Dual layer hollow fiber PVDF ultra-filtration membranes containing Ag nano-particle loaded zeolite with longer term anti-bacterial capacity in salt water

Huyan Shi, Lixin Xue, Ailin Gao and Qingbo Zhou

ABSTRACT

Dual layer polyvinylidene fluoride (PVDF), antibacterial, hollow fiber, ultra-filtration composite membranes with antibacterial particles (silver (Ag) nano-particles loaded zeolite (Z-Ag)) in the outer layer were prepared with high water flux and desired pore sizes. The amounts of Ag⁺ released from the composite membranes, freshly made and stored in water and salt solution, were measured. The result indicated that dual layer PVDF antibacterial hollow fiber containing Z-Ag (M-1-Ag) still possessed the ability of continuous release of Ag⁺ even after exposure to water with high ionic content, showing a longer term resistance to bacterial adhesion and antibacterial activity than membrane doped with Z-Ag⁺ (M-1). Results from an anti-adhesion and bacteria killing test with Escherichia coli supported that the antibacterial efficiency of dual hollow fiber PVDF membranes with Z-Ag was much higher than those with Z-Ag⁺ after long time storage in water or exposure to phosphate buffered saline (PBS) solution. This novel hollow fiber membrane may find applications in constructing sea water pretreatment devices with long term antifouling capability for the desalination processes.

Key words | Ag nano-particle, anti-fouling, desalination process, dual layer hollow fiber membrane, zeolite

INTRODUCTION

Ultra-filtration (UF) membranes are currently used in the pretreatment processes of sea water desalination plants to protect the central desalting components. Building superior and stable long term properties, especially longer term antifouling capacity in salt water, into UF membranes is critical for the successful operation of the whole desalination process. As one of the most popular polymeric membrane materials, polyvinylidene fluoride (PVDF) has attracted much interests due to its excellent properties, such as thermal stability and chemical resistances (Wang et al. 1999; Liu et al. 2011; Edwie et al. 2012). Although the PVDF membranes were widely used (Feng et al. 2008; Wang et al. 2012), membrane fouling and contamination by microorganisms, especially bacteria during the water treatment, are still outstanding issues for researchers and fabricators.

There were methods reported in papers to endow the PVDF membranes with antibacterial properties, such as grafting 2-hydroxyethyl methacrylate and 2-(dimethylamino)ethyl methacrylate brushes and quaternization (Sui et al. 2012), but the reaction steps were complicated. Addition of 4% TiO₂ into PVDF/sulfonated polyethersulfone blend membrane could improve antibacterial results, but it needed the help of UV irradiation (Rahimpour et al. 2011). Furthermore, the research papers of the antibacterial PVDF membranes reported were mostly about flat sheet or single layer hollow fiber membranes.

As is known, silver ions and silver nano-particles own powerful antibacterial activity and broad antibacterial spectrum, and they have been researched extensively (Zhang et al. 2008; Liao et al. 2011; Nagy et al. 2011). The antibacterial mechanism of silver ions and silver nano-particles include the steps of bounding to DNA molecules, disabling their replication abilities (Brunetto & Fromm 2008; Zhang et al. 2008; Shameli et al. 2011), interfering with electron transport (Brunetto & Fromm 2008; Shameli et al. 2011), interacting with thiol groups in protein, and inducing inactivation of bacterial proteins (Zhang et al. 2008). However, silver nano-particles like to aggregate then lead to a non-uniform distribution in samples. Zeolites were proved to be a useful support for antibacterial silver because of their excellent ionic binding capacity and the ability to release Ag⁺ in a
period of time (Liao et al. 2011; Nagy et al. 2011). Antibi-
terial PVD flat UF membrane with Ag\textsuperscript{+} exchanged NaY zeolite particles (Liao et al. 2011), still possessed antibacterial properties against Escherichia coli after kept in distilled water for 60 days.

To develop hollow fiber membranes for the sea water pretreatment steps in the sea water desalination process, it is critical to reduce the amount of the Ag\textsuperscript{+}–zeolite used, and extend the longer term anti-fouling performance in water with high ionic strength.

