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Virus reduction through microfiltration membranes modified with a cationic polymer for drinking water applications

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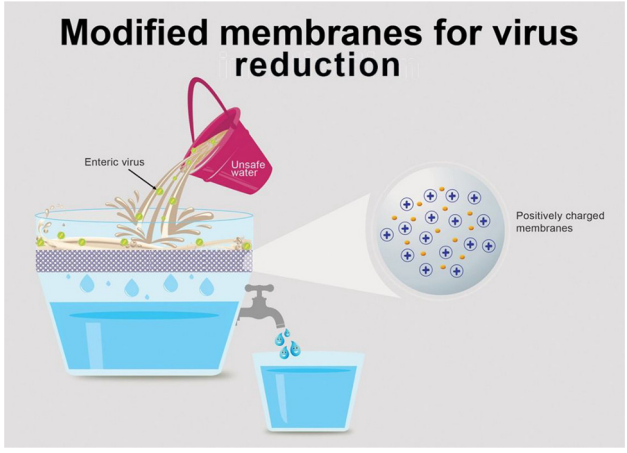
GRAPHICAL ABSTRACT



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ABSTRACT

Virus penetration is a significant problem in water treatment membrane filtration. To effectively remove waterborne viruses nano-filtration, reverse osmosis or ultrafiltration must be used, all of which are high energy filtration schemes. Novel approaches and technologies for the production of virus-free drinking water are therefore warranted. In this study, we modified model surfaces and commercial polyether sulfone, (PES) microfiltration (MF) membranes to achieve a substantial virus reduction under gravity based filtration membranes. The successful modification using the cationic polymer polyethyleneimine (PEI) was confirmed by Fourier transform infrared spectroscopy (FTIR) and zeta potential measurements. MS2 bacteriophages, a surrogate for human pathogenic waterborne viruses like norovirus were used to challenge the modified surfaces. The membrane modification resulted in ~22% loss of the membrane permeability while an increase of ≥3 $\log_{10}$-units (≥99.9%) in MS2 reduction was observed. These reductions were comparable to the reduction of PEI-coated model surfaces tested for contact reduction. This simple modification of a commercially available MF membrane led to substantial viral reductions with a significant flux of 5000 L/m^2 in approximately 2.5 h. This work

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therefore, highlights the potential modified MF membranes for gravity-based filtrations to produce safe drinking water. Further studies should be done to show similarly enhanced reductions of human pathogenic viruses.

1. Introduction

Over 780 million individuals worldwide lack access to clean drinking water and 2.5 billion lack access to adequate sanitation [1]. Consumption of unsafe drinking water, e.g., contaminated with enteric viruses such as gastroenteritis and hepatitis cause a significant global disease burden [2]. Large waterborne outbreaks with enteric viruses include hepatitis E virus [3], rotaviruses [4,5], noroviruses [6–8], amongst others [9–11].

Since very low doses (one infectious viral particle) may be sufficient to cause infection and illness, low concentrations (1 $\log_{10}$-units) of viruses in drinking water constitute a health risk [7,12–14]. Moreover, due to their small size and stable nature, waterborne viruses are among the most difficult enteric pathogens to remove from drinking water sources, such as surface or (vulnerable) groundwater [15].

The use of microfiltration membranes (MF) to remove viruses from water is limited by the small size of viruses and the relatively large pore size of the membranes [16]. These membranes can also have surface imperfections which increase the possibility of virus penetration during filtration [17]. MF membranes are therefore, rarely effective for virus removal without pre-treatment or post treatment procedures. Although, the reliability and ease of operation of membrane-based water filtration systems led to their increasing use in water treatment conventional treatments are usually also employed [18].

Current water treatment and disinfection processes use chemicals such as chlorine and have successfully protected public health against waterborne diseases [19]. However, chemical disinfectants produce potentially toxic disinfectant by-products (DBPs), like bromate and chlorite which can also pose significant health risks and cause problems like bad taste and odour [20]. Additionally, the emergence of waterborne pathogens which are resistant to chemical disinfection has led to the reappraisal of traditional disinfection practices. Alternatively, bottled water is available but only for those who can afford it [21]. UV irradiation and ozonation are potentially suitable alternatives to chemical disinfection, both inactivate enteric pathogens including viruses [22,23]. Nonetheless, they are not without imperfections, such as their overall cost, which is more expensive than traditional treatments, and their far more significant energy consumptions. Also, they too form DBPs, and the most resistant microorganisms to these types of treatments are viruses [24–26].

Another highly relevant alternative to chemical disinfection is membrane filtration, which can remove enteric pathogens as well as other particle-like contaminants by size-exclusion. These processes operate with reduced or no chemical disinfection [27]. Moreover, membrane-based processes driven by gravity would reduce the reliance on energy and the overall cost of operation. The use of membranes avoids the formation of DBPs and can reduce concentrations of other undesirable water constituents such as particles and biopolymers in the drinking water. An added advantage of utilising membranes is the ease with which their surfaces can be functionalized. Membrane modification can endow membranes with additional functionalities and transform them into more valuable final products. Functionalization can improve the overall performance of existing polymeric membranes either by minimising undesired interactions that reduce the performance (antifouling) or by introducing specific interactions [28].

