Novel Regenerable Antimicrobial Nanocomposite Membranes: Effect of Silver Loading and Valence State

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Abstract Silver as a broad spectrum antimicrobial agent has been widely studied as anti-biofouling coatings in membrane surface modifications. Incorporation of Ag in nano-zeolite grafted on the membrane surface was recently demonstrated to provide sustainable and regenerable antimicrobial activity. In this study, we examined two new methods of forming the Ag-zeolite based antimicrobial coating using polyvinyl alcohol (PVA) and polydopamine (PDA), respectively. Both methods achieved a wide range of Ag loading. Silver release and bacterial exposure experiments showed that increasing Ag-zeolite loading accelerated Ag release, which led to stronger antimicrobial activity, but did not significantly extend the antimicrobial lifetime of the membrane. Reduction of Ag⁺ to Ag(0), however, effectively stabilized Ag, reduced Ag release rate, and significantly prolonged the antimicrobial efficacy against bacterial growth on the membrane surface. All coatings strongly inhibited cell attachment and growth on the membrane surface despite the bacterial growth in the suspension, demonstrating the benefit of surface coating versus disinfection pretreatment of feed water. These results show the potential of polymer/Ag-zeolite nanocomposite coatings as versatile approach towards long lasting and regenerable anti-biofouling activity in membrane systems. They also highlight the importance of implementing slow-release mechanism in high Ag-zeolite loading samples for efficient utilization of loaded Ag.

1 **Keywords:** Ag-zeolite, biofouling, antimicrobial, Ag loading, Ag release

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4 1. Introduction

5 Membrane based desalination processes such as nanofiltration and reverse osmosis
6 have gained wide application in water and wastewater treatment [1]. Membrane fouling,
7 however, remains a major hurdle that severely undermines membrane longevity and
8 effectiveness of membrane processes [2]. Biofouling, amongst other types of fouling, is
9 especially problematic due to the lack of effective detection, prevention and post cleaning
10 methods [3, 4]. Severe membrane biofouling will lead to significant decline in membrane
11 flux and effluent quality, as well as membrane damage [5].

12 Commonly used biofouling control strategies include feed water pretreatment,
13 membrane cleaning, and membrane surface modification [3, 6]. Modification of
14 membrane surface to impart fouling resistance has been actively pursued [7] due to its
15 potential to reduce cost related to pretreatment and membrane cleaning. A number of
16 researches have reported membrane surface modification using hydrophilic materials,
17 such as polyvinyl alcohol (PVA), polyethylene glycol (PEG), and various hydrophilic

1 block-co-polymers and dendrimers, to reduce the initial attachment of bacterial cells and
2 biopolymers to the membrane [8]. Although effective in reducing the attachment of
3 bacteria cells to the membrane, hydrophilic modifications do not remove or inactivate
4 attached cells. In long-term operation, biofilms still form.

5 Modification of membrane surfaces using materials with antimicrobial properties is
6 another approach to biofouling control. It inhibits biofilm development by inactivating
7 bacteria on or near the membrane surface [9]. Silver is one of the most common potent
8 broad-spectrum biocides that has very low toxicity to human [10]. It has been utilized in
9 many studies and demonstrated to be effective in inhibiting bacteria growth on membrane
10 surface [11-22]. To better control the release rate of Ag, zero-valent Ag instead of Ag^+
11 ion is preferred, usually in the form of Ag nanoparticles (AgNP) [11]. This can be
12 achieved by physisorption of Ag^+ onto the polymer membrane followed by chemical
13 reduction using reductants such as sodium borohydride or ascorbic acid. [11, 12].
14 Polydopamine can be used as both a polymer linker and a reductant to achieve the same
15 AgNP coating in one step [13, 14]. Upon reduction, AgNPs form on the membrane
16 surface and exerts antimicrobial activity by releasing Ag^+ into the aqueous environment
17 due to dissolution. Preformed AgNPs can also be incorporated into an anti-adhesion
18 surface coating (e.g., polyelectrolyte multilayers and block-co-polymers) on the base
19 membrane [15, 16]. Such coatings have the benefit of being both non-adhesive and
20 antimicrobial. In addition, surface tethering by covalent binding can effectively
21 immobilize AgNPs or Ag loaded nanomaterials on the membrane surface [17-19].
22 However, these approaches suffer from several limitations including the low
23 bioavailability (for Ag embedded in the polymer matrix), short lifetime due to fast
24 dissolution/release of Ag, and fouling of AgNP surface (for AgNPs bound to membrane
25 surface). In many cases, regeneration of the antimicrobial activity is not feasible (for Ag
26 in polymer matrix) or requires complex procedures. In a recent study, Nanozeolite loaded
27 with Ag^+ or AgNPs has also been covalently bound to polyamide membrane surface to
28 provide long-term antibiofouling activity [19]. This method protects Ag from potential
29 passivation by species in water and wastewater, provides slow and stable release of Ag^+ ,
30 and enables easy replenishment of Ag after depletion, enabling long-term biofouling
31 control in practical applications.

