In situ surface functionalization of reverse osmosis membranes with biocidal copper nanoparticles

Desalination

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Abstract

Biofouling may lead to severe operational challenges that can significantly impair membrane desalination processes. In recent years, copper-based nanoparticles (Cu-NPs) have gained increased attention as a potentially viable anti-biofouling agent in membrane processes, due to their strong antibacterial activity and relatively low cost. This study presents a novel and facile method to attach biocidal Cu-NPs on the surface of a thin-film composite reverse osmosis membrane. Herein, we suggest a method for membrane surface functionalization with Cu-NPs that is performed without disassembling the membrane module, which highlights its practicality and potential application for reverse osmosis desalination plants. The loading of Cu-NPs on the membrane was confirmed both by scanning electron microscope imaging and X-ray photoelectron spectroscopy analysis, indicating the deposited nanoparticles were composed of either metallic copper or copper-oxide. The impact of the *in situ* Cu-NPs modification on membrane transport properties was found to be minor, with only a slight increase of the water and salt permeability. Furthermore, except for a slight increase in hydrophobicity, the modified membrane exhibited surface properties comparable to those of the pristine membrane. Finally, the *in situ* formed Cu-NPs imparted strong antibacterial activity to the membrane surface, leading to 90 % reduction in the number of attached live *E. coli* bacteria on the modified membrane compared to the pristine reverse osmosis membrane. This study demonstrates that *in situ* grafting of Cu-NPs on reverse osmosis membranes is an alternative to reduce biofouling.

Keywords: biofouling, copper nanoparticles, reverse osmosis, thin-film composite membrane, surface modification.

1. Introduction

The progress in membrane desalination processes and the worldwide construction of mega desalination plants in the past decades were achieved by intensive scientific and industrial research that tackled many of the engineering process challenges [1, 2]. However, biofouling continues to be a severe operational hurdle in membrane-based water treatment membrane processes [3-5]. Biofouling refers to the formation of a biofilm composed of bacteria and extracellular polymeric substances on the membrane surface and/or membrane spacer. It is a spatial-temporal problem [6], exacerbated in warm waters [7] that contain high nutrient concentrations such as secondary effluent [8], tropical seawater [9], or water during seasonal algal blooms [10, 11].

A densely formed biofilm inside the membrane module may result in negative operational consequences, such as an increase in the pressure drop along the module, exacerbation of concentration polarization effects, and deterioration of permeate water quality.[12-15] Mitigating biofilm formation during desalination of feed waters with high biofouling potential requires high chemical cleaning procedures, which lead to shorter membrane life span and significant increase of process cost. Thus, there is a need for the development of innovative methods that will improve and expand the engineering toolbox for combating biofouling in membrane desalination processes.

In reverse osmosis (RO), a multi-barrier approach is usually used to abate the potential development of biofouling inside the membrane module during the desalination process. Pretreatment methods of filtration and disinfection are employed to significantly reduce the number of live bacteria that are conveyed to the membrane module [16, 17]. Also, frequent chemical cleaning is applied in an attempt to prevent the development of a biofilm at early stages [18]. However, to date, there is no effective method of bacteria inactivation that will complement pretreatment and frequent chemical cleaning by continuous disinfection of the feed water inside the membrane module during the desalination process. This lack stems mostly from the high sensitivity of the predominant type of desalination membranes (i.e., polyamide thin-film composite (PA-TFC) membrane) to commonly used oxidants, such as chlorine, chlorine derivatives, and ozone [19], which degrade the polyamide layer of the membrane [20].

Consequently, there is a critical need for a novel, non-destructive, and cost effective method to effect biocidal activity inside the membrane module during the separation process.

Among different proposed anti-biofouling approaches in desalination technologies, the use of biocidal metal nanoparticles (Me-NPs) has gained substantial momentum in the past decade [21-23]. The strong antibacterial activity of some Me-NPs (e.g., silver [24, 25], copper [26], zinc [27, 28]) toward many diverse types of bacteria and other microorganisms is well established. However, an understanding of the potential to apply these biocidal Me-NPs for biofouling mitigation in reverse osmosis has not been well established, mostly because of the lack of a practical method to load the biocidal Me-NPs inside the membrane module. Controlled localized loading of the biocidal Me-NPs in the vicinity of the module spacers and the membrane surface will require less metal and therefore will be more cost effective than injecting the Me-NPs directly into the feed tank before the RO module.

The inevitable dissolution of Me-NPs during module operation must also be considered. Particle dissolution will likely be aggravated during periodic cleaning operations. Therefore, localized loading of biocidal Me-NPs on the membrane must also be able to replenish the Me-NPs after their dissolution. Such reloading must be performed in the desalination site without disassembling the membrane module, requiring minimal loading time and limited chemical reagents.

Recently, we have demonstrated a new method for loading silver nanoparticles on TFC RO membranes, via *in situ* formation of Ag-NPs [29]. The method is simple and rapid (a 15-minute procedure), requiring the use of a very low concentration of silver salt and reductant (sodium borohydride). However, the relatively high price of silver will increase the operating cost [30], leading to the adjustment of the *in situ* formation method to incorporate other less expensive alternative Me-NPs with efficient anti-biofouling properties.

In recent years, the use of copper nanoparticles (Cu-NPs) as biocides in membrane filtration has received increasing interest [22, 31, 32]. Compared with silver, copper is relatively inexpensive and abundant [30], and is extensively utilized as a wood preservative [33], as a fungicide for vineyards or other agricultural crops [34], as a disinfectant in swimming pools [35], and as a major component of anti-biofouling paint to protect ship hulls [36]. The use of copper

1 salt as an anti-biofouling agent in desalination processes also has been reported [37, 38]. Thus,
2 copper-based NPs offer a potential agent for biofouling mitigation in reverse osmosis, if a
3 practical loading method for NPs inside the membrane module could be introduced.

