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Silver-Enhanced Block Copolymer Membranes with Biocidal Activity

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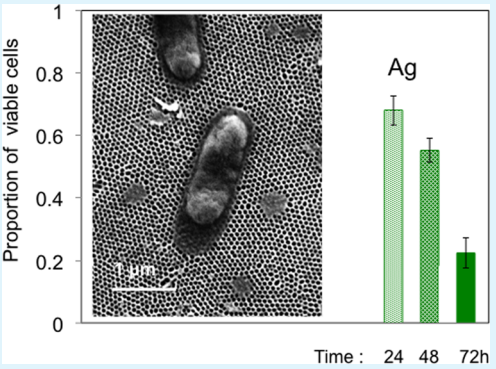
ABSTRACT: Silver nanoparticles were deposited on the surface and pore walls of block copolymer membranes with highly ordered pore structure. Pyridine blocks constitute the pore surfaces, complexing silver ions and promoting a homogeneous distribution. Nanoparticles were then formed by reduction with sodium borohydride. The morphology varied with the preparation conditions (pH and silver ion concentration), as confirmed by field emission scanning and transmission electron microscopy. Silver has a strong biocide activity, which for membranes can bring the advantage of minimizing the growth of bacteria and formation of biofilm. The membranes with nanoparticles prepared under different pH values and ion concentrations were incubated with *Pseudomonas aeruginosa* and compared with the control. The strongest biocidal activity was achieved with membranes containing membranes prepared under pH 9. Under these conditions, the best distribution with small particle size was observed by microscopy.



KEYWORDS: *polystyrene, poly(4-vinylpyridine), block copolymer membranes, pH responsive, biofouling, silver nanoparticles*

INTRODUCTION

The combination of block copolymers self-assembly and phase inversion[1−7] has been demonstrated as a successful method to manufacture asymmetric membranes with high porosity, high water flux and very sharp pore size distribution. Diblock copolymers have a rich variety of morphologies, ranging from disperse spheres, cylinders and gyroids to lamellae in equilibrium, which have been widely investigated in bulk[8] and thin films.[9] Block copolymers have been explored for membranes as dense films[10] or as porous materials, prepared for instance by selective etching[11] one of the blocks. We have been manufacturing porous block copolymer membranes by first promoting micelle formation and assembly in solution, followed by solvent−nonsolvent exchange and phase separation induced by immersion in water. The mechanism of pore formation has been proposed[1] and further discussed[2−4,12] in previous reports of our group. Relevant for potential applications are the membranes' excellent properties. Water fluxes far above 1000 L m^{-2} h^{-1} bar^{-1} could be achieved with selectivity enough to separate proteins with very close molecular weights like bovine albumin (66 kDa) and hemoglobulin (65 kDa).[7] This combination of flux/selectivity was not possible before with polymeric phase inversion membranes and makes the new class of membranes unique with the perspective of application in many fields varying from biomedical devices to separation of valuable products in aqueous media. Having a biocide activity can be advantageous in different applications for which bacteria growth has to be avoided. In water-based separations processes, biofouling is a common issue, which strongly affects the performance in operation. Even membranes with exceptional initial permeation characteristics can experience dramatic flux decrease after short time of operation due to surface adhesion of micro-organisms, forming biofilms, which partially block the pores. The main goal of this work is to propose a strategy to minimize biofouling to be specifically applied to the new class of block copolymer membranes.

Biofouling can have serious consequences in membrane operation, as well as in other fields varying from medicine, food industry to marine transportation.[13−24] The formation of biofilms occurs in different steps. The initial step usually consists of bacteria adhesion on surfaces and is followed by microbial growth.[8,16] Micro-organisms embedded in biofilm produce extracellular polymeric substances, which form the biofilm structure and ensure its cohesion. Biofilm formation is a serious issue in water treatment, being evident in pipe distribution networks. Membrane systems for water treatment have a growing market, but also in this field biofouling or biofilm formation on the membrane surface, with consequent reduction of operation performance, is a major problem that is challenging to control. Aggressive cleaning procedures are normally applied to recover membrane performance. Reducing biofilm formation has better chances of success if the first steps of micro-organism adhesion can be avoided. Biocides can be an effective agent for that.