Dual layer hollow fiber membranes with a reduced amount of silver used (Shi et al. 2013, 2014) and excellent mechanical properties, containing Ag\textsuperscript{+} loaded zeolite, had exhibited excellent antibacterial ability, but it was observed that Ag\textsuperscript{+} tended to be lost in a short period of time in water containing a high concentration of salts, resulting in the loss of antibacterial activity. In this paper, to extend the antibacterial activity of hollow fiber membranes and slow down the release of Ag\textsuperscript{+} ions in sea water, Ag nano-particle loaded zeolites were prepared and incorporated into the outer layer of dual layer PVDF hollow fiber membranes. The Ag\textsuperscript{+} releasing profiles and antibacterial property of the novel polymer-matrix composite membranes were studied and compared with dual layer hollow fiber membranes containing Ag\textsuperscript{+} loaded zeolite under various conditions. With longer term anti-bacterial capacity in salt water, this novel hollow fiber membrane may find applications in constructing sea water pretreatment devices with long term antifouling capability for the desalination processes.

**MATERIALS AND METHODS**

**Materials**

PVD (301F) was supplied by CMDIC Xiamen Import & Export Co. Ltd, China. NaY zeolite were provided by Anhui Mingmei MinChem Co. Ltd, China. Nutrient agar and *E. coli* (ATCC 25922) were purchased from Guangdong Huankai Microbial Science & Technology Co. Ltd, China. 4',6-diamidino-2-phenylindole (DAPI) was supplied by Beijing Solarbio Science & Technology Co. Ltd, China. Other chemicals were analytical reagents and used as received. Deionized water was used during the whole experiment.

**Preparation of Ag\textsuperscript{+} loaded zeolite (Z-Ag\textsuperscript{+}) and Ag nano-particle loaded zeolite (Z-Ag)**

The Z-Ag\textsuperscript{+} was prepared by ion exchange reaction. 5 g NaY zeolite particles were added into silver nitrate (AgNO\textsubscript{3}) solution (50 mL, 0.1 M) and then the solution was stirred continuously at 60°C for 4 h in the dark. After that, the particles were centrifuged with deionized water until Ag\textsuperscript{+} in the supernatant fluid could not be detected by hydrochloric acid (HCl) titration. Then the prepared Z-Ag\textsuperscript{+} was dried in a vacuum oven at 60°C for 24 h before any characterization tests.

The Z-Ag was prepared as follows: 3 g Z-Ag\textsuperscript{+} particles were added into 100 mL deionized water and sonicated for 30 min, then stirred continuously at 60°C for 1 h, and 0.1 M sodium hydroxide (NaOH) aqueous solution was added under vigorous stirring to adjust the pH to 9.0. After stirring for another 1 h, formaldehyde solution (20 mL, 0.4 M) was added into the mixed solution. The reduction reaction was permitted to proceed for 2 h under stirring. The Z-Ag was collected by centrifugation, washed with deionized water, and dried at 60°C in a vacuum oven.

**Preparation of hollow fiber membranes**

The PVDF composite hollow fibers were fabricated through a dry-jet wet-spinning process via co-extruding by a tri-orifice spinneret. The spinning solutions were prepared as follows: for the outer dope solution, the pre-calculated quantity of PVDF powders, polyvinylpyrrolidone (PVP K-30) and Z-Ag were added in N,N-dimethyl acetamide (DMAC), and stirred at 60°C for 24 h. For the inner dope solution, PVDF and PVP were mixed and stirred in DMAC at 60°C for 24 h. At last, the dope solutions were degassed at room temperature under negative pressure. The spinneret used and the spinning parameters were described in detail in our previous work (Shi et al. 2014). The compositions of the spinning solutions are listed in Table 1.

**Table 1** | The compositions (wt%) of the dual layer hollow fiber membranes

<table>
<thead>
<tr>
<th>Sample</th>
<th>Compositions (wt%)</th>
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<tbody>
<tr>
<td></td>
<td>PVDF</td>
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<tr>
<td>M-1</td>
<td></td>
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<tr>
<td>Inner layer</td>
<td>15</td>
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<tr>
<td>Outer layer</td>
<td>15</td>
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<tr>
<td>M-1-Ag</td>
<td></td>
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<tr>
<td>Inner layer</td>
<td>15</td>
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<td>Outer layer</td>
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Characterization of Z-Ag⁺ and Z-Ag

The morphology of the zeolites, Z-Ag⁺ and Z-Ag were studied by transmission electron microscopy (TEM, JEOL2100 HR, Japan). Their crystalline structures were characterized by powdery X-ray diffraction (XRD, Bruker AXS D8 Advance, Germany). The silver content was measured by the inductively coupled plasma-atomic emission spectrometer (ICP-AES, Perkin-Elmer Optima 2100, USA).