1 In these previous studies, the longevity of the antimicrobial activity was not
2 addressed. Because the antimicrobial activity of Ag loaded membranes originates from
3 the Ag⁺ ions released from the membrane [20, 21], the efficacy and life time of the
4 biofouling resistance are expected to depend strongly on the initial loading of the Ag and
5 the rate of Ag⁺ release [22]. Sufficient Ag loading as well as stable Ag release are both
6 important for long-term efficacy. However, most previous studies only evaluated the
7 short-term antimicrobial activity [13, 15, 21]. The influence of initial Ag loading,
8 especially for AgNPs, on the long term anti-biofouling efficacy of the membrane is not
9 well understood. Furthermore, different experimental protocols were employed in
10 previous researches to determine antimicrobial efficacy, making it difficult to compare
11 across different studies and draw insight regarding the effect of Ag loading.

12 In this study, we examined two simple methods of forming Ag-zeolite based
13 antimicrobial coating with a wide range of Ag loading, and investigated the effect of total
14 Ag loading and Ag oxidation state on Ag release kinetics as well as the strength and
15 longevity of the antimicrobial activity of the membranes.

17 **2. Materials and Methods**

18 **2.1. Materials**

19 ACS grade AgNO₃, NaCl, acetic acid, 99% NaBH₄, isopropanol, NaOH, Tris-HCl,
20 glutaraldehyde, dopamine hydrochloride, and polyvinyl alcohol (PVA, MW 89,000 –
21 98,000) were purchased from Fisher Scientific (Pittsburg, PA). Ethanol (200 proof) was
22 purchased from Decon Laboratory, Inc. (King of Prussia, PA). 3-
23 aminopropylmethyldiethoxysilane (APMDES, 95%) was purchased from Gelest, Inc.
24 (Morrisville, PA). FAU type X nanozeolite particles (~150 nm in diameter with 7Å pore
25 opening according to the vendor) were purchased from Nanoscape (Munich, Germany).
26 All solutions and suspensions were prepared using ultrapure water (≥18.0 MΩ-cm)
27 generated by an E-pure system (Barnstead, IL).

28 *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC# 700829) was used as a model
29 biofouling bacterium. Growth media, including Trypticase Soy Broth (TSB), Luria-

1 Bertani (LB), phosphate buffered saline (PBS), and agar were purchased from Fisher
2 Scientific (Pittsburg, PA).

3 The membrane used was a commercial thin film composite nanofiltration membrane
4 Filmtec NF 90 (Dow Water and Process Solutions, Minneapolis, MN). The membrane
5 was cut into small pieces, rinsed thoroughly with ultrapure water, and stored in ultrapure
6 water in dark at 4 °C for at least 24 hr before use.

7 **2.2. Methods**

8 **2.2.1. Membrane Modification**

9 Membrane samples were modified with nanozeolite particles loaded with Ag⁺ or
10 Ag(0) with a wide range of total Ag loading.

11 **Silver Loading.** Silver was loaded in nanozeolite particles by adding the nanozeolite
12 particles (0.05 wt%) into 20 mL 0.1N AgNO₃ solution, sonicating for 30 min at 40 Watts
13 using a sonication cup horn (Vibra-cell VCX 500, Sonics & Material, Newtown, CT),
14 followed by 24 hr of shaking on an orbital shaker (OS-500, VWR, Randor, PA) at 90 rpm
15 to allow adsorption. The suspension was then centrifuged at 11,000 rpm (~14,600×g) for
16 20 min to recover the Ag⁺ loaded nanozeolite (Ag⁺-zeolite). The Ag⁺-zeolite particles
17 were washed three times using ultrapure water by centrifugation at the same rate, and
18 dried overnight at 50 °C in a vacuum oven (Isotemp Model 281A, Fisher Scientific).

19 **Grafting of amine functional groups on nanozeolite.** To attach nanozeolite to the
20 membrane surface through the polydopamine coating, the nanoparticles were grafted with
21 amine functional groups, which react with the quinone groups on polydopamine [23]. For
22 the grafting reaction, 0.05 g nanozeolite was mixed with 5 mL isopropanol, sonicated for
23 30 min at 40 Watts using the sonication cup horn, and transferred into a round bottom
24 flask. APMDES was added drop-wise to reach a concentration of 26 mM. Constant
25 stirring was provided. A condenser was then put on the flask and the mixture was purged
26 with Ar gas for 4 hr. After Ar purging, the system was sealed and the mixture left to
27 react for another 20 hr at ambient temperature. After the reaction, the mixture was
28 centrifuged at 7,500 rpm (~ 6,680×g) to recover the nanozeolite. The nanozeolite

particles were washed twice with 200 proof ethanol, and dried overnight at 80 °C in a vacuum oven (Isotemp Model 281A, Fisher Scientific).