4 This study demonstrates a novel *in situ* protocol for loading biocidal Cu-NPs on TFC-RO
5 membrane. The modified membrane was extensively characterized to confirm the successful
6 loading of Cu-NPs on the membrane surface and to assess the impact of the Cu-NP *in situ*
7 formation reaction on the water and salt transport properties. Surface properties such as
8 hydrophilicity, roughness, and surface charge (zeta potential) were evaluated prior to and after
9 surface functionalization. Finally, we demonstrate the imparted strong antibacterial activity to
10 the membrane surface by the *in situ* formed Cu-NPs.

11 12 **2. Materials and Methods**

13 *2.1. Chemicals*

14 Copper sulfate (CuSO_4), nitric acid (HNO_3), and potassium chloride (KCl) were
15 purchased from Sigma Aldrich (St. Louis, MO). Sodium chloride (NaCl), sodium hydroxide
16 (NaOH), and 2-propanol (isopropyl alcohol, $\text{CH}_3\text{CHOHCH}_3$) were purchased from J. T. Baker
17 (Phillipsburg, NJ). Potassium bicarbonate (KHCO_3), sodium bicarbonate (NaHCO_3), and sodium
18 borohydride (NaBH_4) were obtained from Fisher Scientific (Fair Lawn, NJ). Potassium
19 hydroxide (KOH) was purchased from Aventor Performance Materials (Center Valley, PA).
20 Bacto agar and Luria-Bertani (LB) broth were received from BD Biosciences (Sparks, MD) and
21 American Bioanalytical (Natick, MA), respectively. Unless specified, all solutions were prepared
22 in deionized (DI) water produced by Milli-Q ultrapure water purification system (Millipore,
23 Billerica, MA).

24 25 *2.2. Model bacteria strain*

26 Kanamycin resistant *Escherichia coli* (*E. coli*) BW26437 was received from the Yale Coli
27 Genetic Stock Center (New Haven, CT). The *E. coli* culture was maintained on LB agar plates

1 and grown in LB media 25 mg/L with kanamycin sulfate (American Bioanalytical) prior to any
2 experiments.

2.3. *In situ* formation of Cu-NPs on the membrane

Dried sheets of seawater TFC -RO membrane (Dow Filmtec SW30XLE) were received from OctoChem, Inc. The dried membrane coupons were wetted through immersion in a mixture of 20 v/v% isopropanol in DI water for 20 minutes. The membranes were rinsed and soaked three times in DI water for 24 hours and stored at 4 °C until use.

The following protocol for *in situ* formation of Cu-NPs is modified from our previous report of *in situ* formation of Ag-NPs [29]. A schematic diagram of the modification procedure is shown in Figure 1. Initially, the membrane was placed between a glass plate and a rubber frame (inner hole size: 7.5 cm × 12 cm) designed to hold the solutions on the active layer of the membrane. Ten mL of CuSO₄ solution (50 mM) reacted with the isolated active layer for 10 minutes (Fig. 1B). Subsequently, the CuSO₄ solution was discarded, leaving only a thin layer of CuSO₄ solution on the membrane surface (Fig. 1C). Then, the membrane active layer was allowed to react with a NaBH₄ (50 mM) solution for 15 minutes (Fig. 1D), forming copper nanoparticles. After formation of Cu-NPs, the NaBH₄ solution was removed from the membrane (Fig. 1E), and the prepared *in situ* Cu-NPs modified membrane was rinsed for approximately 10 seconds with DI water. Unless otherwise specified, all the modification experiments were conducted at an ambient temperature of 23 °C.

2.4. *Confirmation of the Cu-NPs loading on the membrane surface*

Visual characterization of the modified and the pristine membrane surfaces was carried out with scanning electron microscopy (SEM; Hitachi Ultra-High-Resolution Analytical Field Emission Scanning Electron Microscope, FE-SEM, SU-70). Prior to SEM measurement, the membrane was dried and sputter-coated (Denton Vacuum, DESK V) with a chromium layer of approximate 10 nm. X-ray photoelectron spectroscopy (XPS; Surface Science Instruments model SSX-100-SPECS) measurements for surface elemental analysis were performed with monochromatized Al-Kα X-ray source.

2.5. Characterization of membrane properties

The transport properties of the membrane were examined on a bench-scale RO filtration system [39]. Two measurements were taken for each type of membrane. The membrane (area of 20.02 cm²) was compacted overnight under 31 bar (450 psi), and subsequently the pressure was reduced to 27.6 bar (400 psi) to determine the pure water permeability measurement, *A*. The salt rejection was measured at 400 psi with 50 mM NaCl as feed solution. The salt permeability coefficient, *B*, was calculated from the water fluxes and salt rejections according to the method described previously [40]. All the filtration experiments were carried out at 25.0 ± 0.5 °C with a cross-flow velocity of 21.4 cm/s.

Water contact angle (VCA Video Contact Angle System; AST Products, Billerica, MA) measurements were performed to evaluate the membrane surface hydrophilicity. To account for variations in the measurements, eight measurements were performed on each of two samples. The zeta potential of the membranes was calculated from streaming potential measurements performed with an electro-kinetic analyzer (EKA, Brookhaven Instruments, Holtsville, NY) following procedures previously reported [41]. Prior to the measurements, the membrane samples were equilibrated with the testing solution (1 mM KCl) for at least 45 minutes. Measurements were performed at room temperature (23 °C) at pH 4-9, adjusted with aliquots of 1 M HCl and KOH. For each type of membrane, two separate samples were characterized to account for any system variation.

Surface roughness was determined using atomic force microscopy (AFM, Bruker, Digital Instruments, Santa Barbara, CA) in tapping mode with a silicon probe. The probe had a spring constant of 40 N/m, a resonance frequency of 300 kHz, a tip radius of 8 ± 4 nm, and a cantilever length of 125 ± 10 μm. Micrographs were captured from three different locations with an area of 5 μm × 5 μm on each membrane sample. Three samples of each membrane type, i.e., pristine and modified, were characterized to calculate average roughness.

