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Biofouling performance of silver-based PES ultrafiltration membranes

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ABSTRACT

Polyethersulfone (PES) polymer is widely used in membrane fabrication due to its good mechanical and chemical stability. However, fouling and in particular biofouling leads to reduced efficiency due to the hydrophobic characteristics of the membrane. In this work, we report on the fabrication of antimicrobial ultrafiltration membranes from PES and a range of silver additives. Commercial silver additives in the form of silver zinc zeolites and silver zinc glasses as well as some common organic silver salts were used. The effect of silver additives on membrane biofouling was extensively studied by both static and dynamic methods using bacteria culture. These tests demonstrated the anti-biofouling properties of modified membranes in comparison with control membranes. The issue of silver leaching during membrane fabrication is of critical importance from both sustainability of membranes and environmental aspects. This was studied in detail for PES–silver zinc zeolite membranes. The treatment of membrane with sodium hypochlorite had the most detrimental effect on silver retention in membrane. Leaching of silver was higher in seawater due to the ion exchange.

Keywords: Biofouling; Silver additives; UF membranes; Antimicrobial

1. Introduction

One of the major factors influencing the economic feasibility of membrane separation technologies is fouling. Membrane fouling affects the overall performance of membrane filtration as it decreases permeate flux and reduces membrane selectivity [1,2]. Biofouling is one of the most common type of fouling in ultrafiltration systems which can lead to shortened membrane life, increased operational and maintenance costs, and reduced efficiency [3,4]. Chemical properties of the membrane at the surface, its roughness, pore shape, and pore size distribution are found to be the main factors controlling the biofouling.

Polyethersulfone (PES) is one of the widely used polymers in membrane preparation due to its good chemical, mechanical, and thermal stability. However, its hydrophobicity makes the membrane prone to fouling. There have been many studies to modify PES membranes to mitigate fouling [5–7]. These approaches were studied in detail [4] and can be divided into "anti-adhesion" approaches, based on the

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inhibition of conditioning film and attachment of organisms and "antimicrobial" approaches which kill, disperse, or suppress the activity of attached organisms. The focus of this work is on antimicrobial approach to address the biofouling.

Three broad classes of materials have been used in the development of antimicrobial surfaces: contact-active amphiphilic polymers, microbe-repelling anti-adhesive polymers, and polymeric or inorganic composite materials loaded with slow-releasing biocides such as heavy metals. Among the third class of antimicrobials, silver-based materials are of special interest. The silver ion exhibits broad-spectrum biocidal activity toward many different bacteria by damaging the bacterial cell walls, leading to increased permeability and cell death. The efficacy of silver as a bactericidal agent depends on its bioavailability and its form in the specific environment.

There are some contradictory reports regarding the role of released Ag^+ ions from nanosilver and its bactericidal effect against micro-organism [8]. For example, Navarro et al. [9] reported that nanosilver has an insignificant effect as a bactericidal agent by itself and that the dominating factor in antibacterial effect of silver is the release of silver ions [10,11]. Other researchers reported toxicity effect only for partially oxidised silver nanoparticles [12]. The shape, size, crystallinity, geometry, surface area, surface charge and chemistry significantly change the bactericidal effect of silver nanoparticles [12–14]. One of the main concerns in the application of silver is the risk of affecting the aquatic micro-organism when it is disposed and released [15,16]. Another issue is the agglomeration and precipitation of silver nanoparticles, which has been targeted using surfactant or coating agents during the preparation of silver nanoparticles [17].

Despite the ambiguity around the bactericidal mechanism of silver, silver materials have been used in the modification of membranes for drinking water treatment [18–23]. Chou et al. [24] described the preparation of cellulose acetate hollow fibre membranes with silver using the dry-jet wet spinning technique. To show the durability of antibacterial performance, they record the relative silver residue on the surface and in the bulk material and evaluated activity against *Escherichia coli* and *Staphylococcus aureus*. It was found that even when the silver content reduced to 10% of the initial value on the surface, membrane remained active against bacteria. Elemental silver was used by Hardorfer and Härtel [25] in the preparation of antimicrobial RO membranes. In this case modification was by deposition of elementary silver on the membrane using a galvanic procedure. The membranes modified with silver showed bactericidal effect as assessed by an agar-stamp test, higher permeate flux (up to 45%) and higher permeate recovery. More recently, to improve the stability of membranes hybrid polysulfone ultrafiltration membranes were prepared with Ag–Cu_2O as antimicrobial additive [26] while Huang et al. reported on using dopamine to immobilise silver nanoparticles onto polysulfone membranes to improve the membrane stability [27].

A comprehensive investigation of membrane stability and antimicrobial properties of membranes under filtration conditions and during membrane fabrication, have not been addressed in detail.

In this work, we report on the fabrication of antimicrobial ultrafiltration membranes with addition of silver in the form of commercial antimicrobial additives (Irgaguard B5000, Irgaguard B7000 and Hygate B4000) as well as silver zeolite and organic silver salts. A wide range of experiments were implemented to optimise the fabrication method and to evaluate membranes for their antimicrobial performance. We also looked at the durability of membranes by investigating the release of silver under different environmental conditions involved in membrane cleaning regimes.