Membrane modifications may provide a means of preparing membranes with enhanced antiviral properties. As most viruses have a negative charge in neutral solutions [29], adsorption, when exposed to positive surfaces, could promote their removal. However, most membranes are negatively charged and need to be functionalized with various substances to render them positive [30–32].In recent years, positively charged (cationic) polymers are especially interesting as some have demonstrated antiviral properties [33–35] and are also well suited for the functionalization of polymeric membranes [36,37]. The poly-cationic chains can damage lipid membranes of enveloped viruses such as influenza virus [38].Furthermore, they can also damage the capsids of the more resistant non-enveloped waterborne viruses which we aim to treat. Specific polymers like polyethyleneimine (PEI) have been found to be valid candidates for imparting antibacterial [39] and antiviral properties onto surfaces [35,40]. Hence they are prime candidates for the functionalization of membranes to improve their viral inactivation capabilities. There have been previous studies of the virucidal activities of similar poly-cations painted or coated onto glass slides but not applied to membranes for drinking water [38,41].

Based on the hypothesis mentioned above, we theorise that cationic modification of membranes with PEI can enhance virus reduction during filtration. Several studies have been carried out using PEI to concentrate [42–44] or adsorb viruses and bacteriophages [45,46] as well as for antifouling purposes [47,48] and antiviral surface creation [40]. But the effects of PEI on the virus removal via membrane gravity-based filtration for drinking water production have not been studied [49] as per authors' knowledge.

In this study, PEI was actively coated onto commercially available polyether sulfone (PES), MF membranes with large pore sizes (0.45 μm) to introduce antiviral properties. The polymer coating induced significant viral reductions without compromising the permeability of the membranes. To investigate their ability to remove waterborne viruses the membrane's abilities were investigated with MS2 bacteriophages (30 nm) [50]. MS2 bacteriophages are surrogates for pathogenic viruses such as norovirus due to their similarities in size and structure [51]. This work was, therefore, designed to illustrate the potential of modified MF membranes to reduce virus concentrations, thereby allowing gravity based filtration. This is expected to lead to alternatives in membrane development and application yielding better virus control for resource-limited settings and emergency situations to produce safe drinking water.

2. Materials and methods

2.1. Materials

Branched polyethyleneimine (M_w ~750 kDa 50 wt. % in water and M_w ~ 25 kDa 1 wt. % in water), sulphuric acid (H_2SO_4, ACS reagent, 95–98%), hydrogen peroxide solution (H_2O_2, contains inhibitor, 30 wt. % in water ACS reagent) and Ponceau S red were obtained from Sigma-Aldrich (The Netherlands). All chemicals purchased were used without any purification. Before use, the stock PEI solutions were diluted in demineralised water to obtain the desired concentrations.

2.2. Model surface modification (glass slide preparation)

Microscope slides (75 × 25 × 1 mm, Sigma-Aldrich) cleaned with piranha solution (H_2SO_4: H_2O_2, 3:1), for 1 h and rinsed (3 times) in Milli-Q water. The slides were then dip-coated in a bath of either, 1.3 wt. %, branched PEI (M_w ~ 750 kDa and 25 kDa) for 15 min, rinsed with mili-q water and subsequently dried by air.

2.3. Model surface characterisation technique

The thickness of the PEI layer coated onto the model surface was

measured using ellipsometry. Dry PEI film thickness measurements were performed using spectroscopic ellipsometry (M2000, J.A. Woollam Co., Inc.).

Ellipsometry data was determined upon reflection of white light (370–900 nm) on the polymer-coated silicon wafers in the dry state, resulting in both a relative phase shift, Δ, and a relative amplitude ratio, tan Ψ. Dry film measurements were performed at angles of incidence of 65, 70, and 75°, while a three-layer model consisting of a silicon substrate, a silicon oxide layer, and a polymer layer was used to simulate experimental data.

2.4. Membrane modification

PEI, a cationic polymer was adsorbed onto a negatively charged commercial flat sheet EXPRESS® Plus polyether sulfone (PES) microfiltration (MF) membranes (pore size: 0.45 µm) from Merck Millipore (Diameter 90 mm).

The concentration of the polycationic polymer used varied over a range of 0.3–1.3 wt. %, with increments of 0.3% wt. for both molecular weights (M_w ~ 25 kDa and 750 kDa) of PEI.