2.6. Quantifying copper dissolution rates

In order to examine the residual copper loading on the membrane after dissolution, 3.8 cm² coupons of *in situ* Cu-NPs modified membranes were placed in glass vials filled with 10 mL of 5 mM NaHCO₃ solution (pH 8.3; without HNO₃). The vial with the membrane was agitated.

At predetermined time intervals, the solution was removed, and the empty glass vial containing the membrane was refilled with 10 mL DI water with 0.1 mL of 70% HNO₃ to promote dissolution of the residual copper from the membrane. Subsequently, the dissolved copper concentration in the solution was quantified with an inductive coupled plasma mass spectrometer (ICP-MS, ELAN DRC-e ICP Mass Spectrometer, Perkin Elmer).

2.7. Antimicrobial activity of Cu-NP functionalized membranes

The antimicrobial properties of the membrane were assessed using *E.coli* as model bacteria. An overnight culture of bacteria (1 mL) grown in LB was diluted in 24 mL of fresh LB broth and grown for two to three hours of exponential phase. Viable cell attachment experiments were carried out to quantify the number of live bacteria attached to pristine and *in situ* modified membranes. The bacteria culture was centrifuged for one minute at 5,000 rpm (Eppendorf 5415D) in 1 mL centrifuge tubes, and re-suspended in isotonic saline solution (0.15 M NaCl, 20 mM NaHCO₃, pH 8.2) to an initial OD₆₀₀ of 0.15± 0.09. Circular membrane coupons (3.8 cm²) were placed in sterile plastic tubes with the bacteria in an isotonic (saline) solution. Only the membrane polyamide active layer was exposed to the bacteria suspension during incubation. The tubes were incubated for two hours at 37 °C. After incubation, the membranes were rinsed gently with DI water to remove unattached bacteria. Membrane coupons were then bath-sonicated for seven minutes in 10 mL of isotonic solution to remove the attached bacteria. The suspension was diluted, plated on LB agar plates, and incubated overnight, at which time bacteria colonies were counted. Statistically significant differences ($\alpha = 0.05$) were determined using Student t-test with two-tailed distribution assuming equal variances (homoscedastic).

3. Results and discussion

3.1. Cu-NP *in situ* formation procedure on TFC membrane

The protocol for *in situ* formation of Cu-NPs was initiated when a solution of copper salt precursor was maintained in contact with the membrane surface to promote diffusion of copper ions to the vicinity of the membrane surface. During this step, the native carboxylic and amine groups on the PA-TFC membrane surface [42] are likely to form complexes with the dissolved copper ions, as discussed later. Then, the copper solution was discarded, leaving a thin film of

the copper solution on the membrane surface. When the membrane surface was covered with NaBH₄ solution, the copper ions in the thin film were chemically reduced to metallic copper to form Cu-NPs as described by the following reaction [43]:

During this step, the membrane color slowly changed to dark brown which, upon exposure to air, further oxidized and turned to dark green.

The entire *in situ* formation procedure was conducted at ambient conditions, without a need to move the membrane during reaction. This demonstrates the potential to load Cu-NPs on the membrane surface onsite, i.e., at the desalination plant, and without any need to disassemble the membrane module. DI water was selected as the solvent because of its availability in desalination plants. Also, the high purity of DI water insures that during the chemical reduction by NaBH₄, only the supplemented copper will be reduced on the membrane. The chosen reducer, NaBH₄, demonstrated industrial scale application in the bleaching process of wood pulp [44]. In addition, NaBH₄ is a selective reducer with fast reaction kinetics to reduce metals. Under normal conditions, NaBH₄ does not reduce amide or carboxylic groups, which are the main constituents of the selective polyamide layer of TFC-RO membranes [45]. Thus, the use of NaBH₄ during the chemical reduction lowers the risk of damaging the membrane transport properties during membrane surface modification.

3.2 Evidence for Cu-NP formation on the membrane surface

The formation of Cu-NPs on the membrane surface by the *in situ* formation procedure was confirmed by both visual and quantitative analyses. SEM images (**Fig. 2**) clearly demonstrated the presence of nanoparticles (NPs) over the typical PA-TFC features. These NPs on the PA-TFC were not observed on the surface of the pristine membrane. Unlike the <15 nm *in situ* reduced Ag-NPs described in our previous article, that could be visible only at very high SEM magnification (X 100,000) [29], the Cu-NPs here were clearly seen at a lower magnification of 45,000, implying larger particles in an approximated size range of a few tens of nanometers.

XPS measurements confirmed that the observed NPs in the SEM are Cu-NPs (**Fig. 3**). The peak at binding energy of 932 eV ascribed to Cu 2p was depicted in the spectrum of the *in*

in situ modified membrane, but not in the spectrum of the pristine membrane. Also, other weaker peaks related to copper, such as Cu 2s (1100 eV), Cu 2p (951 eV), Cu 3s (120 eV), and Cu 3p (74 eV), which appeared only in the *in situ* modified spectrum, validated copper loading on the membrane surface. C, N, and O constitute the polyamide layer of the TFC membrane; therefore, their related peaks (at 281, 396, and 527 eV, respectively) appeared on both the modified and pristine membranes. However, analysis of the elemental surface coverage (**Table 1**) revealed that the O/C ratio was increased by about 10 % on the *in situ* modified membrane as compared to the pristine membrane, which likely indicates partial or complete oxidation of the Cu-NPs. The oxidation of Cu-NPs is expected, as the strong oxidation tendency of copper-based NPs in oxygenated aquatic solutions or during exposure to atmospheric air was extensively reported [46, 47]. For sodium and boron from the NaBH₄, at both spectra of pristine and *in situ* modified membranes, no signals were observed at binding energies of 31, 63, and 188 eV related to Na 2p, Na 2s, and B 1s, respectively, indicating that these elements were not incorporated in the NPs. Thus, the observed NPs in the SEM are Cu-NPs composed primarily of metallic copper, and to some extent copper oxide.