Silver is known as biocide in different fields. It has been widely used in traditional medicine, for treatment of wounds before the advent of modern antibiotics, in water sterilization aboard space stations and as a disinfection additive for food.[8−21] The silver biocide activity is in part thought to

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depend on silver ions, which are released from silver particles and strongly bind to electron donor groups in biomolecules, causing defects in the cell walls, forming complexes with proteins and DNA, finally causing death of bacteria or hindering the reproduction.[17−20] The ion positive charge is reported to contribute to the attraction between ions and negative charged cells. But silver nanoparticles even partially negatively charged can accumulate and cause irregular pits in bacterial membranes. Formation of free radicals and radical-induced membrane damage has been also associated with the biocide mechanism.[19] How effectively the silver ion is released depends on the particles morphology. Larger surface area facilitates the release. Well-dispersed nanoparticles are expected to be more effective than larger particles.[21−24]

Silver has been reported as a biocide agent for membranes based on polylactic acid,[21] polyamide,[22] polysulfone[23] and poly(vinylidene fluoride).[24] Immobilization is required to avoid excessive leaching out of silver during operation. Cao et al.[23] immobilized silver ions on polysufone (PES) membranes, by a multistep process, first sulfonating PES and blending it to the nonmodified polymer to cast membranes. Silver ions were added to interact with the sulfonic group on the membrane surface and reduced to elementary silver particles. Shiffmann et al.[25] prepared the first silver nanoparticles capped with poly(ethylene imine). Polysulfone electrospun nanofibers were treated with oxygen plasma to introduce functional groups, like −COOH to the surface and the PEI-capped particles were then attached to the functional groups. Polysulfone-Ag nanoparticles mats displayed high bioactivity against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus anthracis*.[25,26] Although other polymeric membranes with silver nanoparticles have been reported before[27,28] with enhanced antibacterial properties, the modification of an isoporous PS-*b*-P4VP block copolymer membrane with antibiocidal characteristics has not been addressed yet. The method we used is analogous to what we applied before to add catalytic activities to membranes, by incorporation of other metallic particles.[29−31] Here we describe how the incorporation of silver affects the biocide capability of the block copolymer membranes. We take advantage of the complex formation between silver ions and pyridine,[32] which constitute one of the copolymer blocks, to ensure homogeneous and stable metal distribution.

EXPERIMENTAL SECTION

Materials. Polystyrene-*b*-poly(4-vinylpyridine) block copolymer P10900-S4VP (PS-*b*-P4VP 188 000-*b*-64 000 g/mol) was purchased from Polymer Source, Inc., Canada. Dimethylformamide (DMF), 1,4-dioxane and acetone was supplied by Fisher Scientific. Silver nitrate ($AgN0_3$) (99%), sodium borohydride ($NaBH_4$), sodium hydroxide pellets (NaOH) and hydrochloric acid (HCl) 37% were purchased from Sigma-Aldrich.

Membrane Preparation. Membranes were prepared with the block copolymer polystyrene-*b*-poly(4-vinylpyridine) (PS-*b*-P4VP) (molecular weight 188 000-*b*-64 000 g/mol). A solution with 18 wt % block copolymer, 24 wt % DMF, 42 wt % dioxane (42%) and 16% acetone was stirred at room temperature for 24 h and cast using a doctoral blade with a gap of 200 μm on a glass plate or on a polyester nonwoven fabric. The solvent was evaporated during 10 s and the film was precipitated in deionized water. Finally, the membranes were dried at room temperature. Pores are formed by a combination of micelle assembly and phase separation caused by the solvent−water exchange, as discussed before[3,4] and a regular morphology, as shown in Figure 1a, is obtained.

The silver nanoparticles were generated and incorporated by first immersing the prepared membrane into 1.0 mM $AgNO_3$ and adjusting the pH to the desired value by adding drops of 0.1 N NaOH and/or 0.1 N HCl solutions. The membrane was rinsed with deionized water several times and then immersed in 2.0 mM $NaBH_4$. In a series of experiments, the pH was set as 2.1, 4 and 9, keeping $AgNO_3$ 1.0 mM. In a second series, the pH was kept neutral and the $AgNO_3$ concentration was varied from 1.0 mM to 0.5 M.