2.4.1. Coating of the membranes was as follows

Firstly using an active coating method, by an AMICON cell-based dead-end filtration set up see Supporting information, Fig. A.1. 500 mL of PEI solution was flushed through the membrane at a pressure of 0.2 bars. The membranes were later removed and thoroughly rinsed with 500 mL of Milli-Q water using a sterilised AMICON cell at 0.02 bars to remove the excess (bulk) or unbound polymer prior to all experiments. This process of coating the membrane was also sub-divided as the membrane was either coated on the active surface, the back or both using the AMICON cell.

Secondly, a passive coating method or immersion was utilised, where the membranes were immersed in a solution of PEI overnight under agitation.

2.4.2. Membrane characterization techniques

The surface morphology, membrane asymmetry and cross-section of the membrane were observed using a scanning electron microscope (SEM), JSM-6010LA. The samples were vacuum dried and sputtered with gold before introduction to the microscope. For cross-section samples, the membranes were broken with the assistance of liquid nitrogen.

For the determination of the zeta potential of the modified membranes, an electrokinetic analyser SURPASS (Anton Paar, Graz Austria), was used. The zeta potential is calculated by measuring the streaming current versus the pressure four times in a 5 mM KCL solution at room temperature (RT, approximately 20–22 °C unless otherwise stated) which employed the following equation:

$$\zeta = \frac{dI}{dP}\frac{\eta}{\varepsilon \cdot \varepsilon_0} k_B R \quad (1)$$

where ζ is the potential (V), I is the streaming current (A), P is the pressure (Pa), η is the dynamic viscosity of the electrolyte solution (Pa.s), ε is the dielectric constant of the electrolyte (−), ε_0 is the vacuum permittivity (F m^{-1}), k_B is the bulk electrolyte conductivity (S m^{-1}), and R is the electrical resistance (Ω) inside the streaming potential.

2.5. Surface charge and distribution

To observe the distribution of positive charges a fast non-quantitative characterisation method was used in the form of an anionic dye staining test, Ponceau S red. The anionic dye stains positively charged surfaces resulting in a rapid colour change.

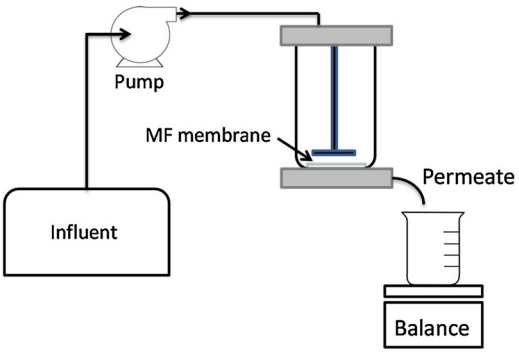

Fig. 1. The schematic diagram of the bench scale filtration unit for virus reduction by flat sheet microfiltration membranes.

2.6. Chemical analysis

To identify the amino groups present in PEI on the modified membrane, Fourier transform infrared spectroscopy (FTIR) was applied using ALPHA FTIR spectrometer, having a resolution between 4000–2000 cm^{-1} each spectrum was collected in mode 40 scans and 4 cm^{-1} resolution.

2.7. Filtration and stability test

An AMICON cell-based dead-end filtration setup schematically shown in Fig. 1 was used to test the performance and the stability of the modified membranes [18,52]. Pure water filtration tests were conducted to study the effect of the modification on the overall permeability of the membrane. Experiments were performed at RT and a pressure of 0.2 bars. Stability tests were also performed using Milli-Q water at normal pH (5.5); pH 4 and pH 3. Lowering of the pH ensured an increase in charge density of the PEI, and thus increased the repulsion between polymer chains.

2.8. Virus detection

The F-specific bacteriophage MS2 (GAP Enviro-microbial services Ltd.) was enumerated by plaque assay [53], using as a host strain *Salmonella typhimurium* WG49 (Culture collections of public health England). The titre of the stock solution MS2 was 10^{11} plaque forming units (PFU/mL) and stored at 4 °C. Before each experiment, a fresh MS2 working stock was generated by diluting the stock in 1x phosphate buffered saline (PBS, pH 7.2 ± 1) or Milli-Q water.

2.9. Virucidal activity and detection on model surfaces

PEI-modified (coated) and piranha cleaned (uncoated) glass slides were exposed to MS2 as described by Haldar et al. [38,54]. In short 10 µL of a 4 ± 0.9 × 10^8 PFU/mL MS2 stock was applied to the coated slide and covered by an uncoated slide. Then gentle manual pressure was applied to spread the droplet. As a control, two uncoated slides were used in the same manner. The slides were placed in a petri dish and after 30 min of incubation with 10 µL MS2 at RT. The top slide was lifted, and the virus exposed sides of both top and bottom slides rinsed thoroughly with 1.99 mL PBS (pH 7.2 ± 1). The collected rinse was used to prepare the 10-fold dilution range in 1xPBS, which was then used for enumeration of MS2 by plaque assay. All experiments were performed in triplicate. Error bars represent the standard deviation for all experiments.

