and nominal pore size) were not evaluated. Bai et al. showed that functionalization of electrospun chitosan nanofibers by a quaternary amine increased porcine parvovirus (<25 nm) removal from 30% to 70% [25]. Park and Kim prepared PVA nanofibers containing a quaternary ammonium compound (benzyl triethylammonium chloride) as an antimicrobial agent and showed that, in addition to bacteria, bacteriophages MS2 and PhiX174 could be removed; the removal was attributed to hydrophobic interactions between quaternary ammonium and viruses although the mechanism of inactivation remained unclear [26].

While virus adsorption to free nanofibers has been explored extensively, to our knowledge, only three studies to date have evaluated ENMs for their ability to remove
viruses from water. Working with bacteriophage MS2, Chu and colleagues first reported 99.99% retention of MS2 by composite fibrous membranes modified with PEI [27]. Wang et. al. showed that two-layered polyacrylonitrile (PAN) / polyethylene terephthalate (PET) ENMs amended with ultrafine (~ 5 nm) cellulose nanofibers achieved 99.99% removal for MS2 [28]. The same degree of removal of this bacteriophage was achieved in another study where by Ma et. al. used poly(1-(1-vinylimidazolium) ethyl-3-vinylimidazolium dibromide/PAN ENMs and attributed the high removal of the virus to its adsorption to the membrane [29].

The goal of the present work was to evaluate the feasibility of electrospinning with in situ crosslinking as a one-step method of fabricating electropositive water-stable membranes with high capacity for virus adsorption. We designed such membranes using PVA and PEI as base polymers. A biodegradable hydrophilic polymer, PVA is easy to process and functionalize and has high chemical and thermal resistance [12, 20]. The hydrophilicity of PVA should help make the resulting membrane more resistant to fouling. PVA is also a highly versatile material whose properties can be adjusted by regulating the degree of hydrolysis [30]. PEI is an aliphatic polyamine containing primary, secondary, and tertiary amines, which make this polymer suitable for producing polycationic nanofibers [19]. PEI is water-soluble and, unmodified, cannot be used as a material for water treatment membranes [17, 18]; thus to fabricate water-stable ENMs, PVA and PEI where photo-crosslinked during electrospinning. We characterized ENMs in terms of their morphological, chemical, and hydraulic properties and evaluated the filters in virus removal tests.
2. Materials and Methods

2.1 Materials

Poly(vinyl alcohol) (PVA; 87-89 % hydrolyzed, 146 -186 kDa), glycidyl methacrylate (GMA; 97%) and tetraethylmethylenediamine (TEMED) were purchased from Sigma Aldrich. Polyethyleneimine (PEI; 60 kDa) was obtained from Alfa Aesar. The radical photoinitiator, 2-hydroxy-2-methyl-1-phenyl-1-propan-1-one (Darocur 1173) was supplied by Ciba Specialty Chemicals. Dimethyl sulfoxide (DMSO), chloroform and ethylene alcohol were purchased from Merck. All chemicals were used as received from vendors without further purification. The polyester (polyethyleneterephthalate (PET); CraneMat® CU 434 UF nonwoven fabric sheets (porosity of 181 1/m²/s at 200 Pa) used as support materials were supplied by Neenah Technical Materials.

2.2 Preparation of membrane casting solutions

Prior to the preparation of membrane casting solutions, PVA and PEI were acrylated with GMA to enable photopolymerization during the electrospinning process. First, PVA was reacted with GMA following the procedure described by Crispim et al. [31]. Briefly, PVA was dissolved in DMSO at ~70 °C to yield 80 g(PVA)/L. GMA was then added dropwise into the solution magnetically stirred in the presence of TEMED as a catalyst at 70 °C for 4 h. The acrylated PVA (a-PVA) was precipitated three times using ethanol as a non-solvent and then dried under vacuum for 1 week. Second, GMA was added drop by drop to a PEI solution in 10 mL of chloroform at 0 °C to prepare acrylated PEI (a-PEI) and the solution was vigorously stirred overnight at room temperature in an flask wrapped in aluminum foil [19]. In the GMA/PEI mixture, the molar ratio of the secondary amine group on PEI to the epoxy group on GMA was 1.69:1. Then the acrylated PEI was precipitated with acetone to remove free GMA. The residue was washed with acetone three times and then filtered, dried, and stored in the fridge. Scheme 1 shows reaction pathways for the synthesis of a-PVA and a-PEI. The membrane casting
solution was prepared by dissolving 5 g a-PVA and 1 g a-PEI (i.e. PVA:PEI mass ratio of 5:1) in 5 mL of ethanol with 3 wt% admix of Darocur 1173.