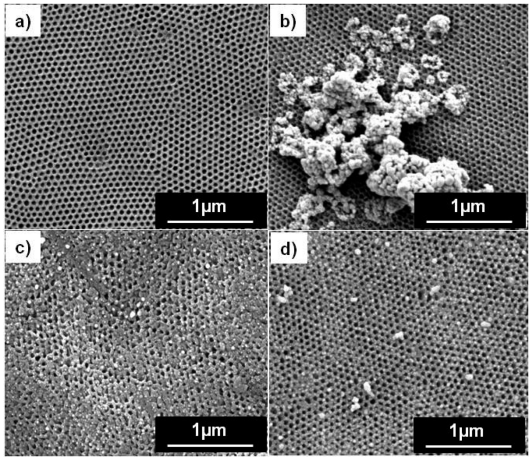

Figure 1. FESEM images of a (a) plain PS-*b*-P4VP membrane and membranes with Ag nanoparticles prepared at (b) pH 2.1, (c) pH 7 and (d) pH 9 with 1.0 mM $AgNO_3$ solution.

Morphological Characterization. The morphology of the membranes was characterized by field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM). The membranes were imaged with a Nova Nano 630 microscope using a voltage of 5 kV. Prior to imaging, the samples were sputtered with platinum using a K575X Emitech sputter coater.

For TEM, the membranes were cut in a LEICA EM UC6 cryo-ultramicrotome after being embedded in a low viscosity resin and imaged in a Titan FEI transmission electron microscope operating at 300 kV.

Bacterial Counting. Cultures of *Pseudomonas aeruginosa* DSM1117 were diluted with 0.85% w/v NaCl to an $OD600_{nm}$ of 0.07. This corresponds to a cell concentration of approximately 1×10^8 cell/mL. The membranes with silver as well as the control membrane were individually immersed into 4 mL of diluted cell suspension, and incubated at 37 °C in an orbital incubator shaker for 24, 48 and 72 h. After incubation, the cell suspension was then aliquot and diluted by 1000-fold with 0.85% w/v NaCl, and then stained with a LIVE/DEAD BacLight bacterial viability kit (Invitrogen, CA) for 10 min at 35 °C prior to flow cytometry on an Accuri C6 (BD Biosciences) instrument. Two milliliters of 0.85% w/v NaCl was added to each of the remaining membranes, and the solutions were ultrasonicated for 3 min by a Q500 sonicator (Qsonica) at 25% amplitude to dislodge the attached bacteria into the suspension. The suspension was then stained with LIVE/DEAD BacLight bacterial viability kit using the above-mentioned protocols.

The leaching out of silver from the membrane was quantified by inductively coupled plasma mass spectrometry (ICP-MS) analysis.

RESULTS AND DISCUSSION

The isoporous membranes were manufactured from block copolymers with pyridine segments exposed to form the pore walls and membrane surface. As early reported,[29] silver ions complex with pyridine. The incorporation of silver could be easily done by directly complexing silver ions and pyridine. This led to an excellent distribution of nanoparticles on the surface and inside the pores, without the need for additional membrane modification. The method was chosen by taking into account previous work we performed on pyridine−metal complexation to induce and stabilize self-assembly (Cu and Co ions) and

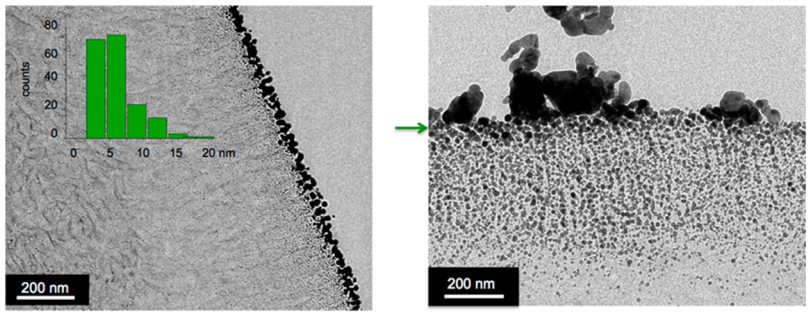

Figure 2. TEM micrographs of PS-*b*-P4VP membrane cross sections with Ag nanoparticles (a) formed at pH 9 with 1.0 mM $AgNO_3$ solution with the corresponding particle size distribution histogram (inset); (b) formed at pH 2.1. Arrows indicate the membrane surface.

impart catalytic activity (Au ion).[1,5] In an analogous approach, we incorporated Ag ions into the pyridine blocks of highly ordered asymmetric copolymer membranes and tested their antibacterial activity.