2.10. Bulk solution

The test was also carried out using 200 µL of bulk PEI solution and 10 µL of a 4 ± 0.9 × 10^8 PFU/mL MS2 stock in a 96 well plate under

agitation using an orbital shaker at 160 rpm for 30 min at RT. Following which, a plaque assay was performed similarly to the slides.

2.10.1. Plaque assay

A 1 mL sample (900 µL of PBS with 100 µL of the virus) at RT was vortexed and diluted 10 folds, then overlaid with 2.5 mL of semi-solid agar (ssTYGA) and 1 mL of *Salmonella* in tryptone-gisextract glucose agar plates (Tritium microbiologie). After 24 h incubation at 37 °C, the agar plates were removed and plaques counted, and results noted.

2.11. Membranes

To determine MS2 reduction by PEI-coated membranes, gravity filtration with a sterilised glass dead-end filtration system was performed. As a control, an identical but uncoated membrane was used. Sterile Milli-Q spiked with MS2 to a final concentration of ($4 \pm 0.9 \times 10^8$ PFU/mL) was used as feed. The permeate was collected in a designated tube and sampled after 1, 5, 10, 20 and 30 L was filtered. The membrane was also collected and was placed in a sterile tube containing 50 mL Milli-Q and sonicated using an ultra-sonication bath. The permeate and membrane rinse were used to prepare 10-fold dilution series for enumeration by plaque assay.

3. Results

Different experimental setups were applied to evaluate virus reduction (removal and inactivation). Initially, we studied the antiviral properties of PEI, both in bulk solution and as a coating on model surfaces (glass slides). Subsequently, we investigated the coating of membranes, studying the resulting membrane properties and the stability of the coating. Finally, the reduction MS2 bacteriophages in a gravity-driven membrane process are described.

3.1. Virus inactivation by PEI on model surfaces

To demonstrate the principle of viral reduction by cationic polymers and to find suitable conditions to modify membranes for water purification we have used model slides. The cationic polymer, PEI was adsorbed to the negatively charged glass surface through a quick and simple dip-coating procedure [55]. The concentration of PEI varied between 0.3–1.3 wt. % as well as the molecular weight 25 and 750 kDa. The thickness of the adsorbed layer was measured using ellipsometry, while the homogeneity of the PEI layer was assessed using Ponceau S staining and the charge was quantified using zeta potential. A layer of approximately 4 ± 1 nm thick was deposited on the glass slides for all types of PEI and concentrations. Ponceau S staining demonstrated that the surface was positively charged as there was a colour change from colourless to reddish/pink when in contact with coated slides and proved an even distribution of the polymer on the surface (Supporting information, Fig. A.3). Finally, zeta potential measurements showed an overall positive charge of +60 mV.

In Fig. 2 the reduction of MS2 by PEI-coated glass slides and by PEI in bulk solution are shown. The uncoated slides showed a reduction between 0.5–1 $\log_{10}$-units, while the modified glass slides showed reductions greater than 3 $\log_{10}$-units. The presence of PEI in bulk solution also leads to high reductions, ≥ 4 $\log_{10}$-units compared to stock MS2 in Milli-Q water. Based on these results we concluded that PEI would be a suitable modifier for membranes to give higher viral reductions.

3.2. Membrane surface modification

Membranes were subsequently coated by PEI, by actively flushing a PEI solution through the PES-based MF membrane (pore size 0.45 µm). FTIR measurements were taken of the modified and unmodified membranes to validate successful modification. Compared to the uncoated membranes there was an additional peak between 3200 and 3600 cm^{-1} this range is indicative of the amine (N—H) stretch of primary and secondary aliphatic amines. Moreover, this peak is also characteristic of a hydroxyl (OH) group stretch. However, tertiary amines do not show within this range [56,57]. FTIR was used to analyse the effect of varying the concentration of the coating solution on the resulting membrane (Fig. 3). After 2000 cm^{-1} no significant peaks were detected.

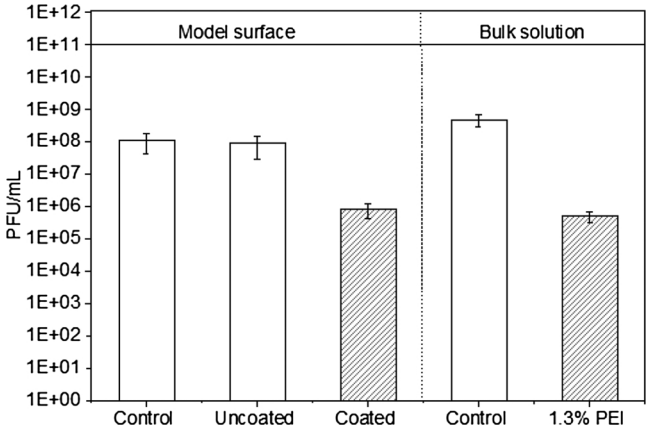

Fig. 2. MS2 bacteriophage reduction by PEI-coated glass slides and PEI in bulk solution. The experiment was performed in triplicate and error bars represent the standard deviation. The control of the surface and bulk experiments represent stock MS2 viral solution which was diluted 10 folds (6 times), and plaque assayed.