PVA/zeolite coating. PVA/zeolite coatings of two different Ag⁺-zeolite loadings, 0.23 (PVA-L+) and 0.43 (PVA-H+) g zeolite/m² membrane, were applied. The PVA stock solution (0.05 wt%) was prepared by dissolving PVA in pure water at 95 °C with vigorous stirring for 8 hr. Ag⁺-zeolite particles, 1.5 and 0.8 mg, were dispersed in 20 mL pure water and sonicated for 10 min. The coating solution was prepared by adding 0.2 mL of the PVA stock solution into the 20 mL Ag⁺-zeolite suspension. The cross-linking solution was prepared by adding 0.1 mL of 10 wt% acetic acid to 20 mL of 5.68 µmol/L glutaraldehyde. Coating was performed in a stainless steel stirred cell, where an NF 90 membrane (effective area of 35.26 cm²) was mounted at the bottom. Ultrapure water (~200 mL) was first filtered through the membrane at 150 psi. Then, 20 mL coating solution was filtered through the membrane at 100 psi, followed by 20 mL cross-linking solution at the same pressure. The membrane sample was then retrieved and heated at 70 °C in an oven for 30 min to allow cross-linking. After cooled to room temperature, the membrane sample was thoroughly rinsed, dried in air and stored in dark before use.

Polydopamine/zeolite coating. To apply the polydopamine/zeolite coating, the membrane sample was mounted on a glass slide with the active layer facing up. A rectangular rubber gasket (effective membrane area 8.5 cm × 5 cm) was installed and secured on the membrane to form a liquid reaction chamber. Dopamine hydrochloride (2 mg/mL) was dissolved in 10 mM Tris-HCl buffer at pH 8, immediately casted into the liquid chamber, left to react with the membrane for 30 min on an orbital shaker (OS-500, VWR, Randor, PA) at ~60 rpm, and rinsed off using ultrapure water [20]. A suspension of amine-grafted Ag⁺-zeolite nanoparticles in 10 mM Tris-HCl buffer was then casted onto the polydopamine coated membrane surface in the liquid reaction chamber. The reaction was carried out at 60 °C for 30 min, and the membrane was thoroughly rinsed [20]. Two different concentrations of Ag⁺-zeolite (0.02 and 0.06 wt%) were used to obtain low (PDA-L+) and high (PDA-H+) Ag-zeolite coating density.

Silver reduction. To prepare the Ag(0)-zeolite coated membrane, both the PVA- and PDA- coated Ag⁺-zeolite membrane samples (2 cm×2 cm) were immersed in 20 mL

0.05 M sodium borohydride for 2 h to reduce the Ag^+ in the zeolite to $\text{Ag}(0)$. The membrane samples were then thoroughly rinsed and used shortly in experiments. These membrane samples were denoted PVA-L0, PVA-H0, PDA-L0 and PDA-H0 depending on their Ag^+ loaded precursors.

2.2.2. Membrane Characterization

Surface Characterization. Surface images of membrane samples were taken with an FEI Quanta 400 FEG SEM operated at 10 kV (FEI, Hillsboro, OR). All samples were sputter-coated with 10 nm of gold before imaging. A KSV CAM 200 Optical Tensiometer (KSV instrument LTD, Helsinki, Finland) was used to measure static water contact angle in air using the sessile drop method. Contact angle values used were the average of left and right contact angles, and each result were average of 5 repeated measurements.

Silver loading measurement. Silver loading on membrane samples was determined by repeated extraction using 10% nitric acid. Prolonged exposure to HNO_3 damages zeolite pore structure by dissolving Al from the crystalline lattice [24], and releases Ag from zeolite pores. Triplicate Ag^+ -zeolite coated membrane samples of $1 \text{ cm} \times 2 \text{ cm}$ were incubated in 3 mL of HNO_3 , and shaken for 24 hr on an orbital shaker at $\sim 90 \text{ rpm}$. The membrane sample was then retrieved and the extraction solution analyzed for Ag concentration using inductively coupled plasma mass spectroscopy (ICP-MS Elan 9000, Perkin Elmer, Waltham, WA). The extraction was repeated three times, after which Ag release became negligible. It was assumed that Ag^+ -zeolite and $\text{Ag}(0)$ -zeolite had the same Ag loading as Ag loss during reduction was negligible.

