Journal of Membrane Science 570–571 (2019) 494–503

Contents lists available at ScienceDirect

Journal of Membrane Science

journal homepage: www.elsevier.com/locate/memsci

Cationically modified membranes using covalent layer-by-layer assembly for antiviral applications in drinking water

T.R. Sinclair[a,c], A. Patil[a], B.G. Raza[c], D. Reurink[a], S.K. van den Hengel[b,c], S.A. Rutjes[b], A.M. de Roda Husman[b,d], H.D.W. Roesink[a], W.M. de Vos[a,*]

[a] Membrane Science & Technology, MESA + Institute for Nanotechnology, University of Twente, Faculty of Science and Technology, P.O. Box 217, 7500 AE Enschede, the Netherlands
[b] National Institute for Public health and the environment (RIVM), A van Leeuwenhoeklaan, 9, 3721 MA Bilthoven, the Netherlands
[c] Wetsus, European Centre of Excellence for Sustainable Water Technology, Oostergoweg 9, 8911 MA Leeuwarden, the Netherlands
[d] Institute of Risk Assessment Sciences, IRAS within the faculties of Veterinary Medicine, Medicine and Sciences of Utrecht University, the Netherlands

ARTICLE INFO

Keywords:
Layer by layer assembly
Single polyelectrolyte
Polyethyleneimine
Water filtration membranes
Antiviral nanoparticles

ABSTRACT

In this work, a new approach towards virus reduction is taken, where modified membranes with large pore sizes (> 450 nm) can reach high $\log_{10}$-unit virus reductions. Polyelectrolyte coatings were used to modify microfiltration (MF) membranes to impart antiviral properties. A stable covalent layer-by-layer (LBL) approach was used to create multilayers from a single polyelectrolyte, polyethyleneimine (PEI). Here terephthalaldehyde (TA) crosslinking was used to create crosslinked multilayers, both on model surfaces and on commercial polyether sulfone, (PES) MF membranes. The substrates were further coated with antiviral silver, and copper nanoparticles (Ag and CuNPs) stabilised with PEI. The specific fabrication during the LBL assembly was stepwise characterised using multi-surface analysis including Fourier transform infrared spectroscopy (FTIR), Atomic Force Microscope (AFM), ellipsometry, zeta potential and contact angle measurements. Model surfaces demonstrated a 4 $\log_{10}$-units reduction of MS2 viral titre, independent of the crosslinked PEI layer thickness. The crosslinked PEI and Ag/CuNPs-modified membranes efficiently reduced 4.5–5 $\log_{10}$-units of infectious MS2 bacteriophages by both adsorption and inactivation of viral particles. This was confirmed by quantitative real-time polymerase chain reaction (qRT-PCR), which showed a stable performance over time. Pure water flux measurements on modified-membranes showed good long-term stability. Thus, 5000 L/m^2 of virus-free water was produced in approximately 2 h, using gravity-based filtration. Furthermore, there was no observable leaching of nanoparticles from the membranes during filtration.

1. Introduction

Approximately 20% of the global mortality rate can be attributed to infectious diseases, where one of the leading causes of such diseases are viruses such as adenovirus and norovirus, they accounting for about one-third of these deaths [1]. The growing demand for safe drinking water is of paramount concern, and therefore investigating alternate approaches for the production of high-quality water is of great importance. Here, especially waterborne viruses are difficult to remove due to their small size (20–100 nm) nm and stable nature [2]. Many approaches are used for the disinfection of contaminated water, each method having its own difficulties and drawbacks [3,4]. Membrane technology, for instance, can remove viruses almost completely (using ultrafiltration (UF)) or significantly (using microfiltration (MF)) under appropriate conditions. For the removal of polioviruses suspensions from contaminated sources of water using hydrophobic MF membrane, retentions of greater than 2 $\log_{10}$-units reduction (≥99%) were achieved [5,6]. Moreover, membranes with large pores (MF) facilitate high fluxes at low pressures, and therefore, could be utilized in gravity-driven household water treatment and safe storage (HWTS) systems. Simple and reliable HWTS systems will be essential in the coming decades to produce safe drinking water in remote and low-income settings.

The removal of viruses depends on membrane characteristics including surface properties [7] and long and short-range interactions with viral particles [8]. An appropriate membrane structure that is thick with a complex porous structure can improve the capacity of the membrane to trap viruses [9]. Interactions between viruses and the

* Corresponding author.
E-mail address: w.m.devos@utwente.nl (W.M. de Vos).

https://doi.org/10.1016/j.memsci.2018.10.081
Received 28 April 2018; Received in revised form 24 October 2018; Accepted 28 October 2018
Available online 30 October 2018
0376-7388/

membrane surfaces can improve the removal efficiency, these interactions include electrostatic repulsion [10] and hydrophobic interactions [11]. Higher virus retentions were, for example, observed with a hydrophobic membrane in comparison to a hydrophilic one [11]. Preconditioning of the viral suspension, for example by inducing aggregation, can further improve the virus removal. Such aggregation can be induced by changing the pH to a value around the isoelectric point of a virus or by adding salt [11].

Manipulating the operating condition like flow [12], or the membrane structure, by for example introducing metallic nanoparticles (NPs) [13], enhances viral removal and reduction from contaminated water sources. Antimicrobial NPs of silver and copper have been used for centuries taking advantage of their antimicrobial properties especially for water storage and purification [14]. For example, silver-impregnated polysulfone membranes showed a significant improvement in viral removal. Additionally, this type of modification leads to reduced biofouling [15]. Also copper nanoparticles (CuNPs), can be incorporated into or on substrates to act as long lasting reservoirs for copper ions for enhanced antimicrobial activity [16,17]. Though metallic NPs can be incorporated in many applications, they have received only little attention for their use in the removal of waterborne viruses from drinking water. It is important to mention that there are ethical issues concerning the use of silver nanoparticles (AgNPs), as their toxicity to human cells is not completely known or fully understood [13].

MF membranes may be combined with other processes to improve viral removal to the same extent as UF membranes [18]. It has also been hypothesised that virus removal by membrane filtration can be improved by inducing repulsive virus-membranes forces to prevent the viruses from approaching the membrane surface using zwitterionic hydrogels [19]. Though this may be true, the authors of this work are convinced that larger pore sizes are essential to reduce energy dependence and to allow gravity-driven HWTS systems. Therefore, an alternative approach is proposed, in this study, to utilise electrostatic attraction between the membrane and viruses, to improve the reduction of pathogenic viral contaminants in drinking water by an initial mechanism of adsorption followed by inactivation.

Cationic polymers have in the past demonstrated antiviral activity against a number of viruses and model viruses and are well suited for the modification of porous polymeric membranes [20,21]. Amid numerous antimicrobial agents, polyethyleneimine (PEI) comprising of polycationic moieties has been widely used to modify various substrates due to (1) long-term antimicrobial activity with no resistance development, (2) the possibility for regeneration upon loss of activity, (3) minimal cytotoxicity to mammalian cells and (4) biocidal and virucidal activity against a broad variety of pathogens in short contact times [22,23]. Cationic polymers could provide a simple electrostatic adsorption mechanism that would remove negatively charged viruses from any permeating water. Such an approach has in the past been used for virus concentration [24,25], but could also potentially lead to MF membranes with the ability to remove waterborne viruses from drinking water.

For HWTS systems, it would be a real breakthrough if MF membranes could be used to remove both bacteria and viruses. It would allow the removal of pathogens from surface water at substantial water production using just gravity as a driving force. Without the need for an additional driving force, the HWTS systems could be produced very cheaply. Still, there are also downsides to expect from these systems if based purely on cationically modified membranes. The membranes could foul more quickly due to adsorption of negatively charged moieties in the water. Moreover the cationic layer might not be stable and lose its function over time.

In our previous work [26], it was demonstrated that the adsorption of a cationic polymer, PEI could increase the viral reduction of MF membrane filtration systems. PEI exerts an attractive electrostatic interaction towards the negatively charged virus and over time also inactivates the virus by causing damage to the virion [27]. Unfortunately, the applied PEI coating was not sufficiently stable, as PEI leached from the membrane. Moreover, while a promising $\geq 3\log_{10}$-units removal was observed, this is not seen as sufficient to produce drinking water free from health risks. Coatings that capture PEI by air drying or electrostatic attraction, easily lose their antiviral ability when the virucidal substance gets released into the surrounding solution or environment [28,29]. In contrast, surfaces with covalently attached PEI can retain virucidal activity through a contact mechanism [30,31]. As the functional groups are covalently attached, the surface retains its antiviral properties even after multiple uses.

In this work, we adopt a chemical-crosslinking methodology to fabricate ultrathin PEI multilayers, first on model surfaces (glass slides) and then on MF membranes. This approach was further optimised by incorporating antiviral metallic silver (Ag) and CuNPs. A detailed study was carried out, both on model surfaces and membranes, to determine the viral reduction (removal and/or inactivation) of MS2 bacteriophages. MS2 is a commonly used surrogate for human pathogenic viruses such as hepatitis E. The study futher establishes whether the removal/reduction of the phages is due to adsorption or a combination of adsorption and inactivation (facilitated by the presence of metallic NPs). This was achieved by using quantitative real-time polymerase chain reaction (qRT-PCR). This work was designed to contribute to the development of new membranes targeting enhanced virus reduction for the production of safer drinking water, at low costs, and under simple operating conditions. Indeed, the study will show here that simple modifications of MF membranes can offer high viral reductions under simple gravity-driven filtration conditions.