While FTIR does not yield qualitative information, one can observe that as the PEI coating concentration increases, so does the absorbance. This result was unexpected, as on model surfaces we did not observe an effect of the concentration on the thickness of the adsorbed PEI layer. Most likely, at higher concentration, more PEI gets trapped in the membrane structure, leading to the observed increase in absorbance. Scanning electron microscope (SEM) pictures of the modified membranes were taken to ensure that the integrity of the membranes was not compromised after coating (Fig. A.2). The pore size distribution was also evaluated by SEM to observe the effects of coating on the pore size distribution. The average distribution was estimated by measuring 30 pores chosen at random using the software image J. Details of the SEM images and average pore size distribution can be seen in the Supporting information, Figs. A.2 and A.4 of the Appendix respectively. There was no significant difference observed by both characterisation techniques.

The zeta potential of the membrane characterised its surface charge. After PEI coating, the negatively charged PES membrane increased from −40 mV to values as high as +70 mV at pH 5 (Fig. 3(b)). This change demonstrates that the polyelectrolyte overcompensates the surface charge, which indicates favourable polycation adsorption onto the membrane [58]. It was observed that as the molecular weight decreased the zeta potential increased due to the surface structure and degree of branching. It should be noted that the zeta potential was measured using streaming potential. The zeta potential represents the potential of the shear plane, where fluid shears over the (polymer coated) surface.

These results show a successful modification of the membrane by a simple coating method. The presence of the additional peak around 3400 cm^{-1}, as well as the change from a negative to a positive zeta-potential, was clear confirmation of successful modification of the membrane. Based on these results we found the optimal conditions for modification to be 1.3 wt. % of 25 kDa PEI, and all further experiments were conducted using these conditions.

3.3. Filtration and stability of modified membranes

Membrane clean water permeation tests were performed to

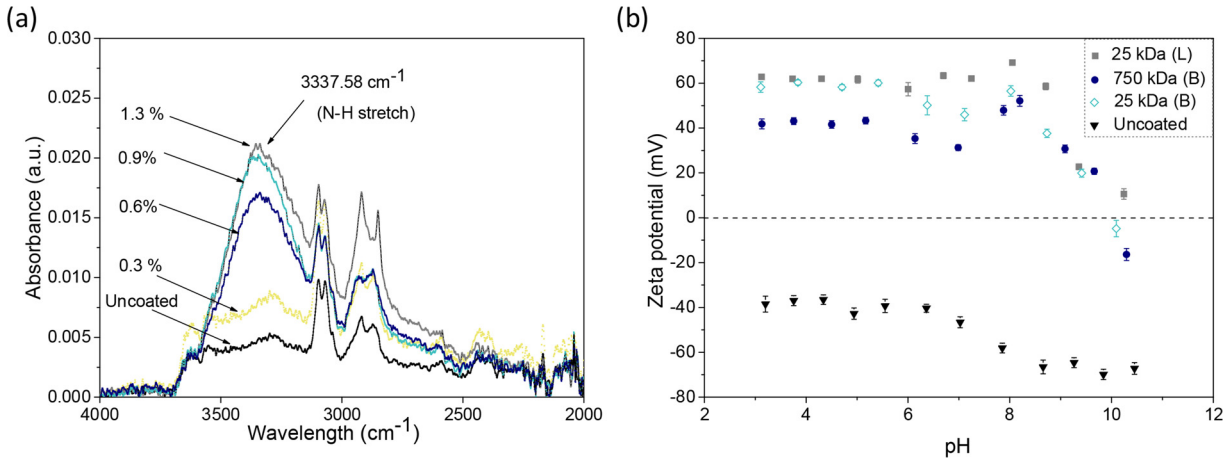

Fig. 3. (a) FTIR spectra of the pristine uncoated membrane (black) and PES MF membranes coated with various concentrations (wt. %) as indicated (other colours) and (b) Zeta potential of the membranes coated with different molecular weights of PEI as indicated. Error bars represent the standard deviation from three separate measurements; some error bars are too small to be seen. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

determine the performance of the newly coated membrane. Permeability before and after the modification are shown in Table 1 below. The PEI adsorbed on the membrane reduces its permeability, by slightly narrowing the membranes pore size. At the same time, the permeability remains high enough to be useful in gravity-driven membrane setups, an explicit goal of this investigation.

Several filtration experiments were conducted using Milli-Q water at pH values between 3 and 5.5. The more acidic pH was used to induce a higher charge in the polymer chains, and membranes stability tests were conducted by assessing FTIR spectra and the membranes zeta-potential. These measurements were carried out before and after long-term filtrations (Fig. 4).