Membrane permeability. The impact of Ag-zeolite coating on membrane permeability was assessed by measuring clean water flux of the control and the modified membranes. Filtration experiments were performed at an applied pressure of 150 psi using a lab-scale stainless steel stirred cell with an effective membrane area of 35.26 cm^2 . Membrane permeability K_w ($\text{m/s} \cdot \text{Pa}$) was calculated by the following equation:

$$K_w = J \Delta P \quad (1)$$

where J is the permeate flux (m/s) and ΔP is the applied pressure (Pa).

2.2.3. Silver release experiments.

Silver release from the membranes was characterized in 154 mM (0.9% w/w) NaCl to simulate the ionic strength of culture media used in the antimicrobial efficacy assessment. Each membrane sample (2 cm × 1 cm) was immersed in 1.5 mL 154 mM NaCl solution in a plastic centrifuge tube. The centrifuge tubes were shaken at ~90 rpm for 24 hr on the orbital shaker. Supernatant from each tube was collected and analyzed for silver concentration using ICP-MS. The membrane samples were rinsed with ultrapure water and subjected to the same procedure 7 times.

2.2.4. Evaluation of Membrane Biofouling Resistance.

The biofouling resistance of the membrane samples was assessed using *P. aeruginosa*, a biofilm forming bacterium commonly used in membrane biofouling studies [26]. The membrane samples were repeatedly exposed to a *P. aeruginosa* suspension in 24 hr cycles. At the end of each cycle, live *P. aeruginosa* concentration in the suspension was measured by plate counting.

P. aeruginosa was grown in TSB for 24 hr. The cell concentration in the culture reached $\sim 10^8$ CFU/mL as determined by plate counting. The bacterial suspension was centrifuged and washed three times with 0.9% NaCl solution, and serially diluted with the same saline solution to $\sim 10^5$ CFU/mL for use in antimicrobial efficacy tests. Membrane samples were cut into 2 cm × 1 cm pieces and put in incubation wells of 6-well cell culture plates. *P. aeruginosa* suspension of 50 μ L of was added to the surface of each membrane sample. The plates were then set aside for 30 min to allow bacteria adhesion, after which 1.5 mL of 1/100 diluted LB in 0.9% NaCl was added to each well and the plates were incubated for 24 hr at 37°C. After each 24 hr exposure period, the membrane samples were thoroughly rinsed with sterilized DI water, and used in the next 24 hr exposure cycle. A freshly prepared bacteria suspension was used in each cycle. Unmodified membranes were used as controls, and a fresh control membrane was used in each cycle.

Fluorescent images of the control and the Ag-zeolite modified membranes were taken using an Olympus IX71 inverted fluorescence microscope (Olympus, Center Valley, PA) after the 1st and 7th 24-hr cycle, respectively, to visualize bacteria attached on the membrane surface. The membranes were stained with propidium iodide (PI) first for 20 min, and then with 4',6-diamidino-2-phenylindole (DAPI) for 10 min. Membrane samples were thoroughly rinsed with ultrapure water between and after the staining steps. The number of total and dead cells on the membrane samples were counted and used to compare the performances of different samples.

3. Results and Discussion

3.1. Membrane characterization

Surface coating density and silver loading. FIG 1 shows representative SEM images of the virgin and modified NF90 membranes. Zeolite NPs are clearly visible on modified membranes. The different coating methods resulted in a range of zeolite surface densities (FIG. 2a), and the zeolite surface coverage (11 to 22 %) is highly consistent with the silver loading measured by HNO₃ extraction (FIG. 2a and 2b). All modified membranes had much higher Ag loading than that prepared by covalently binding zeolite to the membrane surface reported in a previous study (7.18 mg/m²) [19]. PVA-H had the highest zeolite surface coverage (22%) and silver loading (37.5 mg/m²), while PVA-L has a zeolite surface coverage of 13% and silver loading of 18.6 mg/m². The results are fairly consistent with the amount of Ag-zeolite added in the coating suspension. The Ag coating densities of PDA-H and PDA-L were lower at 20.5 and 9.6 mg/m², respectively, with zeolite surface coverage of both less than 15%.

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FIG. 1. SEM images of (a) unmodified membrane; (b) PVA coated membrane; (c) PDA coated membrane; (d) PVA-H membrane; (e) PVA-L membrane; (f) PDA-H membrane; (g) PDA-L membrane;

FIG. 2. (a) Surface coverage of Ag-zeolite on membranes prepared using different methods. (b) Silver loading of the modified membrane samples. The covalent binding sample data was obtained from [19]. Surface coverage were obtained from analysis of SEM images using the ImageJ software¹. Error bars represent standard deviations from three different measurements.

Surface hydrophobicity. Contact angles of the unmodified and modified membranes are summarized in FIG. 3. Both PVA and PDA coatings significantly decreased the membrane surface hydrophobicity, with PDA coating being more hydrophilic. Zeolite also contributed significantly to the increase in surface hydrophilicity; the membrane surface contact angle decreased with increasing zeolite loading.