2. Materials and methods

2.1. Materials

Branched Polyethyleneimine (M_w ~25 kDa), sulphuric acid (H_2SO_4, ACS reagent, 95–98%), hydrogen peroxide (H_2O_2, contains inhibitor, 30 wt% in water ACS reagent) and terephthalaldehyde (TA), sodium borohydride ($NaBH_4$) and silver nitrate ($AgNO_3$) were all obtained from Sigma-Aldrich (The Netherlands). All chemicals were used without any purification, and unless specified, all solutions were prepared in Milli-Q water (Milli-Q, Millipore Billerica, MA).

2.2. Model surface modification

Microscope slides (75 × 25 × 1 mm, Sigma-Aldrich) were cleaned with a piranha solution (H_2SO_4: H_2O_2, 3:1), and after copious rinsing with deionised (DI) water submerged in a PEI solution (1.3 wt% in Milli-Q water) for 15 min (Step 1 in Fig. 1). The slides were subsequently rinsed three times with Milli-Q water to remove any loosely bound polymer chains and were then dried under a stream of nitrogen. The amino-modified slides were then immersed in TA solution (10 mg/mL in ethanol) for 15 min (Step 2 in Fig. 1), and then rinsed thoroughly with pure ethanol followed by drying under a stream of nitrogen. The samples were further incubated in a PEI solution (1.3 wt%, pH 9.6) for 30 min at room temperature (RT~20–22 °C, Step 3 in Fig. 1). After covalent depositions of PEI by crosslinking with TA, one layer of the PEI film was built. This process was repeated to construct 1, 2 and 5 layers as desired.

2.3. Membrane modification

Commercial EXPRESS® Plus polyethersulfone (PES) MF membranes (pore size: 0.45 μm and diameter 90 mm) from Merck Millipore were wetted overnight by immersion in Milli-Q water. The wetted membrane was back-flushed three times with ultra-pure water before modification. Then 500 mL of 1.3 wt% PEI at pH 9.6 was filtered through the membrane using an AMICON cell-based dead-end filtration set up at 0.2

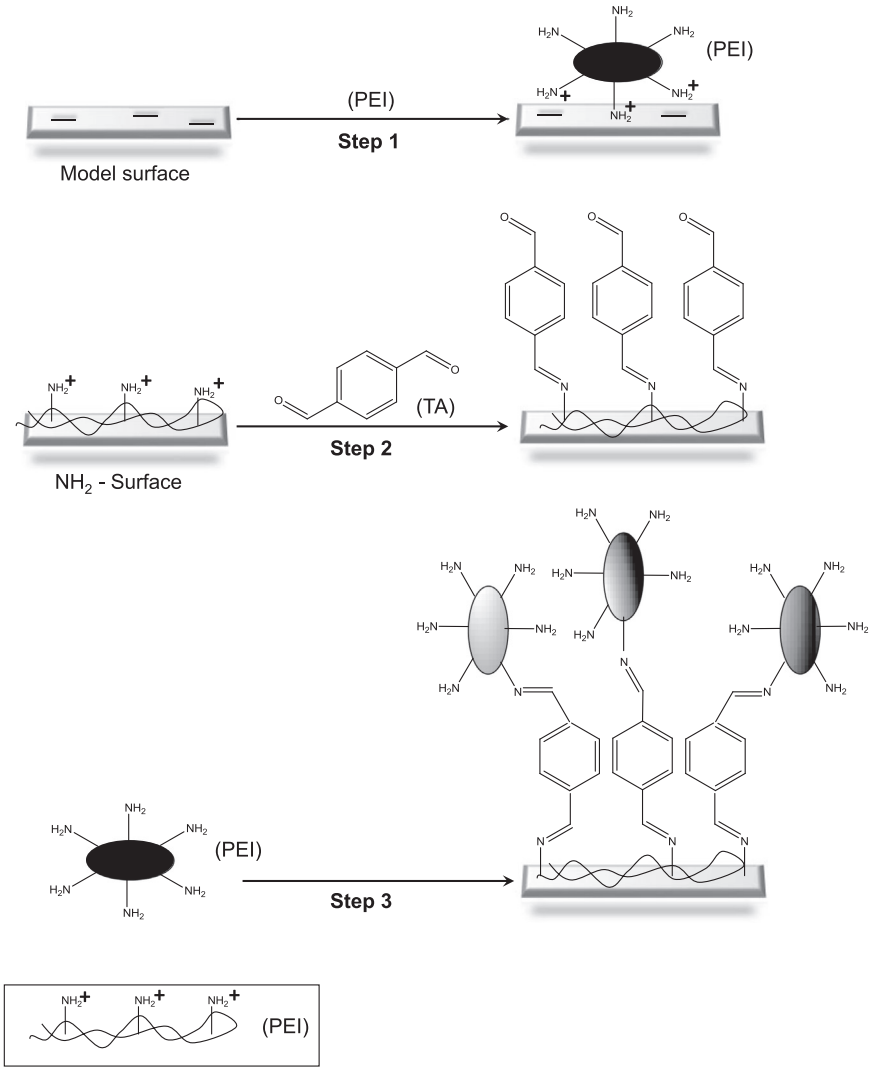

Fig. 1. The Schematic diagram for the covalent LBL assembly of PEI on model (glass) surfaces by chemical crosslinking with TA.

bars. After 10 min the membrane was dipped in 1 wt% TA solution for 15 min, followed by rinsing three times with ethanol and drying by a stream of air. The process as mentioned was repeated for the fabrication of the number of desired PEI layers (2 and 5). All modified membranes were rinsed with 500 mL of Milli-Q water at 0.2 bars before use in all experiments.

2.3.1. Loaded TA/PEI multilayers with metallic nanoparticles

The PEI multilayers supported on commercial PES membranes were loaded with metallic nanoparticles of AgNPs, and CuNPs stabilised with branched PEI by immersion at RT. Modified membranes were immersed in the nanoparticle solution under constant agitation by an orbital shaker for 30 min (500 mL of 0.2 μg/mL AgNPs and 1.3 wt% PEI, 160 rpms). The hybrid membranes were synthesised using an equal concentration and volumes of each metallic nanoparticle. Membranes were then rinsed three times with Milli-Q water via a sterilised glass dead-end filtration AMICON cell set up (see Supplementary information, Fig. A1). The nanoparticle loaded PEI multilayer was then dried under a stream of air and stored for later use. Bare nanoparticles (for comparison) were loaded using an adaptation of the method described by He et al. [32]. In summary, the modified membranes were immersed first in 25 mM $NaBH_4$ in Milli-Q water for 30 min under constant agitation by an orbital shaker (160 rpms) and rinsed three times before drying under a stream of nitrogen. Later the $NaBH_4$ loaded PEI multilayer membranes were dipped in 10 mM $AgNO_3$ solution for another 30 min, and then washed with Milli-Q three times followed by drying with a stream of air.

2.4. Membrane characterisation

2.4.1. Surface characterisation

The surface morphology and cross-section of the membrane were observed using a scanning electron microscopes (SEM), JEOL JSM-6010LA and JSM-6480LV. 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.

2.4.2. Zeta potential

The zeta potential of the modified membranes was obtained using 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 RT, employing 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.

2.4.3. Chemical analysis

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

2.4.4. Membrane property evaluation: filtration and stability tests

An AMICON cell-based dead-end filtration setup was used to test the performance and the stability of the modified membranes. Pure water filtration tests were conducted to study the effect of the modification on the overall permeability of the membrane. Stability tests were also performed using Milli-Q water at pH 7. The stability of the membranes was tested under constant filtration for 24 h after layer deposition. The pure water flux was calculated using the following equation.

$$J_w = \frac{V}{A \cdot \Delta t} \quad (2)$$

where J_w (L/ (m^2 h)) is the pure water flux, V (L) is the permeate volume, A (m^2) is the effective membrane area and Δt (h) is the permeation time.

2.4.5. Contact angles

Static contact angles of water on different PEI/TA crosslinked slides were measured by the sessile drop method at RT (23–25 °C). A 3 μL water droplet was loaded onto the surface of the slide, and after 5 s a measurement was taken using a Dataphysics OCA 35. At least 5 measurements from different regions of each sample were taken. The contact angle measurements on membranes were performed using the captive bubble mode, where a bubble of air is captured under the modified membrane in the aqueous phase. Image analysis for both contact angle measurements was performed using software provided with the measuring instrument.

2.5. Antiviral properties of modified membranes and model surfaces

The F-specific bacteriophage MS2 bacteriophage (GAP Enviro-microbial services Ltd.) was enumerated by the double agar overlay method [33], using as a host strain *Salmonella typhimurium*, WG49 (Culture collections of public health England). The titre of the MS2 stock solution was 4×10^{11} plaque forming units (PFU)/mL and it was stored at 4 °C. Before each experiment, a fresh MS2 working stock was generated by diluting the stock in 1x phosphate buffered saline (PBS) or Milli-Q water.

2.5.1. PEI-coated glass slides

PEI-coated glass slides were exposed to MS2 as described by Haldar et al. [34]. In short 10 μL $4 \pm 0.9 \times 10^8$ PFU/mL MS2 was applied to the coated slide and covered by a second plain (uncoated), piranha cleaned slide. Gentle manual pressure was applied to spread the droplet. As a control two uncoated, piranha cleaned slides were used in the same manner. After 30 min incubation at RT (22 °C), the top slide was lifted, and the virus exposed sides of both slides rinsed with 1xPBS. The collected rinse was used to prepare the 10-fold dilution range in 1xPBS, which was then used for enumeration of MS2 by the double overlay method. All experiments were performed in triplicate. Error bars represent the standard deviation.