The FTIR absorbance and the zeta potential of the membranes treated at pH 5.5 are shown in Fig. 4(a) and (b) respectively. Based on the FTIR data, the concentration of the PEI in the membrane decreased as the volume of filtrate increased, until eventually, the characteristic amine peak (around 3400 cm^{-1}) is no longer visible when the volume of filtrate is substantial (12,000 L/m^2). The zeta potential, however, behaved very differently as there was no observed decline; the zeta potential remained stable between 50–60 mV at pH 5.5. The same effects were observed for filtrations at pH 3 and 4; there was only one difference, the PEI content as estimated from FTIR diminished at a much faster rate. But again, the zeta potential remained stable at the tested pH values. Clearly, some PEI was washed out of the membrane over time, but a single adsorbed PEI layer remains, meaning that the membrane surface remains positively charged.

3.4. MS2 reduction and change in permeate flux during filtration

The reductions of MS2 by the modified and the unmodified membranes as a function of filtration time are shown in Fig. 5.

With the unmodified membrane, the reduction was on average 1 log_{10}-unit. However, from the effluent samples collected with the modified membrane, the MS2 removal was greater than 3 log_{10}-units.

Table 1
Membrane permeability before and after PEI coating using AMICON dead-end filtration set up at 0.2 bars.

Coating	Permeability before coating (L/hm^2 bar)	Permeability after coating (L/hm^2 bar)	Reduction (%)
Uncoated	21×10^3	–	–
25 kDa 1.3 wt. % PEI	20×10^3	16×10^3	22

The modified membrane reduction was, as expected, much higher than that of the unmodified membranes. At the beginning of the filtration, the permeate flux of the modified membrane was 25% lower than the unmodified membrane. This was expected as the pure water permeability of the modified membrane was 22 ± 5% lower than the unmodified membrane (see Table 1). Over time, the fluxes for the modified and unmodified membranes remained rather stable. The ≥3 log_{10}-units viral reduction remained stable for over an hour, allowing at least a production of 1700 L/m^2 of drinking water with a strongly reduced viral content. After the first hour, a more stable 2 log_{10}-units reduction was still present for an additional 3300 L/m^2. Before each virus filtration experiment, the permeate flux of the membrane had been determined with DI water to ensure the consistency in permeate flux between different membranes.

4. Discussion

The simplest design of a membrane-based technology for drinking water production is based on gravity filtration. These systems are cheap to produce, do not rely on the use of energy, and are easy to operate. But to achieve gravity filtration, large pores are required that cannot remove waterborne viruses based on size exclusion. Here we propose the use of PEI-modified MF membranes, which would remove viruses by adsorption of the negatively charged viruses to the positively charged PEI. Such membrane would still operate under gravity filtration and would reduce particles, bacteria and viruses to produce clean drinking water. Indeed, experiments on model surfaces (glass slides) demonstrated how easy it is to coat a negatively charged surface with a thin PEI coating. Moreover, MS2 reduction by these PEI-coated glass slides further indicates the suitability of PEI for the modification of membranes for the reduction of viruses from contaminated water. Synthetic polymers such as PEI are not costly, while there are also reputable and well-established methods in polymer chemistry which enable further modification of their chemical and physical properties, making PEI a very versatile polymer. Here we focus on how PEI in its purest form performs when used to modify commercially available MF membranes.

The zeta potential data are shown in Fig. 3(b) illustrates that while all polymers lead to a positive membrane zeta-potential, the exact zeta potential does depend on the type of PEI used. For polyelectrolyte coatings, a more swollen confirmation would lead to a lower or more negative zeta potential as the cationic groups are less accessible for the measurements [59]. This explains the difference in zeta potential due to molecular weight and branching of the polymer. The smaller

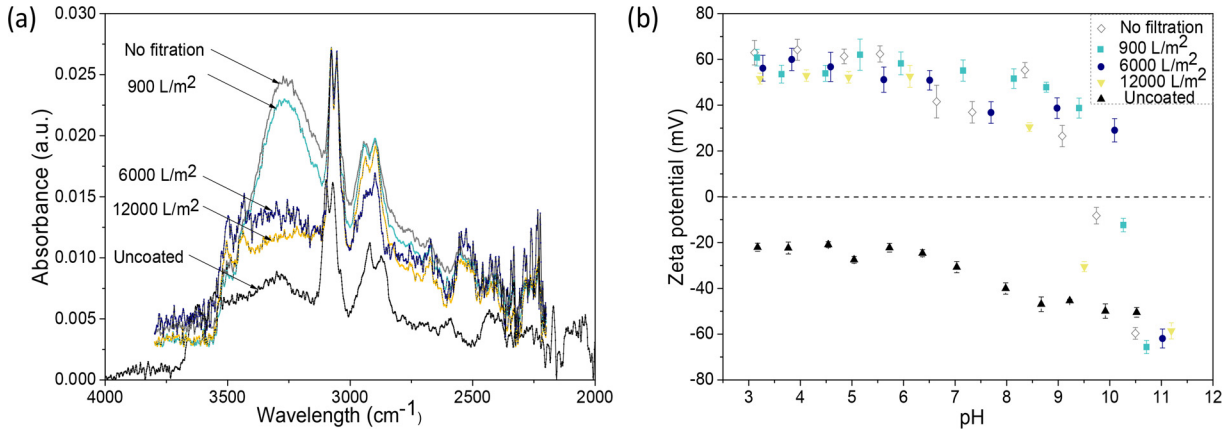

Fig. 4. (a) FTIR absorbance and (b) Zeta potential of the membranes coated with 25 kDa, 1.3 wt. % PEI treated with different volumes of water at pH 5.5; some error bars are too small to be seen.