3.3. Possible mechanism for the attachment of Cu-NPs on the membrane surface

While the chemical reduction of dissolved cupric ions to metallic copper by NaBH₄ is well understood, the tendency of the particles to attach, or more likely to grow, on the membrane surface and not in the bulk solution, is not obvious. The interaction between metal ions, such as copper, and carboxylic surface functional groups was suggested in the past to explain coating of polymeric surfaces by Me-NPs [48]. Such affinity was used to improve coating of polymer surfaces with copper thin-film or NPs, i.e., the process of metallization [49-51].

In several metallization studies, facile wet chemical reduction of Cu-NPs on polyimide was conducted through three main stages [52]. Initially, the polyimide surface was modified through ring cleavage to form carboxylic and amine surface groups that tend to complex with metal ions. Subsequently, the modified polyimide was immersed in a copper solution to promote complexation of copper with the modified surface groups. In the last stage, dimethylamine borane (DMAB) reduced the copper ions to metallic copper, forming copper nanoparticles attached to the polymer surface. Since Cu-NPs were poorly formed on a neat polyimide surface

1 without any modification, copper patterns could be designed through selective modification-
2 etching of the polymer [52].

3 The above discussion emphasizes the important role of the inherent polyamide surface
4 functional groups for the attachment of *in situ* formed Cu-NPs on the polymer surface. The
5 active side of the polyamide layer of the TFC-RO membrane used here already possesses native
6 carboxylic groups [42, 53], and also to some extent amine groups [54], which result from the
7 interfacial polymerization process. These native functional groups will likely interact directly
8 with the copper ions to promote formation of Cu-NPs attached to the membrane surface during
9 the chemical reduction stage.

11 3.4. Effects of *in situ* reaction on the membrane intrinsic transport properties

12 The polyamide selective layer of TFC membranes is highly sensitive to chemical or
13 physical surface modifications that may impact the salt and water transport properties required
14 for effective desalination. Therefore, it is important to understand the impact of the *in situ* Cu-NP
15 formation reaction on the membrane transport properties.

16 A minor increase in the membrane water permeability coefficient (A) was observed after
17 the Cu-NP *in situ* modification, with values of 2.53 ± 0.22 and $2.97 \pm 0.32 \text{ L m}^{-2} \text{ h}^{-1} \text{ bar}^{-1}$ for the
18 pristine and the *in situ* modified membranes, respectively. The salt rejection decreased slightly
19 for the *in situ* modified membrane compared to the pristine membranes ($98.94 \pm 0.23 \%$ and
20 $98.31 \pm 0.32 \%$, respectively). The value of the calculated salt permeability coefficient (B) was
21 higher for the modified membrane ($0.45 \pm 0.11 \text{ L m}^{-2} \text{ h}^{-1}$) than the pristine membrane ($0.26 \pm$
22 $0.04 \text{ L m}^{-2} \text{ h}^{-1}$).

23 By using the same concentration of Cu (50 mM), but decreasing the NaBH_4 concentration
24 (from 50 mM to 20 mM) during the Cu-NPs *in situ* modification, the transport and salt rejection
25 properties of the membrane were comparable, i.e., insignificantly different from those of the
26 pristine membrane ($A = 2.55 \pm 0.18 \text{ L m}^{-2} \text{ h}^{-1} \text{ bar}^{-1}$, $B = 0.34 \pm 0.17 \text{ L m}^{-2} \text{ h}^{-1}$, and $R = 98.76 \pm$
27 0.23%). Thus, by tuning the concentration of NaBH_4 for the *in situ* reaction, the membrane
28 transport properties can remain almost intact.

30 3.5. Effects of *in situ* reaction on the membrane surface properties

During reverse osmosis desalination, different types of fouling may develop from the ubiquitous diverse constituents found in the feed water. This nascent fouling may lead to significant deterioration of the efficiency of the desalination process. The surface properties of the membrane play a critical role in determining the fouling propensity of the membrane. It has been suggested that a membrane with hydrophilic, neutral surface charge and a smooth surface has higher fouling resistance [55-57]. Since the *in situ* reaction modifies the membrane surface, its impact on the membrane surface properties must be thoroughly studied.

Similar zeta potential curves of the *in situ* modified and pristine membranes (**Fig. 5**) suggest an insignificant change in the membrane surface charge after modification. Due to the small size of Cu-NPs, and their monolayer coverage of the membrane surface (**Fig. 2**), the surface roughness parameters did not significantly change for the *in situ* modified membrane compared to that of the pristine membrane (**Fig. 6**). The R_{rms} (root mean square roughness) values were 76.6 ± 6.7 nm and 75.08 ± 9.7 nm; the R_a (average roughness) values were 58.9 ± 6.7 nm and 58 ± 7.9 nm; the R_{max} values (maximum roughness in nm) were 638 ± 205 and 579 ± 131 ; and the SAD (surface area difference in %) were 44.3 ± 8.7 and 27.4 ± 4.7 for the pristine and the *in situ* modified membranes, respectively. Drying the membrane prior to contact angle measurements led to oxidation of the *in situ* formed Cu-NPs. Due to the hydrophobic nature of copper oxide, the *in situ* membrane surface became slightly less hydrophilic, with contact angles increasing from $45.46 \pm 1.68^\circ$ to $59.84 \pm 3.13^\circ$ for the pristine and *in situ* modified membranes, respectively (**Fig. 5**). Thus, overall, the Cu-NPs *in situ* formation reaction only slightly changed the surface properties of the membrane.

3.6. Dissolution of the formed Cu-NPs

Since the feed water composition varies significantly for various desalination applications of TFC membranes, the dissolution experiments were performed with a simple aqueous solution containing bicarbonate as a buffer to achieve the same pH of 8.3 as seawater. The observed amount of residual copper loading on the membrane surface remained steady during the seven days of the dissolution experiments (**Fig. 7**). This demonstrates that the in-situ formed Cu-NPs are not readily dissolved.