![Scheme 1](image)

**Scheme 1.** Synthesis pathways for a-PVA (a) and a-PEI (b).

### 2.3 Synthesis of electrospun nanofiber membranes

The prepared solution was placed in a syringe connected to the positive terminal of a high-voltage (20 kV) power supply. The negative terminal of the power supply unit was connected to a conductive collector positioned 15 cm away from the syringe needle. The solution was spun at the flow rate of 0.10 mL/h for 10 h at room temperature while being irradiated by UV light ($\lambda_{max} = 365$ nm, OSRAM 300W high pressure UV lamp). The fibers were collected on the PET filter support paper (Figure 1). The non-woven PET support helped improve the mechanical stability of the membranes as unsupported ENMs are fragile.

To improve mechanical properties of ENMs they were subjected to compression and heat treatment [24]. Square (50 mm × 50 mm) pieces of the cast ENM mats were cut,
layered and placed in between two 100 mm × 100 mm square dies. For each membrane, 4 layers were compressed by a manual hydraulic press (Specac) at a load of 5 MPa for 1 min [32]. Then the ENM sheets were placed into a preheated oven at 90 °C for 10 min.

**Figure 1.** Schematic diagram of the electrospinning-UV process.

### 2.4 Membrane characterization

The infrared absorption spectra of the prepared ENMs were recorded using a FTIR spectrometer (Nicolet 6700). The morphology of ENMs was assessed using the ultra-high resolution scanning electron microscope (SEM; JEOL 7500F). The pore size of ENMs was measured by capillary flow porometer (Quantachrome 3GWin). Membrane porosity, $\varepsilon$, was determined gravimetrically as follows: first the volume of electrospun membrane ($V_{tot}$) was calculated by multiplying the thickness of the membrane by its surface area. Second, the mass of water was converted to the pore volume, $V_{pore}$, of the membrane in the assumption that all pores were filled with water. Finally, the pore
volume was divided by the total volume to give membrane porosity: \( \varepsilon = \frac{V_{\text{pore}}}{V_{\text{tot}}} \times 100\% \).

The \( \zeta \)-potential of ENMs was measured using Anton Paar Surpaas electrokinetic analyzer. The water contact angle on ENM surface was determined using the sessile drop method within the first 5 s of droplet deposition onto the membrane surface. The permeability of ENMs was measured using a dead-end filtration cell (see Supplementary content (SC); Figure S1). A 25 mm diameter ENM disk was placed in the filtration cell filled with 10 mL of distilled water and permeate flux was measured under three different transmembrane pressures at room temperature.

### 2.5 Propagation and purification of bacteriophage MS2

To examine the filtration performance of ENMs, bacteriophage MS2 (ATCC15597-B1) was used as a model virus. MS2 stock (1 mL) was mixed with 1 mL of suspension of *E. coli* (ATCC 15597) in 10 mL tryptone yeast extract broth and 0.8 mL of this preparation was placed into plate containing trypticase soy agar and incubated for overnight at 37 °C. After incubation, the MS2 suspension was centrifuged at 1000 rpm for 20 min and the supernatant was filtered through a 0.45 μm sterile syringe filter (EMD Millipore). The MS2 concentration in the obtained stock suspension was \( \sim 10^{10} \) plaque forming units (PFU) per 1 mL. The MS2 stock suspension was stored at 4 °C.