complexity of the polymer (lower molecular weight and less branching), gives a flatter layer with less swelling and hence a higher zeta potential. Longer and more branched polymers will adsorb as a more swollen layer, which in turn leads to a lower zeta potential [60].

In Table 1, a 22% decrease in the permeability of the membrane after PEI coating was reported. The adsorbed layer causes the pores to become slightly narrower and hence, there is a reduction in permeability compared to the pristine membrane. While the permeability reduction is of some concern, as a high flux is vital in membrane processes for drinking water, this is the logical consequence of this type of coating procedure. The molecular weight of the polymer could even be used to tune the pore size of the membrane after adsorption. Moreover, the permeability of these modified membranes is still much higher than that of ultrafiltration (UF) membranes typically used for virus reduction [61], and will still allow for a gravity-driven membrane process. FTIR and zeta potential measurements performed before and after coating shown in Fig. 4(a) and (b), showed that even after prolonged filtration, there was still an active PEI layer adsorbed to the PES membrane surface which was validated by stable positive zeta potential. Based on the FTIR data, the concentration of the PEI in the membrane decreases as the volume of filtrate increases, until eventually, the characteristic amine peak (3354 cm^{-1}) is no longer visible when the volume of filtrate is substantial (12,000 L/m^2). It is likely that PEI becomes trapped in the inner porous structure of the membrane and with prolonged filtration, there is the removal of this excess of PEI. However, the active layer; that is electrostatically adsorbed to the PES membrane surface, remains and is so thin that it is not detectable with FTIR. The concentration of PEI in the permeate was too low to be measurable, even after concentration by evaporation. Loss of PEI is also possible due to adsorption on glassware (or polymer tubing). Therefore we estimate that the PEI concentration in the permeate is lower than 10 μg per litre. However, at high pH (> 9.6) the zeta potential decreases due to the deprotonation of PEI which indicates the decrease in stability of the adsorbed layer [62].

At pH 3 and 4 a similar effect is observed with FTIR, but the concentration of PEI diminished at a faster rate. The zeta potential also remained unchanged for all measurements at the tested pH. This result shows that rinsing with a low pH solution is an easy method to remove the excess of PEI and as a post-treatment before use, and in that way it is easy to prevent PEI leakage during drinking water production.

It is likely that viruses can accumulate on the surface of unmodified membranes. Therefore we theorise that virus accumulation on the membrane surface is the dominant factor which results in the 1 log_{10}-unit reduction observed for the unmodified membrane. The significant increase in virus reduction by the PEI modified membranes shown in Fig. 5 can be attributed to the adsorption of the negatively charged virus to the cationic PEI, or to a combination of inactivation and adsorption. Due to the small size of the MS2 (30 nm) in comparison to the pore size of the membrane (0.45 μm) [50,63,64]. The influence of membrane pore size (size based exclusion) was not significant. The PEI-modified membrane was able to reduce at ≥3 log_{10}-units MS2, under gravity filtration. Quantitative analysis using quantitative Polymerase Chain Reaction (qPCR) did not yield precise results, as it appears that PEI leached from the membrane affecting the results.