Surface Characterization. The FESEM images of the pure PS-*b*-P4VP membrane and those with silver nanoparticles at different pH values are compared in Figure 1. Figure 1b shows nanoparticle aggregation on the membrane surface when the deposition process was conducted at pH 2.1. The membranes with 1.0 mM $AgNO_3$ at pH 7 and 9 have uniform distributions of the nanoparticles, unlike the membrane at pH 2.1. The aggregation observed at pH 2.1 is probably due to the protonation of the pyridine group, which takes place at a low pH.[2] When protonated, the membrane acquires a positive charge and the complexation of Ag^+ ions with pyridine is disturbed. A large part of the silver is reduced without being encapsulated by the copolymer segments and is localized as an aggregate on the membrane surface. When $NaBH_4$ is added at a high pH, merging of silver is hindered because the ions are embedded in the copolymer matrix, surrounded by pyridine.

To confirm the fine distribution of Ag in the membranes, transmission electron microscopy (TEM) images were obtained, after sectioning them in an ultramicrotome. The TEM images of PS-*b*-P4VP membranes with Ag nanoparticles deposited at pH 9 and 2.1 are shown in Figure 2. The pores are orthogonally aligned close to the membrane surface and are disordered as it goes deeper. Larger particles are detected on the membrane surface, particularly in the case of pH 2.1, but a large number of smaller Ag nanoparticles (2−7 nm) can be seen on the pore walls in the bulk of the membrane. Smaller particles usually exhibit higher antibacterial activities, mainly due to their larger specific area. Nanoparticles in the range of 1−10 nm attach to the surface of the cell membrane and disturb the functionality of the cell-like permeability and respiration leading to the death of the cell ultimately.[33] The PS-*b*-P4VP membranes are known[2,7] to have a morphology switch around pH 4, closing the pores by protonation of pyridine blocks at lower pH and opening at high pH. The water flux, measured above pH 4−5 without silver, was 1200 L m^{-2} h^{-1} bar^{-1}. After silver incorporation, the flux decreased to 147 L m^{-2} h^{-1} bar^{-1}.The second set of preparation was carried out maintaining a neutral pH, above the switch pH, in which pyridine is not protonated, varying the concentration of $AgNO_3$. The plain membrane was prepared in the same way as before. The membrane was then immersed in 0.5 M $AgNO_3$, at pH 7, rinsed with deionized water to remove uncomplexed silver particles, immersed in 1.0 M $NaBH_4$ and again rinsed with deionized water. The membrane turned brown immediately. Figure 3 shows FESEM micrographs of the membranes. With higher silver concentration, the pores appears to be

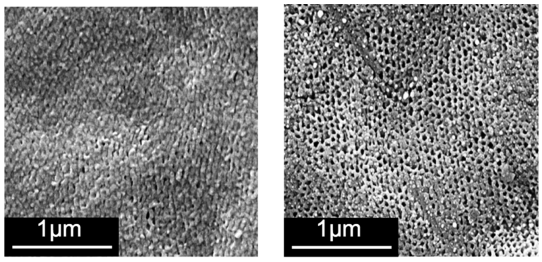

Figure 3. FESEM micrograph of a PS-*b*-P4VP membrane with Ag nanoparticles formed at pH 7 with (a) 0.5 M and (b) 1.0 mM $AgNO_3$.

closed. With 1.0 mM $AgNO_3$, a uniform porosity is still observed after the formation of nanoparticles, which are evenly distributed around the pores.

The TEM image of the membrane bulk with nanoparticles obtained from 1.0 mM $AgNO_3$ solution at pH 7 is shown in Figure 4. The nanoparticles size range is 6−10 nm. Also, in this case, a small particle size with large surface area is expected to promote high biocide activity.

Bacterial Counting. The antibacterial activity of the PS-*b*-P4VP membranes with Ag nanoparticles was evaluated with Gram-negative *Pseudomonas aeruginosa* DSM 1117 bacterium as a model system (Figure 5). The inactivation kinetics on both suspended *P. aeruginosa* cells and those attached on the membranes were observed to be different. For the same time of

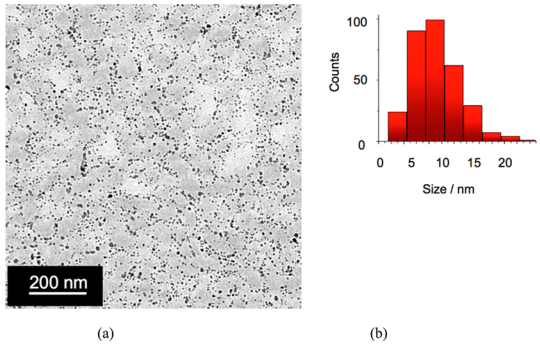

Figure 4. (a) TEM image of PS-*b*-P4VP membrane bulk with Ag nanoparticles prepared from 1.0 mM $AgNO_3$ at pH 7 and (b) the corresponding size distribution histogram.