### 2.6 MS adsorption tests

In adsorption experiments, 10 mg of ENMs was added to 5 mL of MS2 suspension of different concentrations. Contact times up to 60 min were used in each adsorption test. Each adsorption measurement was performed at room temperature and neutral pH and was replicated once.

### 2.7 Filter challenge tests with bacteriophage MS2

The MS2 feed suspension was prepared by diluting 1 mL of the stock (\( \sim 10^{10} \) PFU/mL) in 1 L of sterilized distilled water giving the MS2 concentration in the membrane feed of
~10⁷ PFU/mL. The pH of the feed was ~7.4. The suspension was filtered through an ENM disc using the transmembrane pressure of 2.5 psi (~ 0.17 bar) in the dead-end filtration cell (Figure S1). The permeate was collected in an autoclaved vial and the virus concentration in the permeate was determined by the plaque assay procedure. Serial dilutions of the permeate samples for MS2 assaying were prepared using phosphate buffer solution (pH 7.2). A small volume (0.3 mL) of each permeate sample was added into the glass vial containing approximately 5 mL of soft agar and then 0.1 mL of *E. coli* solution was added. This mixture was poured onto the 100 mm diameter petri dishes with solid agar and allowed to harden. All petri dishes were incubated overnight at 37°C. All experiments were repeated 3 times. After incubation, the plaques were counted to yield the concentration of MS2 phage in PFU/mL. Virus retention by the membranes was calculated as the log reduction values defined as \( LRV = \log \left( \frac{C_f}{C_p} \right) \), where \( C_f \) and \( C_p \) are concentrations of MS2 in the feed and in the permeate, respectively [33].

### 3. Results and Discussion

#### 3.1 Chemical and morphological characterization of ENMs

To obtain water-insoluble ENMs, a-PVA and a-PEI were synthesized first. The FTIR spectrum of a-PVA (Figure 2) presented a broad band at 3432 cm⁻¹ (–OH stretching), a band at 2980 cm⁻¹ (C–H vibrational stretching), and a band at 1023 cm⁻¹ (C–O vibrational stretching). The bands at 1710 cm⁻¹ (C=O stretching) and 1646 cm⁻¹ (C=C stretching) stemmed from methacrylate groups of GMA used to modify PVA [34]. The FTIR spectrum of a-PEI contained bands at 3307 and 3352 cm⁻¹, corresponding to N-H and –OH groups respectively. The carbonyl (C=O) stretching and vinyl (C=C) groups gave rise to 1710 cm⁻¹ and 1647 cm⁻¹ bands. The peak at 1040 cm⁻¹ was attributed to C-N group [19].
Figure 2. FTIR spectra of a-PVA, a-PEI, electrospun polymer solution and ENMs.

Scheme 2. Proposed structure of the ENM polymer synthesized by crosslinking a-PVA with a-PEI.
**Figure 3.** An SEM image of the cross-section of a-PVA:a-PEI (mass ratio of 5:1) the electrospun nanofiber membrane (thickness of ~98 µm) on the non-woven polyethyleneterephthalate support (thickness of ~110 µm).
Figure 4. SEM images of electrospun nanofiber membranes of pure PVA (a, b) and of a-PVA/a-PEI (c, d).
The FTIR analysis of the photo-crosslinked ENMs produced by mixing of these oligomers showed that photo-crosslinking of the nanofibers was followed by the disappearance of the double bond (C=C) band at ~1646 cm\(^{-1}\) (Figure 2). Scheme 2 shows the proposed structure of the synthesized ENMs.

SEM imaging of ENMs revealed a mat of randomly oriented intertwined nanofibers on the non-woven support (Figure 3). The pure PVA nanofibers appeared to be smooth with the fiber diameter of 245 ± 25 nm (Figures 4a and 4b). After acrylation, more intertwined nanofibers with beady inclusions and more apparent crosslinking between the fibers were observed. Parameters, such as the polymer concentration and molecular weight, pH, salt and surfactant contents of the solution, are known to affect the morphology of electrospun PVA fibers [30]. The beading observed in our study was an expected result attributable to the acrylation-induced increase in the molecular weight of the polymer. The diameter of fibers ranged from 90 nm to 350 nm with the average of 228 nm. The modification of PVA and PEI with GMA and crosslinking of those materials may have led to an increase in the molecular weight of the resulting polymer [35]. Based on the SEM images (Figures 4c and 4d), the average thickness of the ENM was ~98 µm.