Previous studies, however, reported inevitable dissolution of Me-NPs in aquatic solutions [58, 59]. Essentially, NPs are expected to exhibit faster dissolution rates than their larger

counterpart particles as theoretically described by the modified Kelvin equation, also known as the Kelvin effect [26, 60]. Solution chemistry may also significantly affect particle dissolution kinetics. The presence of ions such as chloride may expedite Me-NP dissolution while sulfate ions may slow the dissolution [61]. Chelators, ligands, humic substances, and other organics tend to accelerate the dissolution [61, 62]. Also, pH is reported to have a tremendous impact on dissolution of the particles. Another prominent parameter which affects NP dissolution kinetics is the hydrodynamic conditions that surround the NPs. Inside a typical spiral-wound RO membrane module, there is a strong shear force on the membrane surface due to the tangential flow aimed to mitigate concentration polarization effects. Thus, the different and diverse solution chemistries and hydrodynamic conditions found in desalination plants are expected to accelerate the Cu-NPs dissolution.

3.7. Antibacterial activity

The *in situ* formed Cu-NPs imparted strong antibacterial activity to the membrane surface. Using *E. coli* as model bacteria, and after two hours of incubation, the number of live bacteria attached to the membrane surface decreased by 89.6 ± 8.2 % on the Cu-NP *in situ* modified membrane, compared to the pristine membrane (**Fig. 8**). This statistically significant (p value of 0.037) lower number of attached live bacteria can result from one or both of the following: (i) as a result of the *in situ* membrane surface modification, the live bacteria had a weaker attraction to the membrane surface followed by lower attachment rates, and/or (ii) the toxic effect of the bound Cu-NPs killed the bacteria once they approached the membrane surface. Since the modified and pristine membranes exhibited comparable surface properties (as discussed in Section 3.5), the prominent reduction in the number of live colonies was mainly ascribed to the antibacterial effect of Cu-NPs.

While the strong antibacterial properties of Cu-NPs are well established, a long debate in the literature queries whether the solid NPs have unique biocidal capabilities other than the additive effect of the Cu ions that comprise the particles. Several studies claimed that the Cu-NPs serve only as a reservoir for dissolved toxic copper ions [26, 63]. These studies explained an observed stronger antibacterial activity of Cu-NPs compared to Cu ions by the “Trojan horse effect,” meaning the penetration of the Cu-NPs into the bacteria cell and the following intense and concentrated intercellular dissolution of the NPs into toxic ions [64, 65]. Other studies were

not able to explain their observation of Me-NPs toxicity only by toxicity of the matched released ions, and therefore deduced a toxic effect of the solid Me-NPs in addition to the accumulating effect of the ions [62, 66, 67]. This additional intrinsic toxicity of Cu-NPs was recently explained by the NP surface catalytic properties, which induced formation of deleterious reactive oxygen species (ROS) that attack the cell [67, 68].

Thus, the observed biocidal activity of the *in situ* formed Cu-NPs on the membrane surface may result from one or more of the following cytotoxicity pathways: (i) biocidal Cu ions that were released from the attached Cu-NPs diffused and formed a toxic inhibition zone in the vicinity of the membrane surface; (ii) bacteria that contact the membrane surface engulfed the attached Cu-NPs (the “Trojan horse effect”) that later dissolved to toxic Cu ions inside the bacteria cell; and (iii) intrinsic toxic properties of the solid Cu-NPs, such as formation of ROS, that attack the bacteria once they approach the membrane surface.

4. Conclusion

In order to utilize biocidal Me-NPs for biofouling mitigation in membrane desalination, a viable procedure for repeatable loading of the antibacterial agent inside the membrane module must be developed. In our previous work, we demonstrated the potential feasibility of *in situ* formed biocidal Ag-NPs on TFC RO membrane and the rendered antibacterial activity [29]. Here, we extended the *in situ* formation procedure for surface modification of TFC-RO membrane with biocidal Cu-NPs. Cu-NPs have a much lower cost than Ag-NPs, but still have a strong antibacterial activity. We succeeded in achieving *in situ* formed Cu-NPs that coated the surface of TFC-RO membranes by a facile procedure that was performed at ambient conditions, and required only two chemicals (copper salt and reducing agent) without an additional NP stabilizing agent. The loading can be performed *in situ*, implying potential implementation of the method for repeatable loading of Cu-NPs in a desalination plant without disassembling the membrane module. Generally, only a minor impact of the Cu-NP *in situ* procedure was observed on the transport properties of the membrane. Similarly, the surface properties were generally maintained, although the Cu-NPs *in situ* modified membrane demonstrated prominent antibacterial activity compared to the pristine membrane. Therefore, we demonstrated here a potentially viable method for loading biocidal Cu-NPs on TFC-RO membranes.