To evaluate the antimicrobial activity the quantification of biofilm is necessary and this has been carried out by methods such as staining the biofilm with spectroscopic dyes, e.g. crystal violet stain [28], or measuring the amount of extracellular polymeric substance generated by the micro-organisms in the *biofilm* [29] such as polysaccharides (carbohydrate assay), proteins (protein assay) and other biopolymers. However, for growth and estimation of *biofilm* there is no standard experimental technique available in the literature. In our approach, the biofilm was grown using the model micro-organism *Pseudomonas aeruginosa* on different membranes by a batch/batch-flow method and the quantification was carried out by protein and carbohydrate assay of the biofilm. In addition to static tests for evaluation of antibacterial effect of membranes, a more rigorous test was implemented by filtration of bacteria culture in a cross flow cell for 2–6 d.

2. Materials and methods

2.1. Chemicals

Polyethersulfone (PES, E6020p with MW: 58,000 g/mol) was purchased from BASF and polyvinylpyrrolidone (PVP, 40,000 g/mol) as pore former and hydrophilic additive was supplied by Sigma–Aldrich. N-methyl pyrrolidone (NMP) was used as solvent. Irgaguard B5000, Irgaguard B7000 and Hygate B4000 from BASF as well as silver zeolite and organic silver salts were used as antimicrobial additives. Irgaguard has a special glass/

zeolite design which allows for controlled silver-ion release. Bovine serum albumin (BSA, MW: 68 kDa) supplied by Calbiochem Australia and was used as model protein. Sodium hydroxide solution of 0.2 wt.% was used for cleaning of membranes after filtration.

2.2. Membrane preparation

2.2.1. Control PES membrane

The typical procedure to prepare the membrane is given below:

NMP (76 g) was placed in a three-neck flask equipped with mechanical stirrer. PVP (6 g) was then added to the NMP and the temperature was increased to 60˚C. The mixture was stirred until a homogeneous and clear solution was obtained. To PVP solution, PES (18 g) was added and stirring was continued until PES was completely dissolved and the solution was homogeneous. The solution was left overnight (at 70˚C) in oven to degas. The solution was cast on a warm glass plate with a casting knife (~200 μm) using an automated casting machine at speed of 60 mm/s at room temperature and 50% humidity. After 60 s exposure to air, the membrane was immersed in a 25˚C water bath for 10 min. Membranes were rinsed in hot water for 30 min before the post-treatment. The aim of post-treatment was to: deswell/crosslink the PVP, improve the permeability by controlled leaching of PVP and to achieve the controlled drying to avoid the collapse of membrane pores.

Different methods including solvent exchange, immersion in water and sodium hypochlorite treatment were examined. Among these methods, sodium hypochlorite post-treatment was more efficient (shown by better permeability) in deswelling and crosslinking of PVP. For the controlled drying, the immersion in glycerol was more effective than solvent exchange method.

As a result of rigorous optimisation experiments, the following post-treatment methods for stabilisation of PVP and removing the solvent were adopted;

(1) Immersion in water for two days, water was replaced every half day.
(2) Immersion of membrane in 2,000 ppm NaOCl (pH 11.5) for 30 min repeated four times.
(3) Immersion in glycerol treatment (40 vol.%) for 4 h, followed by drying in room temperature.

Prior to water flux test, the membranes were immersed in ethanol solution (about 50%) for about 10 min.

2.2.2. Silver-PES composite membranes

A wide range of silver-based additives including organic and inorganic silver salts as well as commercial silver additive were examined in this study. List of these additives is given in Table 1 with their description.

Silver additives were sonicated in NMP, after complete dissolution or dispersion, additive/NMP was added to PVP solution and then PES was added as the last step of membrane solution preparation. The solution was then thoroughly mixed using probe sonication. Completely homogeneous solution was kept in 70˚C oven prior to casting for complete degassing.

2.3. Characterisation methods

2.3.1. Morphological studies (FESEM and AFM)

Field emission scanning electron microscopy (FESEM) observations of surfaces and cross-sections of membranes were made using a Hitachi S900 scanning electron microscope. For surface morphology observation, a small piece of sample was mounted on carbon tape and sputter coated with chromium. Freeze-fractured cross-sections of membranes were prepared for observation of the bulk structure.

A Dimension® Icon® AFM with ScanAsyst™ in tapping mode was used to study the surface topography of membranes. Phase and three-dimensional images were obtained.

2.3.2. X-ray photoelectron spectroscopy (XPS)

The surface chemistry of membranes was investigated using X-ray photoelectron spectroscopy (XPS) with a VG Scientific (UK) surface analysis system. C1s at 285.0 eV was used for binding energy reference. The untreated samples were mounted on the copper sample stubs by means of double-sided adhesive tape. Under these conditions, no charging effect were observed for samples.

2.3.3. Estimation of silver/zinc content of membrane

Conventional hotplate digestion method was used to evaluate the silver and zinc content of membranes. The membranes were cut into small pieces (2 mm × 2 mm) and digested with 6 mL concentrated HNO_3 in a Teflon beaker on hot plate for ~3 h (~150˚C). The digest was then diluted with Mill-Q water to 30 mL and analysed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). At least three replicates for each sample were analysed.