Table 1. Morphological properties of a-PVA/a-PEI electrospun nanofiber membranes on PET non-woven support.

<table>
<thead>
<tr>
<th></th>
<th>Average fiber diameter, µm</th>
<th>Layer thickness, µm</th>
<th>Porosity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-woven PET support</td>
<td>16.95(^\text{A})</td>
<td>110(^\text{A})</td>
<td>63.0(^\text{C})</td>
</tr>
<tr>
<td>ENM layer</td>
<td>0.23(^\text{A})</td>
<td>98(^\text{A})</td>
<td>91.3(^\text{D})</td>
</tr>
<tr>
<td>Overall membrane</td>
<td>n/a</td>
<td>208</td>
<td>76.3(^\text{B})</td>
</tr>
</tbody>
</table>
The mean pore size and the maximum pore size of ENMs were found to be 0.48 µm and 0.75 µm, respectively. The calculated porosity of the ENM layer (91.3%) is higher than values typically reported for ENMs (typically ~ 80% [13]). The compression that accompanied heat treatment (see section 2.3) was primarily used to improve the mechanical stability of the membrane. However, it likely also reduced both the pore size and the porosity. If larger pore size is desired, it may be possible to avoid compression as heat treatment should be sufficient to render the membranes mechanically stable. Lower pore size and porosity, however, should translate into a higher frequency of virus-fiber collisions [23] and, therefore, higher virus removal. Thus, the decision on whether to employ compression depends on the desired balance between permeability and selectivity of the nanofiber membrane.

The initial water contact angle of ENMs was measured to 52.7 ± 7.7°; the contact angle quickly decreased as the water droplet was absorbed by the ENM. ζ-potential of ENMs at pH 7.4 was measured to be + 7 mV, which can be attributed to the positive charge of PEI; the value is lower than +20 mV reported for cellulose nanofiber membranes modified by polyvinylamine [28]. The relative contents of the electropositive PEI and the electronegative PVA should determine the overall electrical charge on the membrane and, therefore, should be expected to affect virus sorption by such composite membranes.

### 3.2 Water flux performance of ENMs

Clean water flux tests showed that the hydraulic resistance of the ENMs was on average 5.18·10^9 m⁻¹ (i.e. specific permeate flux of 69,290 L/(m²·h·bar)). Given the ENM’s estimated thickness of 98 µm (section 3.1), the measured value of the hydraulic resistance translates into the specific hydraulic resistance of 5.29·10¹³ m⁻² and permeability of 1.89·10⁻¹⁴ m². For homogeneous, isotropic porous media, the square root of its permeability provides an estimate of the average pore size [36]. For the
prepared ENMs, the calculation gives ~ 0.14 µm, which is of the same order of magnitude as the 0.48 µm obtained by porometry (section 3.1).

Four different porous membranes with the nominal pore size in the submicron range – a) commercial polycarbonate track etch membrane ($d_{pore} = 30$ nm), b) commercial polyacrylonitrile membrane ($d_{pore} = 200$ nm), c) PAN/PSf electrospun nanofiber membrane ($d_{pore} = 0.68$ µm), and d) commercial fluoropolymer membrane ($d_{pore} = 800$ nm) were used as comparisons. The average hydraulic resistances of these membranes were $1.90 \times 10^{10}, 1.68 \times 10^{7}, 2.00 \times 10^{8}$ and $6.20 \times 10^{10}$ m$^{-1}$ respectively. (Permeate flux versus transmembrane pressure dependencies for these membranes are shown in SC, Figure S2.) Thus, the resistance measured for the synthesized ENM membrane ($5.18 \times 10^{9}$ m$^{-1}$) is comparable with that of commercially available membranes of similar nominal pore sizes.