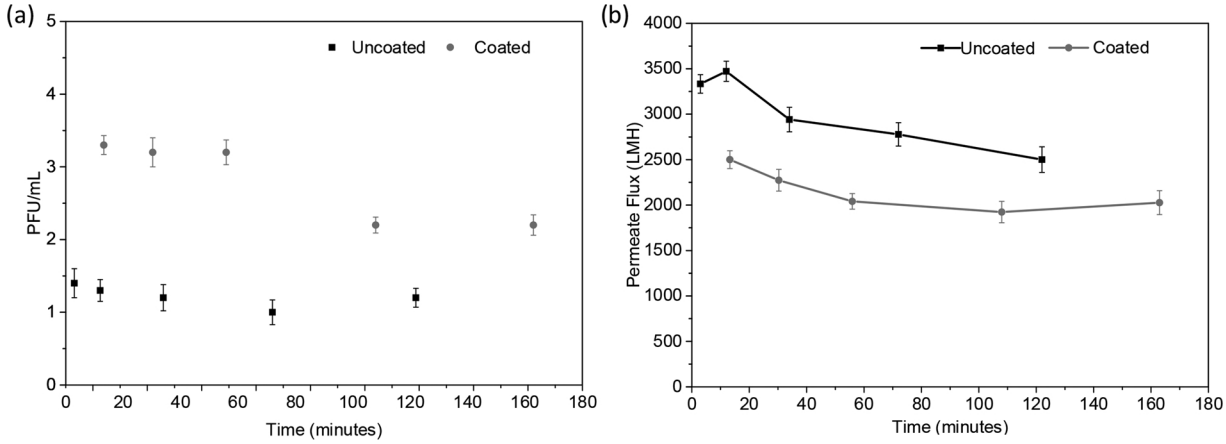

Fig. 5. (a) Virus reduction and (b) The permeate flux were plotted as a function of filtration time for both the modified (grey lines and scatters) and unmodified (black lines, scatters) membranes. The experiments were performed in triplicate, and error bars represent the standard deviation.

Characterization of these surrogate viruses and waterborne pathogenic viruses has shown several similarities concerning size and isoelectric point [29,65,66]. MS2 is an icosahedral, positive-sense single-stranded RNA virus resembling common waterborne pathogenic virus such as hepatitis E viruses [67–71]. Other pathogenic waterborne viruses contain double-stranded RNA such as rotavirus or DNA such as adenovirus; the latter is larger as well in comparison to most surrogates. Influenza viruses often used in polymer inactivation studies contain double-stranded RNA which is segmented and enveloped, which makes these viruses less stable and therefore are not appropriate as surrogates for most of the very persistent waterborne viruses. Though MS2 bacteriophages offer a faster and more straightforward method for testing the efficacy of the PEI modified membranes. There is no one surrogate/indicator virus representative of all human pathogenic waterborne viruses [72]. Therefore, further parallel studies should be undertaken to prove whether modified membrane filtration effectively reduces human pathogenic viruses such as noroviruses, rotaviruses and hepatitis E viruses. Using enhanced MF membranes could be one of the significant technological trends that need to be adopted [73]. Especially since solutions such as modified membranes can avert large waterborne outbreaks caused not only from viruses but also from bacteria such as *Vibrio cholera* and parasites such as *Cryptosporidium* [74,75]. It should be noted that while far exceeding the expectations for such a simple modification, there is a need to optimise and to improve the robustness of the applied membrane coating. Although having ≥ 3 $\log_{10}$-units reduction is highly beneficial, when treating waterborne pathogens however, there could be adverse health risks associated even with this reduction in viral titre. Nevertheless, we demonstrate the critical role played by the immobilised PEI in reducing the titre of the MS2 bacteriophages. It is also advantageous to note additional contaminants in the water, such as bacteria and particles will simultaneously be removed, thus saving time, energy, effort and resources. The observed leaching of PEI is a problem that can be avoided, for example by crosslinking (e.g. by following a method described by He et al. [76]) or as was previously mentioned in Section 2.7 by filtering at a low pH before use. To further improve the antiviral activity of membranes, moieties such as metallic nanoparticles could be incorporated to improve the overall performance of the surfaces [18].

5. Conclusions

We have successfully modified MF membranes for the reduction of waterborne viruses using the cationic polymer PEI. Membrane coating was confirmed by FTIR and zeta potential measurements. Although, FTIR showed some leaching of excess polymer, zeta potential measurements, demonstrated that a single active PEI layer remains adsorbed to the membrane even after prolonged filtration (12,000 L/m^2). The main conclusions of this work demonstrate that modified MF membranes and model surfaces were able to reduce the viral titre of MS2 by ≥ 3 log10-units or 99.9%. No other membrane technology reaches these high levels of viral log reductions and can at the same time be operated under gravity filtration. This unique characteristic enables them to be used in the simplest and cheapest point-of-use (POU) systems to create a good quality drinking. Furthermore, although there is only a 22% reduction in the membrane's permeability after modification, 5000 L/m^2 could be successfully treated in ~ 2.5 h. These modified MF membranes not only save time, but also reduces cost as they do not require any external driving force. Thus, these membranes provide a unique combination of filtration and disinfection, making them promising candidates for gravity-driven POU systems to create safe drinking water. More research, utilising actual surface water and pathogenic viruses, will be needed to establish the full potential of this approach.

Author contributions

Terica R. Sinclair, W. M. de Vos and H. D.W. Roesink conceived and designed membrane and glass slide experiments; Terica R. Sinclair and Dafne Robles performed the experiments with the guidance of Joris de Groot; Terica R. Sinclair, Sanne van den Hengel, S. A Rutjes and A. M. de Roda Husman analysed virology data, Terica R. Sinclair and Brahzil Raza performed the experiments; Terica R. Sinclair and W. de Vos analysed membrane and glass slide fabrication and characterization data; Terica R. Sinclair wrote the paper; all authours reviewed the paper.

Conflicts of interest

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.colsurfa.2018.04.056.

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