2.5.2. PEI/TA crosslinked membranes

The reduction of MS2 by PEI/TA-crosslinked membranes was performed in a gravity-driven filtration with using a sterilised glass dead end, AMICON filtration system. 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 at pH 7 ± 0.2. The permeate was collected in a receiving vessel and sampled after 1 L 5 L, 10 L, 20 L and 30 L were filtered. The membrane was also collected (after 30 L) and was placed in a sterile tube of 50 mL Milli-Q and sonicated using an ultrasonication bath to study the removal of viruses adsorbed to the membrane. The permeate and membrane rinse were used to prepare a 10-fold dilution series for enumeration by the double agar overlay plaque assay. The plaques were counted after 24 h incubation at 37 °C. The reductions were calculated as the logarithm of the ratios of the PFUs in the permeate and those in the influent.

2.6. The effect of modified membranes on virus structure

2.6.1. MS2 RNA extraction

MS2 RNA was extracted with Purelink RNA/DNA mini extraction kit (Thermofisher) according to the accompanying protocol. Next, quantification of the extracted RNA was measured by a Qubit Fluorometer using a Quant-iT RNA Assay Kit (Invitrogen).

2.6.2. Genome quantification with quantitative real-time polymerase chain reaction (qRT-PCR)

The region between nucleotide 657–959 of MS2 genome was used for quantification of genome copies (GC). First, copy DNA (cDNA) of the target sequence of MS2 was generated with the synthetic reverse primer 5´-CGACAACCATGCCAAAC-3´ [35] using Superscript III reverse transcriptase (Invitrogen). For qRT-PCR, iQ SYBR green supermix, from Bio-RAD was used using synthetic primers forward 5´-CCGCTACCTTGCCC TAAAC-3´ and reverse 5´-CGACAACCATGCCAAAC-3´ [35]. In brief, the qRT-PCR reaction mixture contained 2 μL extracted RNA, 5 μM forward and reverse primer, 2.5 μL bovine serum albumin (concentration 4 mg/mL from ROCHE bought through Sigma Aldrich) (BSA), 12.5 μL of 50 x SYBR Green solution 7 μL nuclease-free water. MS2 cDNA was amplified by the following thermocycling conditions 94 °C for 3 min, followed by 30 cycles 94 °C for 45 s and 55 °C for 30 s and 72 °C for 1 min. Finally, the reaction was incubated for an additional 10 min at 72 °C. The qRT-PCR assay was performed on Bio-Rad CFX96 touch thermocycler. All the samples were run in duplicate. For the standard curve, a commercial MS2 RNA (Roche) was used. A relative difference of 3 for the Ct values or less in 10-fold serial dilutions was used as qRT–PCR inhibition.

3. Results and discussion

3.1. Model surface characterisation

3.1.1. Covalent layer-by-layer (LBL) assembly of PEI layers on model surfaces

The full modification route to obtain TA crosslinked PEI multilayers on model surfaces is depicted in Fig. 1. Step 1 shows PEI being attached to the model surface through electrostatic attraction between the positively charged polycation and the negatively charged glass surface. Next a TA crosslinking step is performed, after which a second layer of PEI is added. As described in detail in the materials and methods section, aldehydes are suitable crosslinkers for amine groups by Schiff base reaction. Although glutaraldehyde is one of the most popular bifunctional crosslinkers with amines, TA was chosen for its lower toxicity and higher degree of hydrophobicity [36,37]. The amino (NH_2) groups of the branched polyethyleneimine (BPEI), are converted to aldehyde (-CHO) groups via the formation of imine (C=N) bonds. The covalent LBL process was repeated to grow the desired number of PEI layers. In standard LBL depositions, oppositely charged polyelectrolytes are used in an alternating sequence to make multilayers. However, in this assembly, only a single polycation is used in combination with a crosslinker.

Ellipsometry was used to measure the dry layer thickness of the newly formed PEI layers. Fig. 2(a) shows the thickness after applying 1–5 layers comparing the differences between multiple PEI coatings and

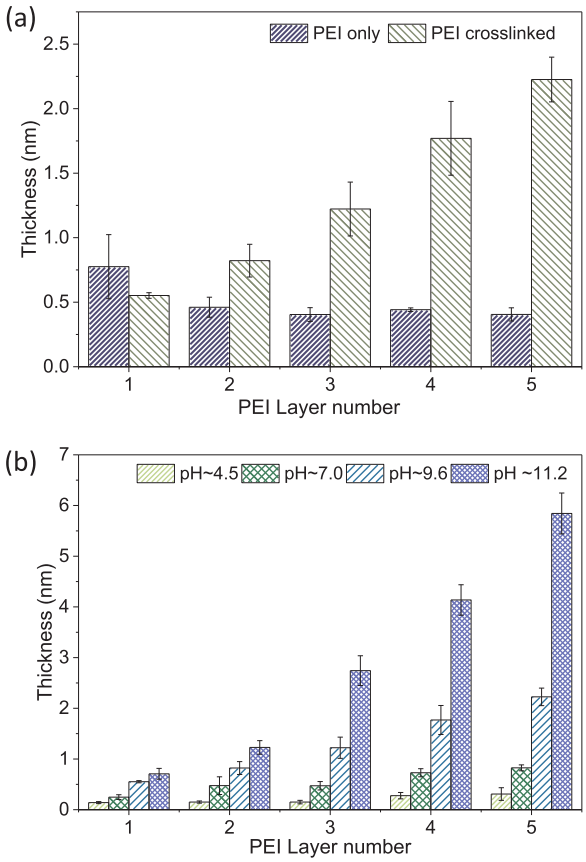

Fig. 2. (a) Layer thickness of PEI layers during LBL assembly using pH 9.6 and a PEI concentration of 1.3 wt% (b) the effect of pH during coating. Experiments were performed by measuring 3 different points on the model surfaces, and error bars represent standard deviations.

multiple PEI coating including based TA crosslinking. As expected, without crosslinking the PEI layer thickness is, within the margin of error, independent of the number of coating steps. Once PEI chains saturate the silica surface, no further adsorption can take place, while thorough rinsing after each adsorption steps removes any unbound chains. However, with crosslinking, it can be seen that the layer becomes thicker with every additional layer. This, confirms that the covalent LBL approach used here allows for the successful formation of thicker PEI (and TA) layers.

During the crosslinking of PEI, TA is dissolved in ethanol, a relatively poor solvent for PEI. As a result, the PEI chains will tend to collapse and self-entangle during the crosslinking reaction with TA. The crosslinked PEI will also contain non-reacted aldehyde groups that allow the binding of the next layer, to create multiple layers. Atomic force microscopic (AFM) analysis, supported the ellipsometry data and also confirmed that the layers were successfully formed, leading to an increase in the roughness of the surface. Details can be found in Supplementary information, Fig. A2.

Since PEI is a weak polyelectrolyte, the coating was expected to be pH dependent. In Fig. 2(b), the effect of coating pH on the formed layer's thickness is shown. PEI, a positively charged cationic polymer has protonated amines over a wide range of pH values (pH ≈ 3–10) [38,39] Evaluations of the growth was analysed under acidic, neutral and basic conditions on silicon wafers. The point of zero charge (PZC) of SiO_2 is between pH 2–4 [40], thus the surface was negatively charged under all conditions studied. The polycation covers the negatively charged SiO_2 surface, and the growth is relatively constant for all pH values. Still, the increase in thickness per layer depends on the pH, with low pH values leading to very thin layers, while at high pH much

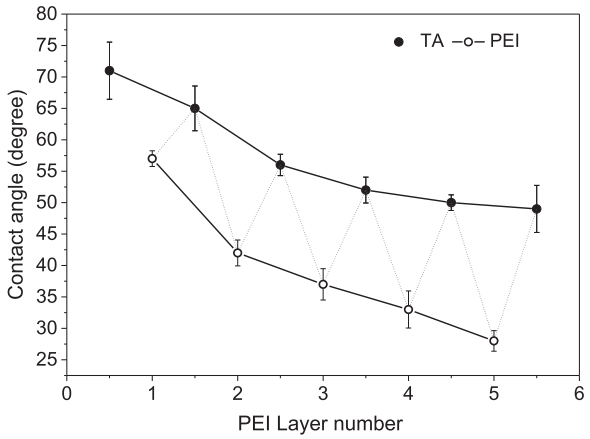

Fig. 3. The water contact angle during the LBL assembly of PEI multilayers (0–5 layers, which is representative of the successful addition of each PEI layer). The points in between PEI layers represent PEI layers after TA treatment (grey dotted line). At least 3 different points were analysed, and the error bars represent standard deviations.

thicker layers are formed. This effect of pH can be understood from the charge of PEI. At low pH, the amine groups that make up the PEI chains will be charged, and PEI chains will repel each other, preventing the chains from coming together to form a thick layer. By increasing the pH, the charge of the PEI chain decreases, facilitating the formation of thicker and more homogeneous layers [36,41]. Therefore, the pH can be a useful parameter to tune the exact nature of the formed coating. For all further coatings in this manuscript, we selected a pH of 9.6, although thicker layers can be achieved with pH 11.2, such a high pH could cause damage to the substrate.

3.1.2. Surface chemistry characterisation of the model surfaces

Contact angle measurements were used to monitor the covalent coupling of the PEI layer and the TA crosslinker (Fig. 3). The unmodified hydrophilic glass surface showed a contact angle of 15°, and after cleaning with piranha solution the surface became even more hydrophilic with the contact angle decreasing to 4°. The addition of PEI to anchor NH_2 groups caused a slight increase to 9°, while after subsequent addition of TA, the contact angle increased substantially to 70°, which indicates that the crosslinking with TA leads to a more hydrophobic surface.