3.3 MS2 adsorption onto electrospun nanofibers

To our knowledge, there have been no published reports with kinetics or isotherms of MS2 adsorption onto membrane polymers. In order to describe the adsorption capability of ENMs with respect to the bacteriophage, batch adsorption experiments were carried out. MS2 adsorption kinetics test showed the time to equilibrium of ~ 30 min (see SC; Figure S3). MS2 adsorption isotherms (Figure 5) followed Freundlich model (eq. (1)) much better than the Langmuir model (see SC):

$$q_e = k_F C_e^{1/n}$$

(1)

The $n^{-1}$ values predicted in two separate isotherm experiments showed favorable adsorption ($n > 1$) and were relatively close: $0.8782 \pm 0.0119$ and $0.9497 \pm 0.0126$. The units of the “capacity” constants, $k_F$, determined in the two isotherm tests are different so that, strictly speaking, the two constants cannot be compared one with another. However, because of the proximity of $n^{-1}$ values in the two tests, we can draw a qualitative comparison of $k_F$ values. The ~ 34% difference ($k_F = 2.63 \pm 1.22$)
(mg/g)(L/mg)$^{1.14}$ in test 1 versus $k_F = 1.97 \pm 1.25$ (mg/g)(L/mg)$^{1.05}$ in test 2) can be interpreted as stemming in part from the difference in units and in part from the experimental error with the concentrations (PFU/mL) of the viable virus.

**Figure 5.** Freundlich isotherm of MS2 adsorption on electrospun nanofibers.

### 3.4 Removal of bacteriophage MS2 by electrospun nanofiber membranes in flow through tests

MS2 is commonly used as a human virus surrogate in virus challenge studies. The hydrophilicity [37] and small size (~ 27 nm [38]) of this bacteriophage make it a suitable conservative tracer [39]. The pI of MS2 ranges from 2.2 to 4.0 depending on the solution chemistry [40]. At pH 7.6 (close to the feed pH of 7.4), the $\zeta$-potential of MS2 was measured to be ~ -32 mV [41]. In the present study, dead-end filtration tests showed ~ 2 LRV of MS2 by ENMs (Figure 6), which compared well with removals achieved by PAN and cellulose-based ENMs (Table 2).
Several factors affect virus removal by membranes. First, when filter pore size is sufficiently small, viruses can be removed by size exclusion (i.e. sieving). Several expressions of varying complexity have been proposed to describe particle removal by filters via the size exclusion mechanism (see Baltus et al [42] for a discussion). In the assumption that only steric particle-pore wall interactions are important, the rejection $\sigma$ of rigid spherical particles of diameter $d_{\text{part}}$ by a membrane with cylindrical pores of diameter $d_{\text{pore}}$ was shown to be accurately described by the following expression [43]:

$$\sigma = 1 - (1 - \lambda)^2 \left( \frac{1 - 3.867\lambda - 1.907\lambda^2 - 0.834\lambda^3}{1 + 1.867\lambda - 0.741\lambda^2} \right)$$

where $\lambda = d_{\text{part}} / d_{\text{pore}}$. Given the large difference between the sizes of MS2 and ENM pores, size exclusion should not make a significant contribution to MS2 removal. Indeed, the removal of MS2 ($d_{\text{part}} = 27$ nm) by the ENM ($d_{\text{pore}} = 480$ nm) as predicted by eq. (2) is ~ 2%, much less than the 99% removal observed experimentally (Figure 6).