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References

- [1] M. Elimelech, W.A. Phillip, The Future of Seawater Desalination: Energy, Technology, and the Environment, *Science*, 333 (2011) 712-717.
- [2] L.F. Greenlee, D.F. Lawler, B.D. Freeman, B. Marrot, P. Moulin, Reverse osmosis desalination: Water sources, technology, and today's challenges, *Water Res*, 43 (2009) 2317-2348.
- [3] A. Matin, Z. Khan, S.M.J. Zaidi, M.C. Boyce, Biofouling in reverse osmosis membranes for seawater desalination: Phenomena and prevention, *Desalination*, 281 (2011) 1-16.
- [4] J. Mansouri, S. Harrisson, V. Chen, Strategies for controlling biofouling in membrane filtration systems: challenges and opportunities, *J Mater Chem*, 20 (2010) 4567-4586.
- [5] M.T. Khan, C.L.D. Manes, C. Aubry, L. Gutierrez, J.P. Croue, Kinetic Study of Seawater Reverse Osmosis Membrane Fouling, *Environ Sci Technol*, 47 (2013) 10884-10894.
- [6] C. Ayache, C. Manes, M. Pidou, J.P. Croue, W. Gernjak, Microbial community analysis of fouled reverse osmosis membranes used in water recycling, *Water Res*, 47 (2013) 3291-3299.
- [7] M. Al-Ahmad, F.A.A. Aleem, A. Mutiri, A. Ubaisy, Biofouling in RO membrane systems Part 1: Fundamentals and control, *Desalination*, 132 (2000) 173-179.
- [8] H. Ivnitsky, I. Katz, D. Minz, E. Shimoni, Y. Chen, J. Tarchitzky, R. Semiat, C.G. Dosoretz, Characterization of membrane biofouling in nanofiltration processes of wastewater treatment, *Desalination*, 185 (2005) 255-268.
- [9] J.S. Baker, L.Y. Dudley, Biofouling in membrane systems - A review, *Desalination*, 118 (1998) 81-89.
- [10] D.R. Vardon, M.M. Clark, D.A. Ladner, The potential of laser scanning cytometry for early warning of algal blooms in desalination plant feedwater, *Desalination*, 277 (2011) 193-200.
- [11] D.A. Caron, M.E. Garneau, E. Seubert, M.D.A. Howard, L. Darjany, A. Schnetzer, I. Cetinic, G. Filteau, P. Lauri, B. Jones, S. Trussell, Harmful algae and their potential impacts on desalination operations off southern California, *Water Res*, 44 (2010) 385-416.
- [12] M. Herzberg, M. Elimelech, Biofouling of reverse osmosis membranes: Role of biofilm-enhanced osmotic pressure, *J Membrane Sci*, 295 (2007) 11-20.
- [13] M. Herzberg, S. Kang, M. Elimelech, Role of Extracellular Polymeric Substances (EPS) in Biofouling of Reverse Osmosis Membranes, *Environ Sci Technol*, 43 (2009) 4393-4398.
- [14] J.S. Vrouwenvelder, D.A. Graf von der Schulenburg, J.C. Kruithof, M.L. Johns, M.C.M. van Loosdrecht, Biofouling of spiral-wound nanofiltration and reverse osmosis membranes: A feed spacer problem, *Water Res*, 43 (2009) 583-594.
- [15] S. Lee, J. Cho, M. Elimelech, Influence of colloidal fouling and feed water recovery on salt rejection of RO and NF membranes, *Desalination*, 160 (2004) 1-12.
- [16] N. Voutchkov, Considerations for selection of seawater filtration pretreatment system, *Desalination*, 261 (2010) 354-364.
- [17] I. Sutzkover-Gutman, D. Hasson, Feed water pretreatment for desalination plants, *Desalination*, 264 (2010) 289-296.
- [18] J.S. Vrouwenvelder, J.C. Kruithof, M.C.M. Van Loosdrecht, Integrated approach for biofouling control, *Water Sci Technol*, 62 (2010) 2477-2490.
- [19] H. Shemer, R. Semiat, Impact of halogen based disinfectants in seawater on polyamide RO membranes, *Desalination*, 273 (2011) 179-183.
- [20] J. Glater, S.K. Hong, M. Elimelech, The Search for a Chlorine-Resistant Reverse-Osmosis Membrane, *Desalination*, 95 (1994) 325-345.

- [21] M.S. Rahaman, H. Therien-Aubin, M. Ben-Sasson, C.K. Ober, M. Nielsen, M. Elimelech, Control of biofouling on reverse osmosis polyamide membranes modified with biocidal nanoparticles and antifouling polymer brushes, *J Mater Chem B*, 2 (2014) 1724-1732.
- [22] M. Ben-Sasson, K.R. Zodrow, G.G. Qi, 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.
- [23] A. Ronen, R. Semiat, C.G. Dosoretz, Impact of ZnO embedded feed spacer on biofilm development in membrane systems, *Water Res*, 47 (2013) 6628-6638.
- [24] A. Dror-Ehre, H. Mamane, T. Belenkova, G. Markovich, A. Adin, Silver nanoparticle-E. coli colloidal interaction in water and effect on E-coli survival, *J Colloid Interf Sci*, 339 (2009) 521-526.
- [25] V.K. Sharma, R.A. Yngard, Y. Lin, Silver nanoparticles: Green synthesis and their antimicrobial activities, *Adv Colloid Interfac*, 145 (2009) 83-96.
- [26] C. Gunawan, W.Y. Teoh, C.P. Marquis, R. Amal, Cytotoxic Origin of Copper(II) Oxide Nanoparticles: Comparative Studies with Micron-Sized Particles, Leachate, and Metal Salts, *Acs Nano*, 5 (2011) 7214-7225.
- [27] K.R. Raghupathi, R.T. Koodali, A.C. Manna, Size-Dependent Bacterial Growth Inhibition and Mechanism of Antibacterial Activity of Zinc Oxide Nanoparticles, *Langmuir*, 27 (2011) 4020-4028.
- [28] N. Jones, B. Ray, K.T. Ranjit, A.C. Manna, Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms, *Fems Microbiol Lett*, 279 (2008) 71-76.
- [29] M. Ben-Sasson, X. Lu, E. Bar-Zeev, K.R. Zodrow, S. Nejati, G.G. Qi, E.P. Giannelis, M. Elimelech, In situ formation of silver nanoparticles on thin-film composite reverse osmosis membranes for biofouling mitigation, *Water Res*, 62 (2014).
- [30] USGS., Metal prices in the United States through 2010., <http://pubs.usgs.gov/sir/2012/5188/sir2012-5188.pdf>.
- [31] N. Akar, B. Asar, N. Dizge, I. Koyuncu, Investigation of characterization and biofouling properties of PES membrane containing selenium and copper nanoparticles, *J Membrane Sci*, 437 (2013) 216-226.
- [32] T.A. Dankovich, J.A. Smith, Incorporation of copper nanoparticles into paper for point-of-use water purification, *Water Res*, 63 (2014) 245-251.
- [33] M.H. Freeman, C.R. McIntyre, A Comprehensive Review of Copper-Based Wood Preservatives, *Forest Prod J*, 58 (2008) 6-27.
- [34] L.A. Brun, J. Maillet, P. Hinsinger, M. Pepin, Evaluation of copper availability to plants in copper-contaminated vineyard soils, *Environ Pollut*, 111 (2001) 293-302.
- [35] B. Eder, Method of disinfecting water, in particular swimming pool water, in, USA, 1985.
- [36] M. Omae, General aspects of tin-free antifouling paints, *Chem Rev*, 103 (2003) 3431-3448.
- [37] M.O. Saeed, A.T. Jamaluddin, I.A. Tisan, D.A. Lawrence, M.M. Al-Amri, K. Chida, Biofouling in a seawater reverse osmosis plant on the Red Sea coast, Saudi Arabia, *Desalination*, 128 (2000) 177-190.
- [38] E.R. Cornelissen, J.S. Vrouwenvelder, S.G.J. Heijman, X.D. Viallefont, D. Van Der Kooij, L.P. Wessels, Periodic air/water cleaning for control of biofouling in spiral wound membrane elements, *J Membrane Sci*, 287 (2007) 94-101.
- [39] E.M.V. Hoek, A.S. Kim, M. Elimelech, Influence of crossflow membrane filter geometry and shear rate on colloidal fouling in reverse osmosis and nanofiltration separations, *Environ Eng Sci*, 19 (2002) 357-372.
- [40] N.Y. Yip, A. Tiraferri, W.A. Phillip, J.D. Schiffman, M. Elimelech, High Performance Thin-Film Composite Forward Osmosis Membrane, *Environ Sci Technol*, 44 (2010) 3812-3818.
- [41] S.L. Walker, S. Bhattacharjee, E.M.V. Hoek, M. Elimelech, A novel asymmetric clamping cell for measuring streaming potential of flat surfaces, *Langmuir*, 18 (2002) 2193-2198.
- [42] A. Tiraferri, M. Elimelech, Direct quantification of negatively charged functional groups on membrane surfaces, *J Membrane Sci*, 389 (2012) 499-508.