The contact angle of the outermost layer increased and decreased after subsequent TA and PEI depositions respectively. The difference in hydrophobicity between plain PEI and TA crosslinked PEI causes this switching. TA modified PEI was, as expected, considerably more hydrophobic than plain PEI as TA is a hydrophobic molecule that is insoluble in water. The change in the contact angle also acts as a confirmation of the change in surface chemistry during the LBL deposition and successful assembly of PEI multilayers. Previous studies have linked an increase in antimicrobial and antiviral activity of PEI with its degree of hydrophobicity. For example, Klibanov et al. repeatedly demonstrated that hydrophobic PEI is more potent than plain PEI for virus reduction. Hence the increase in hydrophobicity could be beneficial to achieve improved virus reductions [42,43].

3.2. Antiviral properties of modified surfaces

Modified PEI/TA coated glass slides were tested against MS2 bacteriophages to determine their antiviral capabilities. The covalent PEI layers have a net positive charge since PEI is positively charged and TA is uncharged. This net positive charge attracts the net negative MS2 viruses, which have an isoelectric point of approximately 3.9 [44].

The $\log_{10}$-unit reductions of MS2 against PEI, crosslinked (CL) PEI and CL PEI with NPs are shown in Fig. 4, and are compared to uncoated

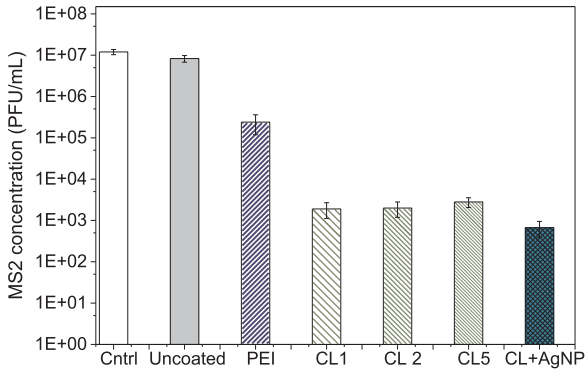

Fig. 4. MS2 bacteriophage reduction by normal PEI and crosslinked PEI (CL) - coated glass slides with 1, 2 and 5 layers. These experiments were performed in triplicate, and the error bars represent the standard deviations. The control represents stock MS2 viral solution which was diluted to the same degree as the slide experiments.

slides.

From Fig. 4, it can be observed that plain uncoated glass slides, used as the control (uncoated), showed MS2 reductions of just 0.5 $\log_{10}$-units while both PEI and CL PEI substantially reduce the viral titre of the MS2 bacteriophages. The effect is much stronger for the CL PEI. By crosslinking PEI with TA, there was a 4 $\log_{10}$-units reduction of MS2 compared to a 2 $\log_{10}$-units reduction with PEI only. The reductions further increased to 4.5 $\log_{10}$-units with the addition of AgNPs. The crosslinked PEI possesses higher (tertiary or quaternary) amine groups which are known to provide improved disinfection activity against microorganisms and viruses [45,46]. Moreover, a more hydrophobic PEI layer, as discussed in the previous Section 3.1, is expected to contribute to increased viral reductions. Finally, the AgNPs add an additional reduction mechanism to the coating, leading to a greater viral reduction.

The antiviral mechanism of cationic polymers such as PEI is not fully understood. It is possible that PEI acts as merely an adsorbent by attracting negative molecules like viruses and other organic substances to its highly positive surface [47]. However, it could also cause structural and or genomic damage to viruses. [21]. These reductions by both the uncoated and (un-crosslinked) PEI-coated slides were also reported by Hsu et al. [42]. They attribute the mechanism of PEI-modified surfaces to a combination of the two previously mentioned mechanisms, inactivation and adhesion, which were demonstrated by SEM images.

3.3. Membrane characterisation

Similar to model surfaces, the building of covalent PEI multilayers on MF membranes is possible through a Schiff base reaction between amines and aldehydes. Membranes were coated using similar concentrations and conditions as those used for the glass slide modifications. The significant difference is that the PEI solutions were actively permeated through the membrane, while TA crosslinking was done under simple stirring conditions. To confirm the successful crosslinking of PEI on commercial PES membranes, FTIR measurements were performed on dried membranes, and the results are presented in Fig. 5(a). The obtained FTIR spectra show an emerging peak between 3200 and 3600 cm^{-1} when PEI is electrostatically adsorbed to the outer and inner surfaces of the membrane. This peak is characteristic of the amine (N-H) stretch of primary, secondary and aliphatic amines [48,49]. However, it is also indicative of hydroxyl (OH) stretch. This peak appears for both PEI modified membranes. Further confirmation of successful modification was demonstrated by the appearance of peaks in the range of 2800–2950 cm^{-1} indicative of an aliphatic (C-H) stretch. The TA is chemically coupled with PEI through an imine bond, between the primary amines and aldehyde groups of TA. As a result, free aldehyde groups of the TA molecules can be seen in this C-H stretch. Reacted

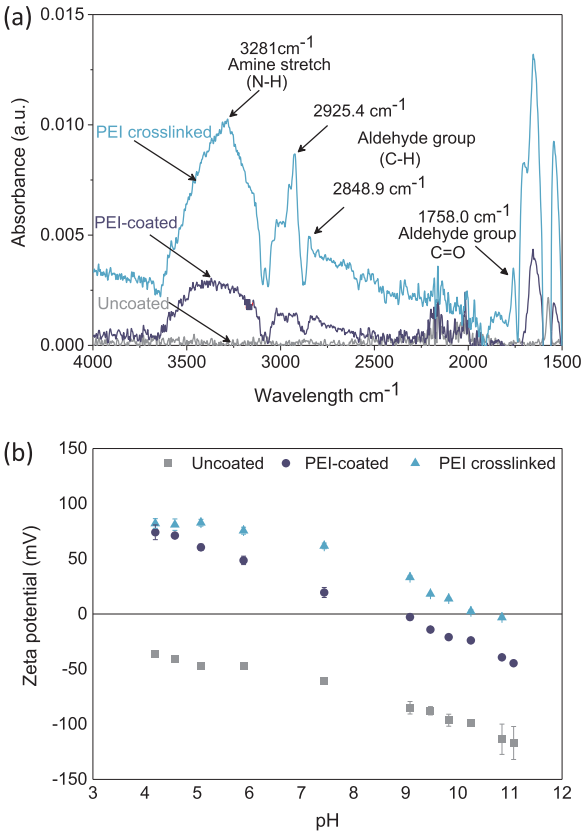

Fig. 5. (a) FTIR spectra of modified membranes with 1) crosslinked PEI, 2) plain hyper-branched PEI and 3) pristine or uncoated membrane, (b) Zeta potential of membranes coated with PEI and crosslinked PEI (PEI + TA). Error bars represent the standard deviation from three measurements; some error bars are too small to be seen.

aldehyde group also appears in an adsorption peak around 1750 cm^{-1} indicative of an oxyl (C=O) group. Both peaks confirm the modification after TA based crosslinking of PEI on the pristine membrane [50].

Zeta potential measurements were used to provide insight about the resulting membrane surface properties. A shown in Fig. 5(b) the uncoated, or pristine, negatively charged commercial PES membrane with a zeta potential of approximately −40 mV at pH 4, and increasingly negative values (-100 mV) at higher pH (pH 10–11). PEI coating leads to charge reversal and brings the zeta potential to +75 mV at pH 4, which also decreases with increasing pH. The crosslinked PEI is overall more positively charged than the just plain PEI-coated membranes. Crosslinking of amine groups changes their pKa value. During crosslinking with TA, a primary amine becomes a secondary amine, a secondary amine becomes a tertiary amine, and a tertiary amine becomes a quaternary amine. Higher amines are stronger bases and retain their charge for longer when moving to a higher pH as clearly observed in Fig. 5(b).

Moreover, the crosslinked PEI membranes were further modified with Ag and CuNPs. The NPs were also stabilised with PEI, making their incorporation in the membranes relatively easy. Membranes were actively coated with a PEI solution containing PEI stabilised nanoparticles of Ag and Cu followed by crosslinking as described in the materials and methods section. From scanning electron microscopy (SEM) images and electron diffraction and energy dispersive spectroscopy (EDS) analysis (see Supplementary information, Fig. A3 and Table A3), it is possible to see the successful distribution of the nanoparticles throughout the matrix and on the surface of the modified membranes.

Table 1
Stability of crosslinked and un-crosslinked membrane (Assembly conditions: PEI concentration 1.3 wt%, TA concentration 1 wt% (in ethanol) PEI pH = 9.6, TA pH = 11.2, 2 layers. All numbers are averages based on results from at least 3 separately prepared membranes, errors provide the standard deviation, rounded up to a minimum of least 1×10^3 L/m2/h/bar.

Coating	Permeability (L/m^2/h/bar)	Permeability after 24 h of continuous permeation (L/m^2/h/bar)
Uncoated	$27 \times 10^3 \pm 1 \times 10^3$	
PEI coated	$16 \times 10^3 \pm 1 \times 10^3$	$24 \times 10^3 \pm 1 \times 10^3$
Crosslinked PEI (1 layer)	$23 \times 10^3 \pm 1 \times 10^3$	$22 \times 10^3 \pm 1 \times 10^3$
Crosslinked PEI (2 layers)	$22 \times 10^3 \pm 1 \times 10^3$	$22 \times 10^3 \pm 1 \times 10^3$

3.4. Stability tests of PEI/TA ultrathin layers

Our subsequent experiments were intended to examine the stability of the chemically crosslinked PEI multilayers. The membranes were analysed before and after a 24 h pressurised filtration experiment, with the results given in Table 1.