**Table 2.** MS2 retention by select ENMs and other microfilters.

<table>
<thead>
<tr>
<th>Membranes</th>
<th>Nominal $d_{\text{pore}}$, $\mu$m</th>
<th>MS2 LRV</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENM: a-PVA/a-PEI</td>
<td>0.48</td>
<td>2</td>
<td>This study</td>
</tr>
<tr>
<td>ENM: cellulose</td>
<td>0.38</td>
<td>~0</td>
<td>[28]</td>
</tr>
<tr>
<td>ENM: cellulose w/ polyvinylamine grafting</td>
<td>0.32</td>
<td>&gt;4</td>
<td>[28]</td>
</tr>
<tr>
<td>ENM: PAN</td>
<td>0.22</td>
<td>2</td>
<td>[15]</td>
</tr>
<tr>
<td>Modified (hydrophilic) PVDF microfilter</td>
<td>0.22</td>
<td>&lt;0.5</td>
<td>[44]</td>
</tr>
<tr>
<td>GS9035 commercial microfilter</td>
<td>n/a</td>
<td>1</td>
<td>[15]</td>
</tr>
</tbody>
</table>

Adsorption of the virus to the membrane material is another possible mechanism of virus removal. In simple water matrices virus adsorption is governed by electrostatic, van der Waals and hydrophobic interactions between the virus and the filter. The hydrophobic interactions between the bacteriophage and the membrane, both of which are hydrophilic, had to be repulsive [45]. The electrical charges on the virus and on the membrane at the pH used in the challenge tests (pH 7.4) were of opposite signs (positively charge membrane and negatively charged MS2) so that the electrostatic
interactions were favorable. (The zeta potential of the membrane was measured to be +7 mV (see section 3.1) while, as mentioned earlier, the charge of MS2 at pH 7.6 is ~32 mV [41]). Thus, we attributed the observed adsorption of MS2 to the dominance of favorable van der Waals and electrostatic interactions over the hydrophilic repulsion.

Figure 6. Log removal of MS bacteriophage by a-PV/a-PEI electrospun nanofiber membranes.

Removing 2 logs of viable MS2 phage from the feed with the concentration of ~$10^7$ PFU/mL means that for each mL of the permeate $10^2$ PFU of MS2 were retained by the membrane. Given that the total volume filtered was 10 mL, ~$10^3$ PFU of MS2 ended up on the surface of ENM fibers. A simple calculation shows that only a very small fraction (~$0.7\cdot10^{-12}$) of the total available membrane surface is occupied by the adsorbed viruses. Based on of the transmembrane pressure applied in the challenge tests (2.5 psi or ~0.17 bar) and the average hydraulic resistance measured for the membrane ($5.18\cdot10^9$ m$^{-1}$; see section 3.2), the average permeate flux is ~$3.28\cdot10^{-3}$ m/s. With the average membrane thickness of ~98 µm (section 3.1), this permeate flux value translates into the detention time of ~30 ms. This is more than 4 orders of magnitude smaller than ~30 min required to achieve adsorption equilibrium (Figure S3). Thus the incomplete (99%) removal of MS2 in the challenge tests was likely limited by the detention time of the virus within the membrane. Optimizing the PEI content of the
membrane to increase the positive charge on the fibers should help increase MS2 removal while maintaining the same permeate flux. We note that due to its small size and hydrophilicity, MS2 provides a very conservative estimate of a filter’s ability to remove viruses. One can expect higher removals for most other viruses.

4. Conclusions

Electropositive nanofiber membranes based on acrylated poly(vinyl alcohol) and polyethyleneimine were produced by electrospinning with *in situ* photo-crosslinking. The resulting membrane was an electropositive (ζ = 7 mV at pH 7.4) and hydrophilic (θw ≅ 53°) microfilter with the mean pore size of 0.48 µm. The hydraulic resistance of the membrane was measured to be 5.18·10⁹ 1/m, which as equivalent to the specific permeate flux of ~ 6.9·10⁴ L/(m²·h·bar). Data from batch adsorption tests with bacteriophage MS2 gave a high quality fit by a Freundlich isotherm pointing to favorable adsorption (n⁻¹ ≅ 0.91). The 99% retention of MS2 demonstrated in flow through virus clearance tests was attributed to adsorption; given the small size and hydrophilicity of MS2, the retention should be expected to be higher for most other viruses. In sum, electrospinning with *in situ* photo-crosslinking was shown to be feasible one-step method of fabricating electropositive nanofiber membranes capable of removing viruses much smaller the membrane pore size at high permeate fluxes.

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