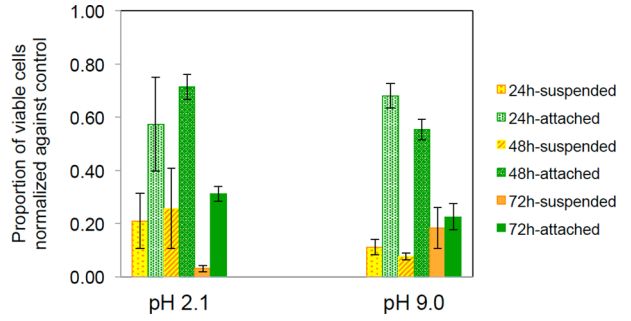

Figure 5. Flow cytometry incubation of *P. aeruginosa*: results for PS-*b*-P4VP membranes with Ag nanoparticles prepared at different pH values from 1.0 mM $AgNO_3$ solutions.

incubation, the inactivation was more effective for suspended cells than for the attached ones forming the biofilms. This behavior is more evident in the first 24 h of incubation and for samples prepared at pH 2.1 even at longer experiments. The proportion of viable cells normalized against the control after 72 h of incubation for membranes prepared at pH 2.1 was lower than 5%, demonstrating high efficiency. The proportion of attached viable cells for the same low pH was around 30%. For pH 9, the proportion of attached viable cells after 72 h was ca. 20% (more effective than pH 2.1). The inactivation of suspended cells was only slightly lower than for attached ones at high pH. The formation of extracellular polymeric substances within the biofilm matrix coating the silver nanoparticles and making the direct contact with the cells can be a likely reason to explain for the lower inactivation of attached biomass. Figures 1 and 2 show that, at high pH, the silver nanoparticles are better and finely dispersed in the membrane pores and, at low pH, silver aggregates are present, loosely attached to the membranes. During incubation, the aggregates might still be effective in deactivating the suspended cells, but the finely distributed silver nanoparticles seen at high pH are more adequate for the inactivation of attached cells.

From thermal gravimetric analysis, an incorporation of 1.74 wt % of Ag related to the total membrane weight could be estimated. Figure 6 gives an idea of how much silver is leached out from the membrane after several hours in water. Seven percent of the incorporated silver is extracted after the first 5 h. After this first period, only 100 μg/L of silver was detected in the permeate, indicating that the remaining silver is strongly connected to the membrane.

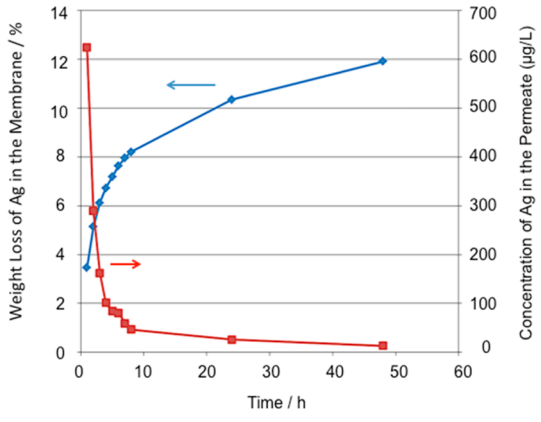

Figure 6. Leaching out of silver from the membrane (pH 9, 1.0 mM $AgNO_3$) measured by ICP-MS.

CONCLUSION

We incorporated for the first time silver nanoparticles into isoporous membranes manufactured from block copolymer self-assembly and phase inversion and investigated the resulting biocidal activity. Well-distributed nanoparticles were obtained by promoting pyridine−silver ion interactions, followed by reaction with a reducing agent, as demonstrated by electron microscopy. Moreover, the Ag nanoparticle deposited membrane prepared at pH 9 with 1.0 mM $AgNO_3$ showed well-distributed membrane morphology, had strong biocidal activity and might be a suitable candidate for practical application. Flow cytometry measurements demonstrated that, after 72 h of incubation of the membrane systems (nanoparticle incorporation at pH 9) with *P. aeruginosa*, the proportion of viable cells compared to the control (in absence of silver nanoparticles) was around 20%. The strong biocidal activity of isoporous block copolymer membrane indicates the possible application in biomedical field requiring a membrane with high water flux and selective removal of components with good antibacterial properties.

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Notes

The authors declare no competing financial interest.

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