From the table, the initial permeability of the uncoated PES membrane was in the region of 27×10^3 L/m^2/h/bar For PEI coated membranes, this decreased by 43%, while for membranes modified with chemically crosslinked PEI a smaller decrease of 22% was observed. It can seen that after 24 h of filtration, the permeability of the un-crosslinked PEI membranes increases, which is attributed to the removal of loosely bound polymer. However, the crosslinked PEI coated membranes maintained similar permeability before and after continuous filtration. This indicates that the crosslinked membrane was more stable than its un-crosslinked counterpart. Though crosslinking initially led to some permeability loss of the membrane, it also endowed the membrane with a lower permeability decline. These results show that crosslinking can lead to more stable membranes with enhanced membrane performance.

Stability tests were also performed on crosslinked membranes containing bare and PEI coated Ag NPs (SI Figs A4 and A8). Where the bare Ag NPs leach out of the membrane, the PEI decorated NPs showed very high stability (no leaching). The PEI coated NP's can be crosslinked to the other PEI chains allowing this high stability.

3.4.1. Antiviral properties of modified membranes

The membranes were then tested for their capacity to reduce the viral titre through filtration. Here results from the plaque assay experiments are also immediately compared to the reduction in GC of the MS2 in the permeate by quantitative polymerase chain reaction (qRT-PCR), to help understand the reduction mechanism.

Fig. 6(a) shows that crosslinked PEI modified membranes have an improved anti-viral activity in comparison to the (uncoated) control membrane, with a reduction of at least 3–3.5 $\log_{10}$-units for MS2. The reduction was found to be nearly identical for either 1 or 2 layers of

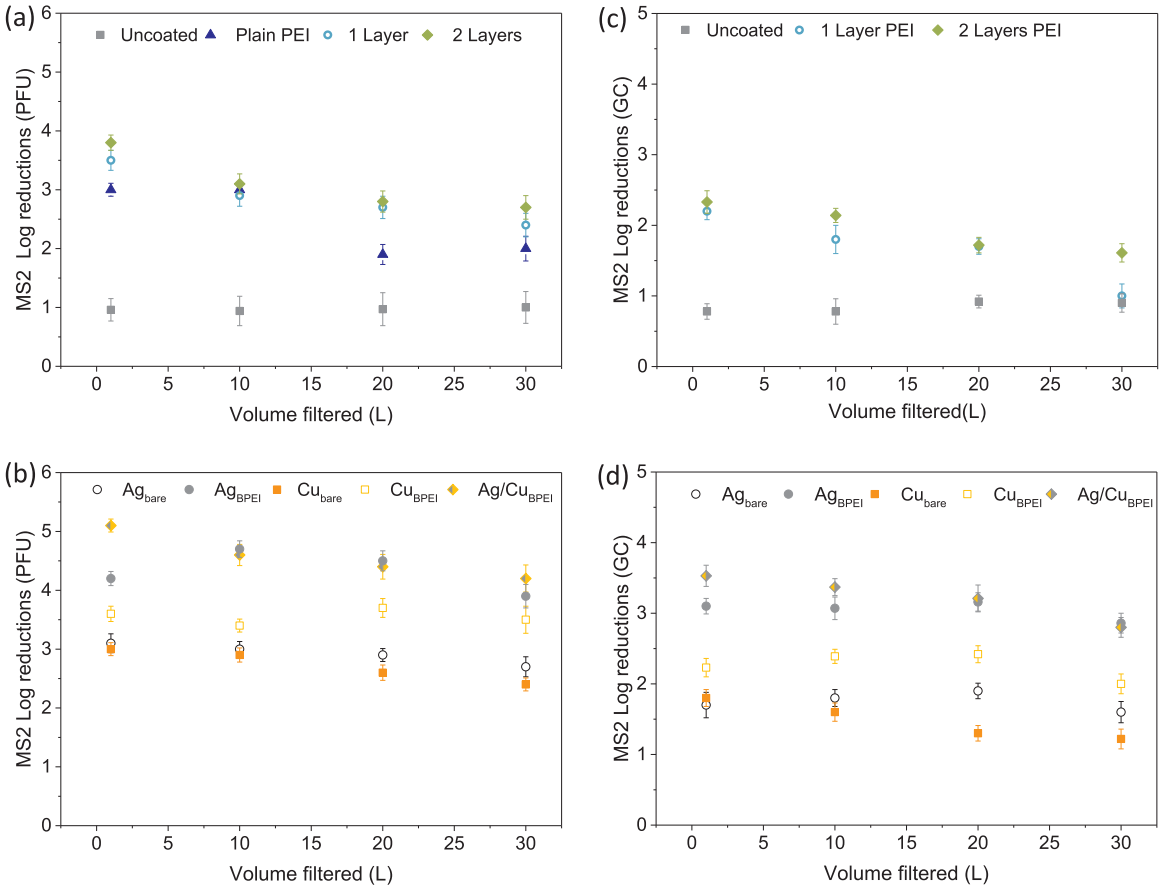

Fig. 6. Viral reduction by membrane filtration, with (a) membranes modified with different forms of PEI, (b) membranes modified with 2 Layers of crosslinked PEI and metallic nanoparticles, Plaques were counted at before filtration (influent/spike) and filtrates, after each test volume, through the modified membrane. qRT-PCR was used to determine the viral reductions regarding genome copies with (c) membranes modified with different forms of PEI and (d) membranes modified with 2 Layers of crosslinked PEI and metallic nanoparticles. The values are expressed as averages and errors represented as standard deviations.

crosslinked PEI, in agreement with the results found in on glass slides (Fig. 4). The crosslinked PEI membranes also performed better than the un-crosslinked PEI membranes.

The enhanced viral reduction is attributed to various mechanisms including a change in permeability, and electrostatic adsorption. It is clear that the negatively charged viruses will be electrostatically attracted to the positive surface of the membranes. Hence adsorption is expected to be the dominant process. MF membranes are also able to retain large aggregates, as viral aggregation is possible during the filtration with MS2 spiked feed water (See supplementary information, Fig. A6 for the stability of MS2 in feed water), but this would also have appeared for the uncoated membrane [11]. Interestingly, qRT-PCR data showed (Fig. 6(c) and (d)), a lower reduction, meaning that viral RNA is present in the permeate. However, this assay does not discriminate between RNA in active or in inactive virus particles. There is an approximate 1.5 $\log_{10}$-units higher reduction in infectious particles (plaque forming unit (PFU)) as seen in Fig. 6(a) and (b) compared to the reduction in GC. This indicates that the MS2 infectious titre reduction can be attributed not just to removal by adsorption, but also to true inactivation of the viruses. It is theorised that there is damage of the viral capsid resulting in RNA being released into the permeate. If not, the damaged capsid (non-infectious particle) still containing RNA which could also be present in the permeate after filtration. Only a short segment of the RNA-genome was analysed by qRT-PCR, no data about damage to the genome was observed. These results show why PEI is such a promising antiviral agent as it serves as an adsorption agent as well as an actual inactivation agent, which causes damage to the capsids of the viruses.

It took an average of 2 h to filter 5000 L/m^2 of MS2 contaminated water operating under dead end gravity filtration. The flux remained relatively constant after an initial drop in both coated and uncoated membranes. However, over time the high flux and virucidal activity remain relatively stable (Supplementary information, Fig. A5). The concentration of viruses used for these experiments was excessive (4×10^8 PFU/mL) in comparison to the concentration found in nature (10^3 PFU/mL) [51–53]. Therefore, it is expected that at lower concentrations of viruses, these membranes could effectively and efficiently treat much more than the 5000 L/m^2 of contaminated water described here.

Further optimisation of modified membranes using PEI-capped Ag and CuNPs was done to increase their overall antiviral activity. Similarly, membrane optimisation using bare nanoparticles (without PEI stabiliser) was done for comparison, and the results are presented in Fig. 6(b). From the figure, there are notably higher $\log_{10}$-unit reductions achieved with the NP containing membranes, the best being those containing a combination of PEI-coated CuNPs and AgNPs.

Inactivation of MS2 by silver ions (Ag^+) and copper ions (Cu^{2+}) has been previously reported by several publications and was validated by batch tests with suspensions of MS2 (10^8 PFU/mL) (Supplementary information, Fig. A7) [54,55]. These experiments suggest that nanoparticles work both by contact as well as diffusion which may have also influenced the resulting enhanced reductions. Membranes coated with nanoparticles showed a 2–2.5 $\log_{10}$-units increase in reduction of MS2 in comparison to membranes modified only with either form of PEI, reaching $\geq$ 5 $\log_{10}$-units reductions. This implies a synergistic effect of PEI and NPs which results in increased virucidal activity of the membrane. Most likely, viruses initially adsorb to the PEI-coated membrane surface and later, gets inactivated due to the presence Ag^+ and Cu^{2+} ions. The membranes with PEI-coated NPs exhibited higher reductions in comparison to membranes modified with bare nanoparticles. This could be due in part to bare nanoparticles tending to aggregate affecting the virucidal activity, as their antiviral activity is inversely proportional to the size of the particle [56]. Moreover, the bare nanoparticles were also found to leach from the membranes (Supplementary information Fig. A8).