- [43] G.N. Glavee, K.J. Klabunde, C.M. Sorensen, G.C. Hadjipanayis, Borohydride Reduction of Nickel and Copper Ions in Aqueous and Nonaqueous Media - Controllable Chemistry Leading to Nanoscale Metal and Metal Boride Particles, *Langmuir*, 10 (1994) 4726-4730.
- [44] S.K. Gulsoy, H. Eroglu, Influence of Sodium Borohydride on Kraft Pulping of European Black Pine as a Digester Additive, *Ind Eng Chem Res*, 50 (2011) 2441-2444.
- [45] M. Periasamy, P. Thirumalaikumar, Methods of enhancement of reactivity and selectivity of sodium borohydride for applications in organic synthesis, *J Organomet Chem*, 609 (2000) 137-151.
- [46] N.L. Pacioni, V. Filippenko, N. Presseau, J.C. Scaiano, Oxidation of copper nanoparticles in water: mechanistic insights revealed by oxygen uptake and spectroscopic methods, *Dalton T*, 42 (2013) 5832-5838.
- [47] P. Kanninen, C. Johans, J. Merta, K. Kontturi, Influence of ligand structure on the stability and oxidation of copper nanoparticles, *J Colloid Interf Sci*, 318 (2008) 88-95.
- [48] J.P. Chen, S.N. Wu, K.H. Chong, Surface modification of a granular activated carbon by citric acid for enhancement of copper adsorption, *Carbon*, 41 (2003) 1979-1986.
- [49] D. Aldakov, Y. Bonnassieux, B. Geffroy, S. Palacin, Selective Electroless Copper Deposition on Self-Assembled Dithiol Monolayers, *Acs Appl Mater Inter*, 1 (2009) 584-589.
- [50] K. Akamatsu, A. Kimura, H. Matsubara, S. Ikeda, H. Nawafune, Site-selective direct photochemical deposition of copper on glass substrates using TiO₂ nanocrystals, *Langmuir*, 21 (2005) 8099-8102.
- [51] J.H.G. Ng, M.P.Y. Desmulliez, M. Lamponi, B.G. Moffat, A. McCarthy, H. Suyal, A.C. Walker, K.A. Prior, D.P. Hand, A direct-writing approach to the micro-patterning of copper onto polyimide, *Circuit World*, 35 (2009) 3-17.
- [52] K. Akamatsu, S. Ikeda, H. Nawafune, H. Yanagimoto, Direct patterning of copper on polyimide using ion exchangeable surface templates generated by site-selective surface modification, *J Am Chem Soc*, 126 (2004) 10822-10823.
- [53] X. Lu, S. Romero-Vargas Castrillón, D.L. Shaffer, J. Ma, M. Elimelech, In Situ Surface Chemical Modification of Thin-Film Composite Forward Osmosis Membranes for Enhanced Organic Fouling Resistance, *Environ Sci Technol*, 47 (2013) 12219-12228.
- [54] A. Pihlajamäki, Y. Shabtai, J. Gilron, Detection of free primary amines in TFC membranes by microscopic fluorescence imaging, *Desalination*, 286 (2012) 80-86.
- [55] E. Ostuni, R.G. Chapman, R.E. Holmlin, S. Takayama, G.M. Whitesides, A survey of structure-property relationships of surfaces that resist the adsorption of protein, *Langmuir*, 17 (2001) 5605-5620.
- [56] E.M. Vrijenhoek, S. Hong, M. Elimelech, Influence of membrane surface properties on initial rate of colloidal fouling of reverse osmosis and nanofiltration membranes, *J Membrane Sci*, 188 (2001) 115-128.
- [57] X. Lu, L. H. Arias Chavez, S. Romero-Vargas Castrillón, J. Ma, M. Elimelech, Influence of Active Layer and Support Layer Surface Structures on Organic Fouling Propensity of Thin-Film Composite Forward Osmosis Membranes, *Environ Sci Technol*, 49 (2015) 1436-1444.
- [58] J.R. Conway, A.S. Adeleye, J. Gardea-Torresdey, A.A. Keller, Aggregation, Dissolution, and Transformation of Copper Nanoparticles in Natural Waters, *Environ Sci Technol*, 49 (2015) 2749-2756.
- [59] A.S. Adeleye, J.R. Conway, T. Perez, P. Rutten, A.A. Keller, Influence of Extracellular Polymeric Substances on the Long-Term Fate, Dissolution, and Speciation of Copper-Based Nanoparticles, *Environ Sci Technol*, 48 (2014) 12561-12568.
- [60] I.A. Mudunkotuwa, T. Rupasinghe, C.M. Wu, V.H. Grassian, Dissolution of ZnO Nanoparticles at Circumneutral pH: A Study of Size Effects in the Presence and Absence of Citric Acid, *Langmuir*, 28 (2012) 396-403.
- [61] Z.Y. Wang, A. von dem Bussche, P.K. Kabadi, A.B. Kane, R.H. Hurt, Biological and Environmental Transformations of Copper-Based Nanomaterials, *Acs Nano*, 7 (2013) 8715-8727.