Characterization of PVDF hollow fiber membranes

Field emission scanning electron microscope (FE-SEM, Hitachi S-4800, Japan) was used to observe the morphologies of the PVDF antibacterial hollow fiber membranes; the accelerating voltage and current were 4.0 kV and 7 μA, respectively.

The pure water flux was measured by a self-made dead-end filtration cell and the deionized water was used as the filtration solution. For each membrane module, the deionized water was forced to permeate from the outside to the inside of the hollow fiber membranes. Before the tests, the membranes were all pre-compressed at 0.12 MPa for 30 min, then the pure water flux was tested at 0.1 MPa. Three modules were tested for each membrane sample, and the average pure water flux was calculated.

The mean pore sizes of the membranes were determined by a liquid–liquid porometer (LLP-1200A, Porous Materials Inc., USA). The experimental procedure can be seen elsewhere (Shi et al. 2013) in detail.

Measurement of Ag⁺ release profile

Each hollow fiber membrane sample with a certain length was immersed in 80 mL of phosphate buffered saline (PBS) solution (containing 4.70 g of anhydrous sodium dihydrogen phosphate and 8.66 g of anhydrous disodium hydrogen phosphate in 1 L of de-ionized water, adjusted to pH 7.0) and shaken at 100 rpm at 37°C. The contents of the Ag⁺ in the solutions were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES, Perkin-Elmer Optima 2100, USA) for five cycles, and every cycle was 24 h. After every sampling, the solutions were replaced with fresh PBS solution to accelerate the release of silver ions. To investigate the long term stability of the antibacterial ability, membrane samples were stored in water for 3 months (M-1(3) and M-1-Ag(3) denote M-1 and M-1-Ag kept in water for 3 months, respectively) were used to perform the same tests. PBS solution was used for the leaching tests for two reasons: (1) the antibacterial tests below were carried out in the PBS solution containing E. coli; (2) research reports showed that the Ag⁺ release can be increased due to the presence of other cations that can exchange the Ag⁺ sites (Lalueza et al. 2011; Boschetto et al. 2012).

Deionized water and 3 wt% NaCl solution, two extreme solutions, were also applied to observe the release of Ag⁺ from the antibacterial membranes.

Determination of the antibacterial activity

Bacteria killing test

The antibacterial activities were tested using E. coli. The E. coli were cultivated in 100 mL of a 3.1wt% yeast–dextrose broth at 37°C with shaking at 100 rpm for 24 h, then harvested through centrifugation and re-suspended in PBS at a concentration of about 10⁶ cells/mL (Yao et al. 2008; Sui et al. 2012).

To test the antibacterial property of the membranes, all the samples were sterilized and presoaked in PBS for 1 h prior to immersing in E. coli suspension. Four pieces of 5 cm hollow fiber membranes were immersed in 50 mL above bacterial suspension at 37°C and shaken at 100 rpm. After shaking for 1 h, 0.1 mL of the suspension was pipetted out and serially diluted, then 0.1 mL of the diluted solution was spread onto triplicate solid agar plates and incubated at 37°C for 24 h. Viable bacterial colonies were counted and expressed as the mean colony forming units per milliliter after multiplying the dilution factor. Another sterile Erlenmeyer flask containing only bacterial suspension was used as the blank group and dealt with the same procedure. The antibacterial property was tested three times and the average value was reported.

Anti-adhesion test

Similar to the bacteria killing test, another four pieces of 5 cm membranes to evaluate the anti-adhesion behavior were handled in the same way, but shaken at 100 rpm for three days. Then the membranes were all taken out, washed with PBS gently and immersed in 5 vol.% glutaraldehyde in PBS for 5 h at 4°C. At last, the membranes were taken out and washed with PBS again. Then they were contacted with 10 μg/mL DAPI solution, stained for 30 min in the dark, and washed gently twice with PBS solution. Confocal laser scanning microscope (Leica TCS SP5II,
RESULTS AND DISCUSSION