3.5. Challenges and future perspectives

Although bacteriophages have been used as the standard for testing membranes in drinking water purification processes, parallel studies with actual pathogenic viruses are needed to confirm the validity of these claims to real situations. However, the current crosslinked PEI modified membrane meets the WHO standards for virus reduction by HWTS systems. The WHO has established criteria for HWTS systems effectiveness to remove microbial contaminants in water. Based on the requirements, the highest rank is 3 stars which should effectively remove at least 5 $\log_{10}$-units of the virus, followed by 2 stars capable of removing at least 3 $\log_{10}$-units of the virus. And lastly, 1 star is given to systems which can meet performance targets for at least 2 stars for any two classes of pathogens (bacteria, virus or protozoa) [57,58]. Membrane-based technologies rank very highly as HWTS technology. From this work it has been demonstrated that crosslinked PEI modified membrane coupled with Ag and CuNPs has a synergistic effect which could potentially be beneficial for use as membranes in multi-barrier HWTS systems. These findings complement our previous results that plain PEI has promising potential to be a potent antiviral agent.

A technological challenge that persists is the silver and copper release from commercial products which contain metallic nanoparticles [59,60]. Future research is needed on the slow release of nanoparticles to reduce the loss of antiviral activity due to depletion of metallic nanoparticles from the membrane. Additionally, regeneration is essential to potentially improve the lifespan of the membrane with sustained and stable virus reduction. Moreover, other challenges could affect the performance of the membrane such as the loss of Ag and CuNPs from the membrane during water disinfection as well as, viruses and other microorganisms may develop resistance to the metals and also affect the water chemistry.

4. Conclusions

In this work, a general strategy was developed to fabricate antiviral MF membranes with coatings from chemically–crosslinked PEI multilayers which used a crosslinker terepthalaldehyde to improve their overall performance and stability thus giving them the ability to be used in gravity-driven filtration. These coatings were first studied on model surfaces where the pH controlled growth was monitored by ellipsometry. Surface characterisation techniques, AFM, FTIR, zeta potential and contact angle measurements were also used to monitor the successful growth of crosslinked PEI layers. Similarly, commercial PES flat sheet MF membranes were successfully modified via an LBL process with a single polyelectrolyte. The main conclusions and observations of this work are summarised in the following points.

- Crosslinked PEI layers can be successfully assembled on both model surfaces and MF membranes. Where pH can be used as a control or tune the thickness of the assembled layer(s). The layers assembled on MF membranes were found to be stable, even after prolonged filtration where only 22% reduction in flux was observed compared to the pristine membrane.
- Both on model surfaces and membranes, high $\log_{10}$-unit reductions (3–4 $\log_{10}$-units) were demonstrated against the bacteriophage MS2, a common surrogate for water borne viruses.
- The anti-viral properties of the PEI coated model surfaces and membranes could be further improved by the incorporation of PEI stabilized Ag and CuNPs. For model surfaces and membranes with both Ag and CuNPs, the virus reduction increased to 4.5 $\log_{10}$-units and 4.5–5 $\log_{10}$-units, respectively.
- The PEI-modified membranes could treat approximately 5000 L/m^2 of MS2 contaminated water in 2 h via gravity-based filtration. Something that is simply not possibly with UF membranes that are commonly used to remove viruses on the basis of size exclusion.
- Viruses are not only removed by adsorption to the coated

membrane, but qRT-PCR also revealed that viruses are removed by true inactivation.

- These membranes thus offer a unique combination of virus removal and inactivation. They can serve as promising candidates for HWTS systems, as they already meet WHO standards (5 $\log_{10}$-units) for viral reduction. Still, more research is needed to assess the full potential of these membranes utilising real surface water and pathogenic viruses.

Acknowledgements

This work was performed in the TTIW-cooperation framework of Wetsus, European Centre of Excellence for Sustainable Water Technology (www.wetsus.nl). Wetsus is funded by the Dutch Ministry of Economic Affairs, the European Union Regional Development Fund, the Province of Fryslân, and the City of Leeuwarden and the Northern Netherlands Provinces. We thank the participants of the research theme "Virus Control" for their financial support and helpful discussions.

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 Akshay Patil performed the experiments with advice from Dennis Reurink; Terica R. Sinclair, Sanne K. van den Hengel, Saskia A Rutjes and Ana Maria de Roda Husman analysed virology data, Terica R. Sinclair and Brahzil G. Raza performed the experiments; Terica R. Sinclair and W. de Vos examined membrane and glass slide fabrication and characterization data; Terica R. Sinclair wrote the paper; and all authours reviewed the article.

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.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.memsci.2018.10.081.

References

[1] R. Lozano, M. Naghavi, K. Foreman, S. Lim, K. Shibuya, V. Aboyans, J. Abraham, T. Adair, R. Aggarwal, S.Y. Ahn, M. Alvarado, H.R. Anderson, L.M. Anderson, K.G. Andrews, C. Atkinson, L.M. Baddour, S. Barker-Collo, D.H. Bartels, M.L. Bell, E.J. Benjamin, D. Bennett, K. Bhalla, B. Bikbov, A. Bin Abdulhak, G. Birbeck, F. Blyth, I. Bolliger, S. Boufous, C. Bucello, M. Burch, P. Burney, J. Carapetis, H. Chen, D. Chou, S.S. Chugh, L.E. Coffeng, S.D. Colan, S. Colquhoun, K.E. Colson, J. Condon, M.D. Connor, L.T. Cooper, M. Corriere, M. Cortinovis, K.C. De Vaccaro, W. Couser, B.C. Cowie, M.H. Criqui, M. Cross, K.C. Dabhadkar, N. Dahodwala, D. De Leo, L. Degenhardt, A. Delossantos, J. Denenberg, D.C. Des Jarlais, S.D. Dharmaratne, E.R. Dorsey, T. Driscoll, H. Duber, B. Ebel, P.J. Erwin, P. Espindola, M. Ezzati, V. Feigin, A.D. Flaxman, M.H. Forouzanfar, F.G.R. Fowkes, R. Franklin, M. Fransen, M.K. Freeman, S.E. Gabriel, E. Gakidou, F. Gaspari, R.F. Gillum, D. Gonzalez-Medina, Y.A. Halasa, D. Haring, J.E. Harrison, R. Havmoeller, R.J. Hay, B. Hoen, P.J. Hotez, D. Hoy, K.H. Jacobsen, S.L. James, R. Jasrasaria, S. Jayaraman, N. Johns, G. Karthikeyan, N. Kassebaum, A. Keren, J.P. Khoo, L.M. Knowlton, O. Kobusingye, A. Koranteng, R. Krishnamurthi, M. Lipnick, S.E. Lipshultz, S.L. Ohno, J. Mabweijano, M.F. MacIntyre, L. Mallinger, L. March, G.B. Marks, R. Marks, A. Matsumori, R. Matzopoulos, B.M. Mayosi, J.H. McAnulty, M.M. McDermott, J. McGrath, G.A. Mensah, T.R. Merriman, C. Michaud, M. Miller, T.R. Miller, C. Mock, A.O. Mocumbi, A.A. Mokdad, A. Moran, K. Mulholland, M.N. Nair, L. Naldi, K.M.V. Narayan, K. Nasseri, P. Norman, M. O'Donnell, S.B. Omer, K. Ortblad, R. Osborne, D. Ozgediz, B. Pahari, J.D. Pandian, A.P. Rivero, R.P. Padilla, F. Perez-Ruiz, N. Perico, D. Phillips, K. Pierce, C.A. Pope, E. Porrini, F. Pourmalek, M. Raju, D. Ranganathan, J.T. Rehm, D.B. Rein, G. Remuzzi, F.P. Rivara, T. Roberts, F.R. De León, L.C. Rosenfeld, L. Rushton, R.L. Sacco, J.A. Salomon, U. Sampson, E. Sanman, D.C. Schwebel, M. Segui-Gomez, D.S. Shepard, D. Singh, J. Singleton, K. Sliwa, E. Smith, A. Steer, J.A. Taylor, B. Thomas, I.M. Tleyjeh, J.A. Towbin, T. Truelsen, E.A. Undurraga, N. Venketasubramanian, L. Vijayakumar, T. Vos, G.R. Wagner, M. Wang, W. Wang, K. Watt, M.A. Weinstock, R. Weintraub, J.D. Wilkinson, A.D. Woolf, S. Wulf, P.H. Yeh, P. Yip, A. Zabetian, Z.J. Zheng, A.D. Lopez, C.J.L. Murray, Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010, Lancet 380 (2012) 2095–2128, https://doi.org/10.1016/S0140-6736(12)61728-0.

[2] M. Carrillo-Tripp, C.M. Shepherd, I.A. Borelli, S. Venkataraman, G. Lander, P. Natarajan, J.E. Johnson, C.I. Brooks, V.S. Reddy, VIPERdb2: an enhanced and web API enabled relational database for structural virology, Nucleic Acids Res. 37 (2009), https://doi.org/10.1093/nar/gkn840.

[3] W.A. Lovins, J.S. Taylor, S.K. Hong, Micro-organism rejection by membrane systems, Environ. Eng. Sci. 19 (2002) 453–465, https://doi.org/10.1089/109287502320963436.

[4] M. Abdel-Jawad, S. Ebrahim, M. Al-Tabtabaei, S. Al-Shammari, Advanced technologies for municipal wastewater purification: technical and economic assessment, Desalination (1999) 251–261, https://doi.org/10.1016/S0011-9164(99)00110-1.

[5] S.S.S. Madaeni, The application of membrane technology for water disinfection, Water Res. 33 (1999) 301–308, https://doi.org/10.1016/S0043-1354(98)00212-7.

[6] S.S.S. Madaeni, A.G. Fane, G.S.S. Grohmann, Virus removal from water and wastewater using membranes, J. Membr. Sci. 102 (1995) 65–75, https://doi.org/10.1016/0376-7388(94)00252-T.