¹ Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, <http://imagej.nih.gov/ij/>, 1997-2014

FIG. 3. Contact angles of the control and different modified samples. Error bars represent standard deviations from five different measurements.

Membrane permeability. The permeability of the original and modified membranes is shown in FIG. 4. The measured water permeability of the NF90 membrane, 2.78×10^{-11} (m/s-Pa) is consistent with that reported by a previous study [26]. The PDA coating had little impact on membrane permeability due to the thin coating and its hydrophilicity [27], but the addition of zeolite resulted in notable decrease in membrane permeability, and the reduction in permeability increased with increasing zeolite loading. The PVA coating resulted in very large decrease in membrane permeability. This is attributed to the dense structure of crosslinked PVA. The incorporation of zeolite in PVA, however, did not add to the resistance of the coating layer.

FIG. 4. Permeability of the unmodified and modified membranes.

3.2. Antimicrobial Efficacy of the modified membranes

FIG. 5 presents the bacterial concentration in the suspensions after each 24 hr exposure to the control or modified membranes. The results are averages of two independent experiments and the error bars represent the range of results from the two experiments.

The control sample showed consistent bacterial concentrations of $\sim 1 \times 10^8$ to 2×10^8 CFU/mL at the end of each 24 hr cycle, while strong inhibition of bacteria growth were measured in all Ag-zeolite modified samples. The higher Ag loading samples exert stronger antimicrobial efficacy. PVA-H+ and PVA-H0 completely inactivated the bacteria in the culture suspension for 5 consecutive cycles, while the PVA-L samples achieved complete inactivation for 4 cycles. Similar results were observed on PDA-Ag-zeolite samples, where the higher loading samples completely inhibited bacterial activity one cycle more than the lower loading samples, but their antimicrobial activities were lower than the PVA-Ag-zeolite samples. After 7 cycles, the bacteria concentration in the solution exposed to the modified membranes was still more than 70% lower than the control. In particular, the PVA-H samples, which had the highest Ag loading, maintained a bacterial concentration 2~3 orders of magnitude lower than the control after 7 cycles.

1 The reduction of Ag seems to have relatively small impact on the inactivation of
2 suspended bacteria than the Ag loading.

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5 FIG. 5. Antimicrobial activity in bulk solution in contact with membrane samples (a)
6 PVA-Ag-zeolite (b) PDA-Ag-zeolite

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8 Because biofouling is caused by growth of bacteria on the membrane surface, the
9 number of bacteria attached on the membrane surface was also examined. FIG. 6
10 summarizes the results of the surface bacteria attachment and inactivation. For the control
11 experiments, fresh NF90, PVA and PDA coated NF90 membranes were used (FIG. 6a
12 and 5b). A large number ($5.4 \times 10^6 \pm 1 \times 10^6$ cells/cm²) of bacterial cells were found on the

1 NF90 membrane surface after 24 hours, and ~90% of the cells were alive. The PVA and
2 PDA coatings significantly reduced cell attachment, presumably due to the hydrophilicity
3 of the coatings. In subsequent cycles, however, the number of attached cells continued to
4 increase, reaching a cell density of 4.4×10^6 and 4.2×10^6 cells/cm² after 3 days for PVA
5 and PDA coating, respectively. Little bacterial inactivation was found on the PVA and
6 PDA coated membranes.

7 Cell attachment on the modified membrane surfaces was measured after 9 exposure
8 cycles, when there was already significant bacterial growth in the suspension. Cell
9 attachment on the Ag-zeolite membranes was much lower than that on the control
10 samples even after 9 days of repeated incubation, during which significant biopolymer
11 adsorption and cell debris attachment could enhance cell adhesion. As shown in FIG. 6c,
12 no bacteria cells were found on PVA-Ag/zeolite coated membranes on day 9, except for
13 PVA-L⁺, which had a very small number of dead cells attached. Live cells were only
14 found after 17 days for PVA-H0 and 15 days for other PVA-Ag/zeolite coated
15 membranes, and a large number of cells attached were dead. The sample that showed the
16 highest efficacy is PVA-H0, which had a total cell attachment of 1.4×10^6 cells/cm² and a
17 surface inactivation rate of ~70% on day 17. These results show that even though the Ag
18 release was no longer able to control bacterial growth in the suspension (FIG. 4), these
19 was still strong antimicrobial activity on the membrane surface that inhibited attached
20 growth.

21 The PDA-Ag/zeolite coated membranes (FIG. 6e and 6f) showed overall similar
22 magnitude of cell attachment as the PVA-Ag/zeolite membranes, but the surface
23 inactivation was lower due to the lower Ag loading in these samples.