Morphology and crystalline structure of zeolite

After the ion exchange reaction and reduction reaction, the Ag loaded zeolite powders were characterized with TEM. As shown in Figure 1, there were many Ag nano-particles that could be identified in Z-Ag, but none in zeolite and Z-Ag⁺. The size of the Ag nano-particles synthesized in Z-Ag was about 10–20 nm. There were characteristic peaks of zeolite at 2θ = 15.7°, 23.7°, 27.2°, and 31.5° in the XRD patterns of the powders (Tayade et al. 2006; Liao et al. 2011) in Figure 2. No new peak was found after ion exchange reaction, but the intensity of the peaks changed slightly because of the replacement of Na⁺ by Ag⁺ (Tayade et al. 2008; Zhang et al. 2008). According to the ICP-AES results, synthesized Z-Ag⁺ and Z-Ag contained about 6.40 wt% and 6.38 wt% of silver, respectively. Typical peaks of metallic silver particles at 2θ = 38.1°, 44.3°, 64.4°, 77.5° (Zhang et al. 2012) could be found only in Z-Ag, implying Ag⁺ has been reduced to the state of Ag.

Structures and performance of PVDF dual layered hollow fiber membranes

No de-lamination was observed between the inner and outer layer, according to the cross-sectional images for the PVDF hollow fiber membranes displayed in Figure 3. The cross sections contained a thin outer layer with shorter finger-like pores and a well-developed thicker inner layer with longer finger-like pores. Ag-nano-particle loaded zeolite could be identified only in the outer layer of the of the dual layered hollow fiber membrane.

The formation of the structures could be attributed to the rapid precipitation that occurred at both the inner and outer walls (Deshmukh & Li 1998; Yeow et al. 2004; Fontanano et al. 2006). In general, the fast coagulation rate resulted in a formation of large finger-like macro-voids (Deshmukh & Li 1998; Fontanano et al. 2006). The PVDF membranes prepared using DMAc as solvent usually possessed a large finger-like structure (Deshmukh & Li 1998; Yeow et al. 2004). Furthermore, the addition of 7% PVP, a hydrophilic polymer, in the outer dope solutions also accelerated the precipitation rate, and contributed to a formation of a large finger-like porous structure near the outer and the inner skin surface (Deshmukh & Li 1998). According to the enlarged images in Figure 3, there was only zeolite distributed in the outer layer of the membranes. It is also shown that the uses of zeolite as a carrier effectively avoided potential aggregation of Ag nano-particles and facilitated their even distribution in the outer layer of the cross section.

Both samples owned porous outer and inner surfaces, which facilitated water permeation through the membranes. The water flux and mean pore size of M-1 and M-1-Ag hollow fiber membranes are both in the range of 520–530 L/m².h, and 0.23–0.30 nm, respectively, which may be further adjusted according to needs (Figure 4). The outer surfaces were much denser than the inner surfaces because of the stretching caused by gravity, and the dope solutions became more concentrated as the solvent volatilized during the air gap.

Ag⁺ release profiles of the dual layered hollow fiber membranes

The release of Ag⁺ into PBS solution from the dual layer hollow fiber membranes freshly made and stored in water...
for 3 months at 37 °C are shown in Figure 5. From the left diagram, freshly prepared dual layer hollow fiber M-1 with Ag⁺ loaded zeolite, Z-Ag⁺, quickly release theirs in the first three cycles while freshly prepared M-1-Ag with Ag nano-particle loaded zeolite, Z-Ag, showed a slower releasing profile with more release in the last two cycles. From the right diagram, after being kept in water for 3 months, membrane M-1 containing Z-Ag⁺ nearly released no Ag⁺ after two cycles, while the hollow fiber M-1-Ag with Z-Ag could still release a large amount of Ag⁺.

In Z-Ag⁺, Ag⁺ balanced structural charges and was easily released when the Na⁺ cations in another cation took its place. In the experiments, the rapid release of Ag⁺ in Z-Ag⁺ could be well accelerated in two conditions: (1) at 37 °C and 100 rpm, this would provide the membranes enough opportunities to contact with other cations; (2) with Na⁺ as high as 3.7 × 10³ ppm in PBS solution, this would accelerate the exchange of Ag⁺. However, the situation was different for Z-Ag which contained metallic Ag nano-particle, which required a much slower dissolution–oxidation process to generate Ag⁺ (Lalueza et al. 2011). Therefore, Ag⁺ released from M-1-Ag was much lower.

To assess the impact of salts in sea water, the Ag⁺ releasing profiles of the dual layered fiber membranes were compared in deionized water and 3 wt% NaCl solution. As shown in Figure 6 left, in de-ionized water, the Ag⁺ released for both M-1 and M-1-Ag were both very low when the ionic content was low. In Figure 6 right, 3 wt% NaCl obviously accelerated the rates of Ag⁺ release for both samples. However, M-1-Ag showed much longer term release than M-1, showing detectable Ag⁺ release in the four to five cycles due to the slower releasing profile of Z-Ag.