[7] J. Langlet, F. Gaboriaud, J.F.L. Duval, C. Gantzer, Aggregation and surface properties of F-specific RNA phages: implication for membrane filtration processes, Water Res. 42 (2008) 2769–2777, https://doi.org/10.1016/j.watres.2008.02.007.

[8] J. Langlet, L. Ogorzaly, J.C. Schrotter, C. Machinal, F. Gaboriaud, J.F.L. Duval, C. Gantzer, Efficiency of MS2 phage and Qβ phage removal by membrane filtration in water treatment: applicability of real-time RT-PCR method, J. Membr. Sci. 326 (2009) 111–116, https://doi.org/10.1016/j.memsci.2008.09.044.

[9] T. Hongo-Hirasaki, K. Yamaguchi, K. Yanagida, K. Okuyama, Removal of small viruses (parvovirus) from IgG solution by virus removal filter Planova®20N, J. Membr. Sci. 278 (2006) 3–9, https://doi.org/10.1016/j.memsci.2005.10.057.

[10] C.M. Schaldach, W.L. Bourcier, H.F. Shaw, B.E. Viani, W.D. Wilson, The influence of ionic strength on the interaction of viruses with charged surfaces under environmental conditions, J. Colloid Interface Sci. 294 (2006) 1–10, https://doi.org/10.1016/j.jcis.2005.06.082.

[11] E.M. Van Voorthuizen, N.J. Ashbolt, A.I. Schäfer, Role of hydrophobic and electrostatic interactions for initial enteric virus retention by MF membranes, J. Membr. Sci. 194 (2001) 69–79, https://doi.org/10.1016/S0376-7388(01)00522-1.

[12] D.L. Grzenia, J.O. Carlson, S.R. Wickramasinghe, Tangential flow filtration for virus purification, J. Membr. Sci. 321 (2008) 373–380, https://doi.org/10.1016/j.memsci.2008.05.020.

[13] S. Galdiero, A. Falanga, M. Vitiello, M. Cantisani, V. Marra, M. Galdiero, Silver nanoparticles as potential antiviral agents, Molecules 16 (2011) 8894–8918, https://doi.org/10.3390/molecules16108894.

[14] R.L. Davies, S.F. Etris, The development and functions of silver in water purification and disease control, Catal. Today 36 (1997) 107–114, https://doi.org/10.1016/S0920-5861(96)00203-9.

[15] K. Zodrow, L. Brunet, S. Mahendra, D. Li, A. Zhang, Q. Li, P.J.J. Alvarez, Polysulfone ultrafiltration membranes impregnated with silver nanoparticles show improved biofouling resistance and virus removal, Water Res. 43 (2009) 715–723, https://doi.org/10.1016/j.watres.2008.11.014.

[16] U. Vainio, K. Pirkkalainen, K. Kisko, G. Goerigk, N.E. Kotelnikova, R. Serimaa, Copper and copper oxide nanoparticles in a cellulose support studied using anomalous small-angle X-ray scattering, Eur. Phys. J. D 42 (2007) 93–101, https://doi.org/10.1140/epjd/e2007-00015-y.

[17] M. Ben-Sasson, K.R. Zodrow, Q. Genggeng, Y. Kang, E.P. Giannelis, M. Elimelech, Surface functionalization of thin-film composite membranes with copper nanoparticles for antimicrobial surface properties, Environ. Sci. Technol. 48 (2014) 384–393, https://doi.org/10.1021/es404232s.

[18] L. Fiksdal, T. Leiknes, The effect of coagulation with MF/UF membrane filtration for the removal of virus in drinking water, J. Membr. Sci. 279 (2006) 364–371, https://doi.org/10.1016/j.memsci.2005.12.023.

[19] R. Lu, Q. Li, Z. Yin, I. Xagoraraki, V.V. Tarabara, T.H. Nguyen, Effect of virus influent concentration on its removal by microfiltration: the case of human adenovirus 2, J. Membr. Sci. 497 (2016) 120–127, https://doi.org/10.1016/j.memsci.2015.08.065.

[20] A.M. Larson, B.B. Hsu, D. Rautaray, J. Haldar, J. Chen, A.M. Klibanov, Hydrophobic polycationic coatings disinfect poliovirus and rotavirus solutions, Biotechnol. Bioeng. 108 (2011) 720–723, https://doi.org/10.1002/bit.22967.

[21] N.M. Milović, J. Wang, K. Lewis, A.M. Klibanov, Immobilized N-alkylated polyethylenimine avidly kills bacteria by rupturing cell membranes with no resistance developed, Biotechnol. Bioeng. 90 (2005) 715–722, https://doi.org/10.1002/bit.20454.

[22] J.C. Tiller, S.B. Lee, K. Lewis, A.M. Klibanov, Polymer surfaces derivatized with poly(vinyl-N-hexylpyridinium) kill airborne and waterborne bacteria, Biotechnol. Bioeng. 79 (2002) 465–471, https://doi.org/10.1002/bit.10299.

[23] J. Haldar, D. An, L. Alvarez de Cienfuegos, J. Chen, A.M. Klibanov, Polymeric coatings that inactivate both influenza virus and pathogenic bacteria, Proc. Natl. Acad. Sci. USA 103 (2006) 17667–17671, https://doi.org/10.1073/pnas.0608803103.

[24] K. Satoh, A. Iwata, M. Murata, M. Hikata, T. Hayakawa, T. Yamaguchi, Virus concentration using polyethyleneimine-conjugated magnetic beads for improving the sensitivity of nucleic acid amplification tests, J. Virol. Methods 114 (2003) 11–19, https://doi.org/10.1016/j.jviromet.2003.08.002.

[25] L.A. Ikner, C.P. Gerba, K.R. Bright, Concentration and recovery of viruses from