24 In general, higher Ag loading resulted in stronger antimicrobial effects shown by the
25 higher fraction of surface inactivation in the last few days of the antimicrobial testing
26 (FIG. 6d and 6f). It is noteworthy that although Ag⁺ and Ag(0) loaded samples showed
27 similar inactivation of suspended cells (FIG. 5), Ag(0) samples showed notably stronger
28 and longer-lasting effect on cell attachment and growth on the membrane surface. This
29 can be attributed to the slower Ag release from the Ag(0)-zeolite coated samples as

discussed in the next section, which can sustain a significant surface concentration of Ag for a longer time.

Current membrane samples showed very strong and long lasting antimicrobial efficacy, with total Ag-loading comparable or lower than other studies [11-13, 18]. Complete surface bacterial inactivation of up to 15 days exceeded the performance of most other membrane samples that showed long-term activity [11, 28].

FIG. 6. Density of attached bacteria cells and fraction of inactivation on the surface of controls (a and b), PVA-Ag/zeolite (c and d), and PDA-Ag/zeolite (e and f) samples. The error bars are the standard deviations from three measurements.

3.3. Silver release behavior of the modified membranes

FIG. 7 presents silver release from the modified membranes in 0.9% NaCl (154 mM) solution measured in 7 consecutive 24-hr cycles, and FIG. 8 compares the initial Ag release rates from different samples. Ag^+ -zeolite samples exhibited significantly faster initial Ag release than $\text{Ag}(0)$ -zeolite samples, and the release rate decreased to very low levels after 4 (for high Ag^+ loading) or 5 days (for low Ag^+ loading), with a significant portion of the loaded Ag (31 to 54%) remaining. The $\text{Ag}(0)$ -zeolite samples had slower release, resulting in more than 10% less release compared with their Ag^+ -zeolite counterparts over the 7-day period (FIG. 7c and 7d). This is because $\text{Ag}(0)$ must first be oxidized to $\text{Ag}(I)$, whose dissolution and subsequent ionic exchange leads to release of Ag^+ . In our test solution (154 mM NaCl), the solubility of Ag is $6.1 \mu\text{M}$ ($660 \mu\text{g/L}$) (FIG. 9), significantly higher than the measured amount of Ag release. This indicates that Ag release rate from the $\text{Ag}(0)$ -zeolite samples is controlled by the oxidation reaction. Therefore, reduction of Ag^+ to $\text{Ag}(0)$ in zeolite provides an effective way to control Ag release.

There have been very few studies to show membrane efficacy in high ionic strength solution conditions comparable to the current study. Nevertheless Ag release as high as 30% to 60% of the total loading in less than 24 hr have been observed even in DI water,

for both physisorbed Ag on membrane as well as Ag within polymer coating [11, 29]. The current results demonstrated that utilizing nano-zeolite as carriers effectively stabilized the Ag release under high ionic strength solution conditions.

It was also found that higher Ag-zeolite loading resulted in higher release rate, but did not significantly prolong the active period of Ag release. In the initial 4 days, the higher Ag loading samples generally released $>10 \text{ mg/m}^2$, much higher than their lower loading counterparts. Over the 7 days the higher loading samples also had significantly higher total release than the lower samples. However, each high and low loading pair had comparable percentage release over the 7-day period (FIG. 7c and 7d). This was observed for both Ag^+ and $\text{Ag}(0)$ loaded samples. These results indicate that the effect of total Ag loading will depend on the solution chemistry. A higher loading could be beneficial for feed water with high concentration of anions or organic matter that scavenge Ag^+ ; for feed waters with low concentrations of bacteria and Ag scavengers, higher loading will increase cost per unit volume of water treated.

The PVA/Ag-zeolite membranes showed significantly lower Ag release rate compared to the PDA/Ag-zeolite membranes (FIG. 7c and 7d) even though the initial Ag loading in the PVA/Ag-zeolite membranes was almost twice as high as that in the PDA/Ag-zeolite membranes. It is postulated that dispersing the Ag-zeolite in PVA resulted in PVA coating on Ag-zeolite surface, causing kinetic hindrance to Ag release.

FIG. 7. (a), (b) Daily Ag release from the modified membranes; (c), (d) cumulative Ag release behavior from the modified membranes. Percentage number indicates the percentage of silver released from each sample after 7 days.

Although high Ag-zeolite loading (both Ag^+ and $\text{Ag}(0)$) in general led to higher Ag release and hence stronger and slightly longer lasting antimicrobial activity, the measured Ag release did not seem to correlate well with the antimicrobial activity. There are two potential reasons. First, the presence of bacterial cells may interfere with Ag release as well as Ag concentration measurement because Ag^+ strongly interacts with thiol groups in bacterial surface and extracellular proteins [28]. Second, the measured bulk concentration of Ag may be much lower than that on the membrane surface and therefore not a good indicator of the antimicrobial activity on the surface. After 7 days, although the released bulk Ag concentration became very low ($< 10 \mu\text{g/L}$), the membrane samples continued to exert strong antimicrobial activity on the surface (FIG. 6c – 6f). Similar discrepancy between Ag release and antimicrobial activity was observed in a previous study [19].