- [62] K. Midander, P. Cronholm, H.L. Karlsson, K. Elihn, L. Moller, C. Leygraf, I.O. Wallinder, Surface Characteristics, Copper Release, and Toxicity of Nano- and Micrometer-Sized Copper and Copper(II) Oxide Particles: A Cross-Disciplinary Study, *Small*, 5 (2009) 389-399.
- [63] M. Heinlaan, A. Ivask, I. Blinova, H.C. Dubourguier, A. Kahru, Toxicity of nanosized and bulk ZnO, CuO and TiO₂ to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*, *Chemosphere*, 71 (2008) 1308-1316.
- [64] A.M. Studer, L.K. Limbach, L. Van Duc, F. Krumeich, E.K. Athanassiou, L.C. Gerber, H. Moch, W.J. Stark, Nanoparticle cytotoxicity depends on intracellular solubility: Comparison of stabilized copper metal and degradable copper oxide nanoparticles, *Toxicol Lett*, 197 (2010) 169-174.
- [65] T. Gomes, J.P. Pinheiro, I. Cancio, C.G. Pereira, C. Cardoso, M.J. Bebianno, Effects of Copper Nanoparticles Exposure in the Mussel *Mytilus galloprovincialis*, *Environ Sci Technol*, 45 (2011) 9356-9362.
- [66] H.L. Karlsson, P. Cronholm, J. Gustafsson, L. Moller, Copper oxide nanoparticles are highly toxic: A comparison between metal oxide nanoparticles and carbon nanotubes, *Chem Res Toxicol*, 21 (2008) 1726-1732.
- [67] G. Applerot, J. Lellouche, A. Lipovsky, Y. Nitzan, R. Lubart, A. Gedanken, E. Banin, Understanding the Antibacterial Mechanism of CuO Nanoparticles: Revealing the Route of Induced Oxidative Stress, *Small*, 8 (2012) 3326-3337.
- [68] R.J. Griffitt, R. Weil, K.A. Hyndman, N.D. Denslow, K. Powers, D. Taylor, D.S. Barber, Exposure to copper nanoparticles causes gill injury and acute lethality in zebrafish (*Danio rerio*), *Environ Sci Technol*, 41 (2007) 8178-8186.

Table 1. Relative elemental surface coverage (%) as analyzed from the XPS measurements of pristine and Cu-NPs *in situ* modified membranes.

	O	C	N	Cu
Pristine	21.37 ± 1.9 %	70.25 ± 0.29 %	8.37 ± 1.62 %	0
Cu-NPs membrane	22.61 ± 0.05 %	67.15 ± 0.38 %	7.49 ± 0.35 %	2.75 ± 0.02 %

Figure 1. A schematic diagram of *in situ* formation of Cu-NPs on a TFC RO membrane: pristine RO membrane (A) is covered with CuSO_4 solution (pale blue; 50 mM) for 10 minutes (B), at that point the CuSO_4 solution is discarded leaving a thin layer of the CuSO_4 solution on the surface (C), next, the membrane is recoated with NaBH_4 solution (50 mM) for 15 minutes (D) to cover the membrane surface with Cu-NPs (E).

Figure 2. SEM images of the polyamide active layer of TFC-RO membrane. (A, C) pristine and (B, D) Cu-NPs *in situ* modified membranes. A, B are micrographs at 45,000 X magnification and C, D are at 100,000 X magnification.

Figure 3. XPS spectra of pristine (red) and modified TFC membrane with *in situ* formed Cu-NPs (green). The peaks at 932 eV are related to the presence of copper.

Figure 4. Water permeability coefficient (A), salt permeability coefficient (B), and salt rejection (R), of pristine and Cu-NP *in situ* modified membranes. Experimental conditions: hydraulic pressure of 27.5 bar (400 psi), crossflow velocity of 21.4 cm/s, temperature of 25.0 $\pm$ 0.5 $^{\circ}$ C, and a feedwater of 50 mM NaCl.

Figure 5. Membrane surface charge represented by zeta potential (A); and membrane surface hydrophilicity represented by contact angle measurements (B). Black – pristine membrane; blue – Cu-NPs *in situ* modified membrane.

Figure 6. Three-dimensional AFM images of: (A) pristine polyamide membrane, and (B) *in situ* Cu-NPs modified membrane. C) calculated surface roughness parameters (R_{rms} , root mean squared roughness; R_a , average roughness; R_{max} , maximum roughness; and SAD, surface area difference). Note that the X and Y dimensions in A and B are both 5 μm (2.5 μm /division), while the Z-scale is 300 nm (150 nm/division).

Figure 7. Residual copper loading on Cu-NP *in situ* modified membrane after dissolution for several days in 10 mL of 5 mM NaHCO₃ solution (pH 8.3).

Figure 8. Number of attached live bacteria colonies (CFU) on pristine (black) and Cu-NPs *in situ* modified membranes (blue) for gram negative *E. coli* bacteria. Experimental conditions: pristine and *in situ* modified membranes were contacted and incubated with isotonic solution (0.15 M NaCl; 20 mM NaHCO₃ as buffer, pH 8.2) with bacteria (OD₆₀₀: 0.15 ± 0.01) for 2 h at 37°C; after incubation the membranes were sonicated with sterile isotonic solution and the detached bacteria were plated on LB agar.