As discussed above, the uses of zeolite as a carrier not only had effectively avoided potential aggregation of Ag nano-particles, but also had facilitated their long term stability in the membrane surfaces. In the experiments, the silver content on the membrane surface was also compared to PVDF-Ag, as prepared according to Damm et al. 2007, before and after ultrasonic for 4 h at a power of 500 W. As shown in Figure 7, the initial silver content on M-1-Ag was 1.09 wt%, and this value changed little to 1.05 wt% after ultrasonic treatment, but the silver content for PVDF-Ag dropped dramatically, from 0.22 wt% to 0.02 wt%, so that nearly no silver was detected. Obviously,
the silver on the surface of M-1-Ag with zeolite carrier was much more stable.

**Antibacterial activity of the membranes**

In addition to pure water flux, another important parameter for antibacterial hollow fiber membranes is antibacterial efficiency. As shown in Figure 8, sample M-1 tended to lose part or all of its anti-bacterial activity after being kept in water for 3 months or soaked with 80 mL of PBS solution in three depleting cycles. On the contrary, M-1 retained full antibacterial activity after both treatments. These results agree well with the Ag\(^+\) releasing profile tests shown in Figure 5. It
could be concluded that the M-1-Ag possessed a longer term antibacterial ability, in which silver nano-particles served as a longer term reservoir for the release of silver ions. In contact with water and dissolved oxygen, metallic Ag nano-particle could slowly release silver ions (Damm et al. 2007). In certain cases, the dissolved oxygen would be reduced by the oxidation of silver and generated \( \text{O}_2^- \), which also played roles in the antibacterial activities.

As is known, the nature of PVDF was hydrophobic, which was favorable to the adhesion of bacteria (Mansouri et al. 2010). Many researchers reported methods to improve the hydrophilicity of PVDF membranes (Chen et al. 2007; Chang et al. 2009) and reduce fouling. Chemical modification was difficult due to inertness of PVDF, while adding the hydrophilic inorganic particles may enhance its hydrophilicity but not antibacterial property (Liu et al. 2011). The addition of Z-Ag\(^+\) and Z-Ag not only endowed the membranes with antibacterial property, but also increased the ability to resist the adhesion of bacteria. The silver ions, released into the surroundings from Z-Ag\(^+\) and Z-Ag, could deactivate cellular enzymes and DNA by reacting with electron-donating groups such as thiol group and generated reactive oxygen species (Choi et al. 2008).
gain a better perspective on the situations of the membranes adhered bacteria, fluorescence photomicrographs were taken. As shown in Figure 9, the black background stood for the membrane surface without bacteria, while blue dots indicated the *E. coli* adhered to the surfaces. More *E. coli* adsorbed on the surface of the PVDF membrane without Z-Ag⁺ or Z-Ag, but the bacteria adhered to M-1 and M-1-Ag were reduced dramatically, which meant that they owned a much better resistance to the adhesion of bacteria. The bacteria on the surfaces of M-1 and M-1-Ag differed little, indicating they had the similar ability to resist the adhesion of bacteria. However, after being immersed in water for 3 months, there were more bacteria adhered to M-1(3) than M-1, which indicated that M-1(3) became much easier to be adhered to by bacteria, and also meant it almost lost the antibacterial property. Meanwhile, M-1-Ag(3) had much less bacteria on its surface than M-1(3), and its antibacterial ability was still comparable with M-1-Ag, which was caused by the controlled-release of Ag⁺ in the membranes.

**CONCLUSIONS**

Dual layered PVDF hollow fiber UF membranes with Ag nano-particle loaded zeolite in the outer layer have been successfully prepared to have high water flux and desired pore distribution. The uses of zeolite as carrier not only had avoided potential aggregation of Ag nano-particles, but also facilitated their long term stability in the membrane surfaces. The longer term control release of Ag⁺ ions from these membranes has provided longer term antibacterial ability, especially in water with high ionic content such as sea water. This particular method renders the possibility of uniformly distributing and fixing a limited amount of silver nano-particles in the outer surface of PVDF UF membranes for longer term anti-fouling effects in sea water, providing additional venues to extend the service life of the current desalination devices.

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