FIG. 8. Initial silver release rates of different membrane samples. The initial release rate was determined by linear regression of the cumulative release data in the initial 5 (for low Ag loading) or 4 days (for high Ag loading).

FIG. 9. Silver speciation as a function of total Ag concentration in 0.9% NaCl computed using Visual MINTEQ. Temperature was assumed to be 25 °C. Ionic strength and pH were not fixed.

4. Conclusions

Slow release of antimicrobial agents is an effective way to achieve long-term biofouling resistance in membrane system. The two coating techniques reported in this

1 study allows easy control of total Ag loading over a wide range in the form of Ag^+ or
2 $\text{Ag}(0)$, and were able to achieve slow, relatively steady release of Ag for strong and long
3 lasting antimicrobial activity, a major improvement from previous reported methods.[11 -
4 13, 18] Easy replenishment of Ag upon depletion make this method particularly
5 attractive for long-term biofouling resistance [19]

6 Interestingly, although higher Ag-zeolite loading led to higher level of inactivation of
7 both planktonic and sessile bacteria, it did not proportionally extend the active lifetime of
8 the coating because increased Ag-zeolite loading resulted in higher Ag release rate.
9 Reduction of Ag^+ to $\text{Ag}(0)$, however, was able to decrease Ag release rate and hence
10 prolong the antimicrobial efficacy against cells attached on the membrane surface. With
11 the two coating methods evaluated in this study, the PVA/ $\text{Ag}(0)$ -zeolite coating exhibited
12 slower and more stable Ag release, likely due to the PVA coating on the zeolite particle
13 surface that hindered Ag diffusion. As a result, it maintained antimicrobial activity longer.
14 However, the PVA coating significantly increased water transport resistance of the
15 membrane, while the PDA coating only had slight impact on membrane permeability.
16 Future research should consider PDA coating with modified, e.g., polymer coating, Ag-
17 zeolite nanoparticles, to better control the Ag release rate while maintaining the water
18 permeability of the membrane.

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2 **References**

- 3 [1] S.S. Shenvi, A.M. Isloor, A.F. Ismail, A review on RO membrane technology: Developments
4 and challenges, *Desalination*, 368 (2015) 10-26.
- 5 [2] M. Elimelech, W.A. Phillip, The Future of Seawater Desalination: Energy, Technology, and
6 the Environment, *Science*, 333 (2011) 712-717.
- 7 [3] R.A. Al-Juboori, T. Yusaf, Biofouling in RO system: Mechanisms, monitoring and controlling,
8 *Desalination*, 302 (2012) 1-23.
- 9 [4] A. Subramani, E.M.V. Hoek, Biofilm formation, cleaning, re-formation on polyamide
10 composite membranes, *Desalination*, 257 (2010) 73-79.
- 11 [5] M.J. Boorsma, S. Dost, S. Klinkhamer, J.C. Schippers, Monitoring and controlling biofouling
12 in an integrated membrane system, *Desalin. Water Treat.*, 31 (2011) 347-353.
- 13 [6] J.S. Baker, L.Y. Dudley, Biofouling in membrane systems - A review, *Desalination*, 118
14 (1998) 81-89.
- 15 [7] D.S. Zhao, S.L. Yu, A review of recent advance in fouling mitigation of NF/RO membranes
16 in water treatment: pretreatment, membrane modification, and chemical cleaning, *Desalin. Water
17 Treat.*, 55 (2015) 870-891.
- 18 [8] G.D. Kang, Y.M. Cao, Development of antifouling reverse osmosis membranes for water
19 treatment: A review, *Water Res.*, 46 (2012) 584-600.
- 20 [9] X.L. Qu, J. Brame, Q.L. Li, P.J.J. Alvarez, Nanotechnology for a Safe and Sustainable Water
21 Supply: Enabling Integrated Water Treatment and Reuse, *Accounts Chem. Res.*, 46 (2013) 834-
22 843.
- 23 [10] A. Melaiye, W.J. Youngs, Silver and its application as an antimicrobial agent, *Expert
24 Opinion on Therapeutic Patents*, 15 (2005) 125-130.
- 25 [11] X.Y. Zhu, R.B. Bai, K.H. Wee, C.K. Liu, S.L. Tang, Membrane surfaces immobilized with
26 ionic or reduced silver and their anti-biofouling performances, *J. Membr. Sci.*, 363 (2010) 278-
27 286.
- 28 [12] M. Ben-Sasson, X.L. Lu, E. Bar-Zeev, K.R. Zodrow, S. Nejati, G.G. Qi, E.P. Giannelis, M.
29 Elimelech, In situ formation of silver nanoparticles on thin-film composite reverse osmosis
30 membranes for biofouling mitigation, *Water Res.*, 62 (2014) 260-270.
- 31 [13] L. Tang, K.J.T. Livi, K.L. Chen, Polysulfone Membranes Modified with Bioinspired
32 Polydopamine and Silver Nanoparticles Formed in Situ To Mitigate Biofouling, *Environ. Sci.
33 Technol. Lett.*, 2 (2015) 59-65.
- 34 [14] Z. Yang, Y. Wu, J. Wang, B. Cao, C.Y. Tang, In Situ Reduction of Silver by Polydopamine:
35 A Novel Antimicrobial Modification of a Thin-Film Composite Polyamide Membrane,
36 *Environmental Science & Technology*, 50 (2016) 9543-9550.
- 37 [15] G.M. Nisola, J.S. Park, A.B. Beltran, W.J. Chung, Silver nanoparticles in a polyether-block-
38 polyamide copolymer towards antimicrobial and antifouling membranes, *RSC Adv.*, 2 (2012)
39 2439-2448.
- 40 [16] L. Tang, K.A. Huynh, M.L. Fleming, M. Larronde-Larretche, K.L. Chen, Imparting
41 antimicrobial and anti-adhesive properties to polysulfone membranes through modification with
42 silver nanoparticles and polyelectrolyte multilayers, *J. Colloid Interface Sci.*, 451 (2015) 125-133.