water: a comprehensive review, Food Environ. Virol. 4 (2012) 41–67, https://doi.org/10.1007/s12560-012-9080-2.
[26] T.R. Sinclair, D. Robles, B. Raza, S. van den Hengel, S.A. Rutjes, A.M. de Roda Husman, J. de Grooth, W.M. de Vos, H.D.W. Roesink, Virus reduction through microfiltration membranes modified with a cationic polymer for drinking water applications, Colloids Surf. A Physicochem. Eng. Asp. 551 (2018), https://doi.org/10.1016/j.colsurfa.2018.04.056.
[27] K.R. Wigginton, T. Kohn, Virus disinfection mechanisms: the role of virus composition, structure, and function, Curr. Opin. Virol. 2 (2012) 84–89, https://doi.org/10.1016/j.coviro.2011.11.003.
[28] S.A. Koplin, S. Lin, T. Domanski, Evaluation of the antimicrobial activity of cationic polyethylenimines on dry surfaces, Biotechnol. Prog. 24 (2008) 1160–1165, https://doi.org/10.1002/btpr.32.
[29] Z. Shi, K.G. Neoh, S.P. Zhong, L.Y.L. Yung, E.T. Kang, W. Wang, In vitro antibacterial and cytotoxicity assay of multilayered polyelectrolyte-functionalized stainless steel, J. Biomed. Mater. Res. - Part A 76 (2006) 826–834, https://doi.org/10.1002/jbm.a.30597.
[30] J. Lin, S. Qiu, K. Lewis, A.M. Klibanov, Mechanism of bactericidal and fungicidal activities of textiles covalently modified with alkylated polyethylenimine, Biotechnol. Bioeng. 83 (2003) 168–172, https://doi.org/10.1002/bit.10651.
[31] D. Park, J. Wang, A.M. Klibanov, One-step, painting-like coating procedures to make surfaces highly and permanently bactericidal, Biotechnol. Prog. 22 (2006) 584–589, https://doi.org/10.1021/bp0503383.
[32] T. He, V. Chan, Covalent layer-by-layer assembly of polyethyleneimine multilayer for antibacterial applications, J. Biomed. Mater. Res. - Part A 95 A (2010) 454–464, https://doi.org/10.1002/jbm.a.32872.
[33] M. Adams, Bacteriophages, Bacteriophages (1959) 620 〈http://www.cabdirect.org/abstracts/19602204111.html〉.
[34] J. Haldar, A.K. Weight, A.M, Klibanov, Preparation, application and testing of permanent antibacterial and antiviral coatings, Nat. Protoc. 2 (2007) 2412–2417, https://doi.org/10.1038/nprot.2007.353.
[35] B.M. Pecson, L.V. Martin, T. Kohn, Quantitative PCR for determining the infectivity of bacteriophage MS2 upon inactivation by heat, UV-B radiation, and singlet oxygen: advantages and limitations of an enzymatic treatment to reduce false-positive results, Appl. Environ. Microbiol. 75 (2009) 5544–5554, https://doi.org/10.1128/AEM.00425-09.
[36] B. Xia, C. Dong, Y. Lu, M. Rong, Y.Z. Lv, J. Shi, Preparation and characterization of chemically-crosslinked polyethyleneimine films on hydroxylated surfaces for stable bactericidal coatings, Thin Solid Films (2011) 1120–1124, https://doi.org/10.1016/j.tsf.2011.09.056.
[37] J. Biscarat, B. Galea, J. Sanchez, C. Pochat-Bohatier, Effect of chemical cross-linking on gelatin membrane solubility with a non-toxic and non-volatile agent: terephthalaldehyde, Int. J. Biol. Macromol. 74 (2015) 5–11, https://doi.org/10.1016/j.ijbiomac.2014.11.022.
[38] Z. Adamczyk, A. Michna, M. Szaraniec, A. Bratek, J. Barbasz, Characterization of poly(ethylene imine) layers on mica by the streaming potential and particle deposition methods, J. Colloid Interface Sci. 313 (2007) 86–96, https://doi.org/10.1016/j.jcis.2007.04.005.
[39] M. Morga, Z. Adamczyk, Monolayers of cationic polyelectrolytes on mica - electrokinetic studies, J. Colloid Interface Sci. 407 (2013) 196–204, https://doi.org/10.1016/j.jcis.2013.05.069.
[40] R.H. Yoon, T. Salman, G. Donnay, Predicting points of zero charge of oxides and hydroxides, J. Colloid Interface Sci. 70 (1979) 483–493, https://doi.org/10.1016/0021-9797(79)90056-0.
[41] Y. Kayaba, H. Tanaka, S.S. Ono, PH-dependent selective thickness control of polyelectrolyte nanolayers on SiO2 and Cu surfaces, J. Phys. Chem. C 119 (2015) 22882–22888, https://doi.org/10.1021/acs.jpcc.5b04939.
[42] B.B. Hsu, S. Yinn Wong, P.T. Hammond, J. Chen, A.M. Klibanov, Mechanism of inactivation of influenza viruses by immobilized hydrophobic polycations, Proc. Natl. Acad. Sci. USA 108 (2011) 61–66, https://doi.org/10.1073/pnas.1017012108.
[43] H. Liu, Y. Kim, K. Mello, J. Lovaasen, A. Shah, N. Rice, J.H. Yim, D. Pappas, A.M. Klibanov, Aerosol-assisted plasma deposition of hydrophobic polycations makes surfaces highly antimicrobial, Appl. Biochem. Biotechnol. 172 (2014) 1254–1264, https://doi.org/10.1007/s12010-013-0593-4.
[44] S.E. Dowd, S.D. Pillai, S. Wang, M.Y. Corapcioglu, Delineating the specific influence of virus isoelectric point and size on virus adsorption and transport through sandy soils, Appl. Environ. Microbiol. 64 (1998) 405–410.
[45] E. Tuladhar, M. Bouwknegt, M.H. Zwietering, M. Koopmans, E. Duizer, Thermal stability of structurally different viruses with proven or potential relevance to food safety, J. Appl. Microbiol. 112 (2012) 1050–1057, https://doi.org/10.1111/j.1365-2672.2012.05282.x.
[46] Y. Xue, H. Xiao, Antibacterial/antiviral property and mechanism of dual-functional quaternized pyridinium-type copolymer, Polymers 7 (2015) 2290–2303, https://doi.org/10.3390/polym7111514.
[47] F. Gelman, K. Lewis, A.M. Klibanov, Drastically lowering the titer of waterborne bacteriophage PRD1 by exposure to immobilized hydrophobic polycations, Biotechnol. Lett. 26 (2004) 1695–1700, https://doi.org/10.1007/s10529-004-3737-3.
[48] J. Coates, Interpretation of infrared spectra, a practical approach, Encycl. Anal. Chem. (2000), https://doi.org/10.1002/9780470027318.
[49] F. Wang, P. Liu, T. Nie, H. Wei, Z. Cui, Characterization of a polyamine microsphere and its adsorption for protein, Int. J. Mol. Sci. 14 (2013) 17–29, https://doi.org/10.3390/ijms14010017.
[50] Paula Yurkanis Bruice, Organic Chemistry, 5th ed., Prentice Hall, University of California, Santa Barbara, 2006〈https://www.pearson.com/us/higher-education/product/Bruice-Organic-Chemistry-5th-Edition/9780131963160.html〉.
[51] W.J. Lodder, A.M. De Roda Husman, Presence of noroviruses and other enteric viruses in sewage and surface waters in The Netherlands, Appl. Environ. Microbiol. 71 (2005) 1453–1461, https://doi.org/10.1128/AEM.71.3.1453-1461.2005.
[52] A. Steyer, K.G. Torkar, I. Gutiérrez-Aguirre, M. Poljšak-Prijatelj, High prevalence of enteric viruses in untreated individual drinking water sources and surface water in Slovenia, Int. J. Hyg. Environ. Health 214 (2011) 392–398, https://doi.org/10.1016/j.ijheh.2011.05.006.
[53] X.Y. Ye, X. Ming, Y.L. Zhang, W.Q. Xiao, X.N. Huang, Y.G. Cao, K.D. Gu, Real-time PCR detection of enteric viruses in source water and treated drinking water in Wuhan, China, Curr. Microbiol. 65 (2012) 244–253, https://doi.org/10.1007/s00284-012-0152-1.
[54] A.M. Armstrong, M.D. Sobsey, L.M. Casanova, Disinfection of bacteriophage MS2 by copper in water, Appl. Microbiol. Biotechnol. 101 (2017) 6891–6897, https://doi.org/10.1007/s00253-017-8419-x.
[55] G. Rao, K.S. Brastad, Q. Zhang, R. Robinson, Z. He, Y. Li, Enhanced disinfection of Escherichia coli and bacteriophage MS2 in water using a copper and silver loaded titanium dioxide nanowire membrane, Front. Environ. Sci. Eng. 10 (2016), https://doi.org/10.1007/s11783-016-0854-x.
[56] L. Wang, C. Hu, L. Shao, The antimicrobial activity of nanoparticles: present situation and prospects for the future, Int. J. Nanomed. 12 (2017) 1227–1249, https://doi.org/10.2147/IJN.S121956.
[57] V. Curtis, S. Cairncross, R. Yonli, R.K. Sivanappan, A.K. Daoud, K.M. Swaileh, R.M. Hussein, M. Matani, R. Few, I. Lake, P.R. Hunter, P.G. Tran, WHO, Unicef, P. Pham-Duc, H. Nguyen-Viet, J. Hattendorf, P.D. Cam, C. Zurbrügg, J. Zinsstag, P. Odermatt, United Nations, WHO, UNICEF, WHO/UNICEF, P. Kær Jensen, J.H.J. Ensink, G. Jayasinghe, W. Van der Hoek, S. Cairncross, A. Dalsgaard, A. Fallis, United Nations, 国立保健医療科学院, S. Rufener, D. Mäusezahl, H.J. Mosler, R. Weingartner, J.H. Botero-Garcés, G.M. García-Montoya, D. Grisales-Patiño, D.C. Aguirre-Acevedo, M.C. Álvarez-Uribe, L. Liu, H.L. Johnson, S. Cousens, J. Perin, S. Scott, J.E. Lawn, I. Rudan, H. Campbell, R. Cibulskis, M. Li, C. Mathers, R.E. Black, S. Uga, N.T.V. Hoa, L.K. Thuan, S. Noda, Y. Fujimaki, F. Berrilli, R. D'Alfonso, A. Giangaspero, M. Marangi, O. Brandonisio, Y. Kabor, C. Cianfanelli, R. Lauro, D. Di Cave, M.E. Hoque, V.T. Hope, R. Scragg, T. Kjellström, V. Curtis, S. Cairncross, T.K. Fischer, D.D. Anh, L. Antil, N.D.L. Cat, P.E. Kilgore, V.D. Thiem, R. Rheingans, L.H. Tho, R.I. Glass, J.S. Bresee, H.H. Dib, S.Q. Lu, S.F. Wen, S.M. Baker, J.H.J. Ensink, M.S.H. Al-Mekhlafi, M. Azlin, U. Nor Aini, A. Shaik, A. Sa'iah, M.S. Fatmah, M.G. Ismail, M.S. Ahmad Firdaus, M.Y. Aisah, A.R. Rozlida, M. Norhayati, H.M. Al-Mekhlafi, M.T. Al-Maktari, R. Jani, A. Ahmed, T.S. Anuar, N. Moktar, M.A.K. Mahdy, Y.A.L. Lim, R. Mahmud, J. Surin, C.L. Fischer Walker, J. Perin, M.J. Aryee, C. Boschi-Pinto, R.E. Black, M. Hussein Gasem, W.M.V.W.M. V Dolmans, M.M. Keuter, R.R. Djokomoeljanto, WHO (World Health Organization), D.J. Lye, J.S. Heyworth, G. Glonek, E.J. Maynard, P.A. Baghurst, J. Finlay-Jones, T. Clasen, W.-P.P. Schmidt, T. Rabie, I. Roberts, S. Cairncross, W. Ahmed, T. Gardner, S. Toze, N. Pathak, H. Heijnen, V. Garrett, P. Ogutu, P. Mabonga, S. Ombeki, A. Mwaki, G. Aluoch, M. Phelan, R.E. Quick, D.T. Trang, B.T.T. Hien, K. Mbak, P.D. Cam, A. Dalsgaard, WHO, L. Fewtrell, R.B. Kaufmann, D. Kay, W. Enanoria, L. Haller, J.M. Colford, S. Cairncross, C. Hunt, S. Boisson, K. Bostoen, V. Curtis, I.C.H. Fung, W.-P.P. Schmidt, C.A. Evans, P.J. Coombes, R.H. Dunstan, Results of round 1 of the who international scheme to evaluate household water treatment technologies, Trop. Med. Int. Health 106 (2012) 155–162, https://doi.org/10.3329/jhpn.v28i1.4521.
[58] WHO, Evaluating household water treatment options: health-based targets and microbiological performance specifications, NML Classif. WA 675, 2011, pp. 1–68.
[59] T. Benn, B. Cavanagh, K. Hristovski, J.D. Posner, P. Westerhoff, The release of nanosilver from consumer products used in the home, J. Environ. Qual. 39 (2010) 1875, https://doi.org/10.2134/jeq.2009.0363.
[60] D.M. Mitrano, E. Rimmele, A. Wichser, R. Erni, M. Height, B. Nowack, Presence of nanoparticles in wash water from conventional silver and nano-silver textiles, ACS Nano 8 (2014) 7208–7219, https://doi.org/10.1021/nn502228w.