- [17] M.S. Mauter, Y. Wang, K.C. Okemgbo, C.O. Osuji, E.P. Giannelis, M. Elimelech, Antifouling Ultrafiltration Membranes via Post-Fabrication Grafting of Biocidal Nanomaterials, *ACS Applied Materials & Interfaces*, 3 (2011) 2861-2868.
- [18] J. Yin, Y. Yang, Z. Hu, B. Deng, Attachment of silver nanoparticles (AgNPs) onto thin-film composite (TFC) membranes through covalent bonding to reduce membrane biofouling, *J. Membr. Sci.*, 441 (2013) 73-82.
- [19] J.J. Wu, C. Yu, Q.L. Li, Regenerable antimicrobial activity in polyamide thin film nanocomposite membranes, *J. Membr. Sci.*, 476 (2015) 119-127.
- [20] X. Cao, M. Tang, F. Liu, Y. Nie, C. Zhao, Immobilization of silver nanoparticles onto sulfonated polyethersulfone membranes as antibacterial materials, *Colloids and Surfaces B: Biointerfaces*, 81 (2010) 555-562.
- [21] J.S. Taurozzi, H. Arul, V.Z. Bosak, A.F. Burban, T.C. Voice, M.L. Bruening, V.V. Tarabara, Effect of filler incorporation route on the properties of polysulfone-silver nanocomposite membranes of different porosities, *J. Membr. Sci.*, 325 (2008) 58-68.
- [22] M. Zhang, K. Zhang, B. De Gusseme, W. Verstraete, Biogenic silver nanoparticles (bio-Ag0) decrease biofouling of bio-Ag0/PES nanocomposite membranes, *Water Res.*, 46 (2012) 2077-2087.
- [23] H. Lee, N.F. Scherer, P.B. Messersmith, Single-molecule mechanics of mussel adhesion, *Proc. Natl. Acad. Sci. U. S. A.*, 103 (2006) 12999-13003.
- [24] R.L. Hartman, H.S. Fogler, Understanding the dissolution of zeolites, *Langmuir*, 23 (2007) 5477-5484.
- [25] M. Herzberg, S. Kang, M. Elimelech, Role of Extracellular Polymeric Substances (EPS) in Biofouling of Reverse Osmosis Membranes, *Environmental Science & Technology*, 43 (2009) 4393-4398.
- [26] C.Y. Tang, Y.-N. Kwon, J.O. Leckie, Effect of membrane chemistry and coating layer on physiochemical properties of thin film composite polyamide RO and NF membranes: II. Membrane physiochemical properties and their dependence on polyamide and coating layers, *Desalination*, 242 (2009) 168-182.
- [27] B.D. McCloskey, H.B. Park, H. Ju, B.W. Rowe, D.J. Miller, B.J. Chun, K. Kin, B.D. Freeman, Influence of polydopamine deposition conditions on pure water flux and foulant adhesion resistance of reverse osmosis, ultrafiltration, and microfiltration membranes, *Polymer*, 51 (2010) 3472-3485.
- [28] H.L. Yang, J.C.T. Lin, C. Huang, Application of nanosilver surface modification to RO membrane and spacer for mitigating biofouling in seawater desalination, *Water Res.*, 43 (2009) 3777-3786.
- [29] 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, *Journal of Materials Chemistry B*, 2 (2014) 1724-1732.