Table 1
Characteristics of silver additives

Company code	Appearance	Chemical composition	Supplier
Irgaguard B5000	Fine white powder	Silver-zinc zeolite	Ciba
Hygate 4000	Fine black powder	Elemental silver	Bio-Gate
Irgaguard B7000	Fine white powder	Silver-containing glass	Ciba Specialty Chemicals Corporation, Tarrytown NY
Zinc free silver zeolite	Light-grey granular mini marbles	Silver zeolite	Aldrich
Silver benzoate 99%	White powder (70% fine)	Silver benzoate	Aldrich
Silver p-toluene sulphonate	White 70% fine flakes	Silver p-toluene sulphonate	Aldrich
Silver acetate	White cotton-like powder	Silver acetate	Sigma Aldrich

2.3.4. Leaching studies

A sample of membrane (3 cm × 3 cm) was soaked in 100 ml of Milli-Q water in clean glass bottles. To remove the loosely attached particles, samples were sonicated for 2 min and water was replaced. After a specific period (at least every 3 d), bottles were inverted for 5 times for homogenising the solution and then 5–10 mL samples were taken from the bottles and to these 2 drops of 50/50 (v/v) nitric acid/Milli-Q water were added to stabilize the silver. Silver and/or zinc in water were analysed by a Perkin Elmer Elen 6100 Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) analyser. After each sampling, water was replaced with fresh water. Measurements were carried out for up to 32 d.

2.4. Performance studies

2.4.1. Water flux and fouling studies

Water flux and fouling evaluation of membranes were performed in a dead-end filtration cell (110 ml capacity, membrane area 1.59×10^{-3} m^2). The experiment consists of four main stages: initial water flux at constant pressure, fouling at constant flux, water rinse and chemical cleaning (Fig. 1). In a typical run, membrane was pre-compacted at 125 kPa until a constant flux was obtained. Then the pressure was reduced to 100 kPa and the initial water flux (J_i) was measured (for about 1 h). Then the ultrafiltration experiments were performed with 0.5 wt.% BSA solution (pH was adjusted to 4.9–5.1 by dropwise addition of 0.1 M HCl) at constant pressure of 50 kPa and constant flux of about 41 ± 3 L/m^2 h for 90 min. Constant flux was achieved using a Gilson pump in the permeate line. Then the membrane was rinsed with water for 15 min (1 L/min) and pure water flux was measured (J_{w1}). Chemical cleaning was done by rinsing membrane with a 0.2 wt.% NaOH solution for 15 min and after buffering the cell, water flux was measured again (J_{c1}). The cycle was repeated for three times. Flux recovery and resistance removal for different membranes were estimated. Samples from permeate were withdrawn for analysis by UV spectrometer to estimate the rejection. For modified membranes, samples were taken from permeate/retentate to examine the release of the active component mainly silver.

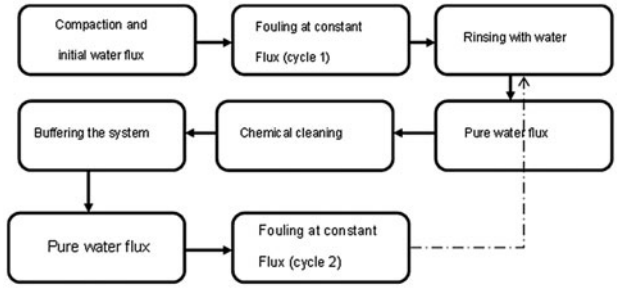

Fig. 1. Flow diagram of the test protocol for fouling and cleaning experiments.

2.4.2. Biofouling studies

Antimicrobial membranes were tested by two types of biofilm tests as follows:

(1) Biofilm was formed on disks in 24-well Titre Plates (TPP) [30]. The 24-well plate format is a "batch culture" method. As each well provides a separate compartment and there is no replacement of the media, the biocidal effect can be increased compared to the open system which is run as a once-through flow culture over the second 24 h period.

(2) A cross-flow filtration test was developed to provide a more realistic evaluation of membranes' performance. The method challenges the selected membrane over 6 d with the same bacterial culture as the short-term assay but under cross-flow filtration conditions. Either one or two membrane samples depending upon the configuration was tested. Performance of the membranes was assessed primarily in terms of the amount of biofilm on the surface measured by carbohydrate and protein assays. Fig. 2 shows the schematic of cross-flow rig (single) and (double) used in these studies.

Due to possible build-up of additive leached from the modified membranes the permeate flow was discarded to waste rather than being returned to the feed circuit.

In the single cross-flow cell, the feed was pumped around a single circuit (1 L/min) with a constant pressure applied on the membrane via back-pressure i.e. valve P1. To compensate for the volume lost from the system via permeate flow and to maintain a level of nutrients, sterile nutrient media (tryptic soy broth; 100 mg/L) was pumped from a carboy (R1) to the in-line reservoir (R2) at a rate to compensate for the volume lost via permeate flow. To maintain the

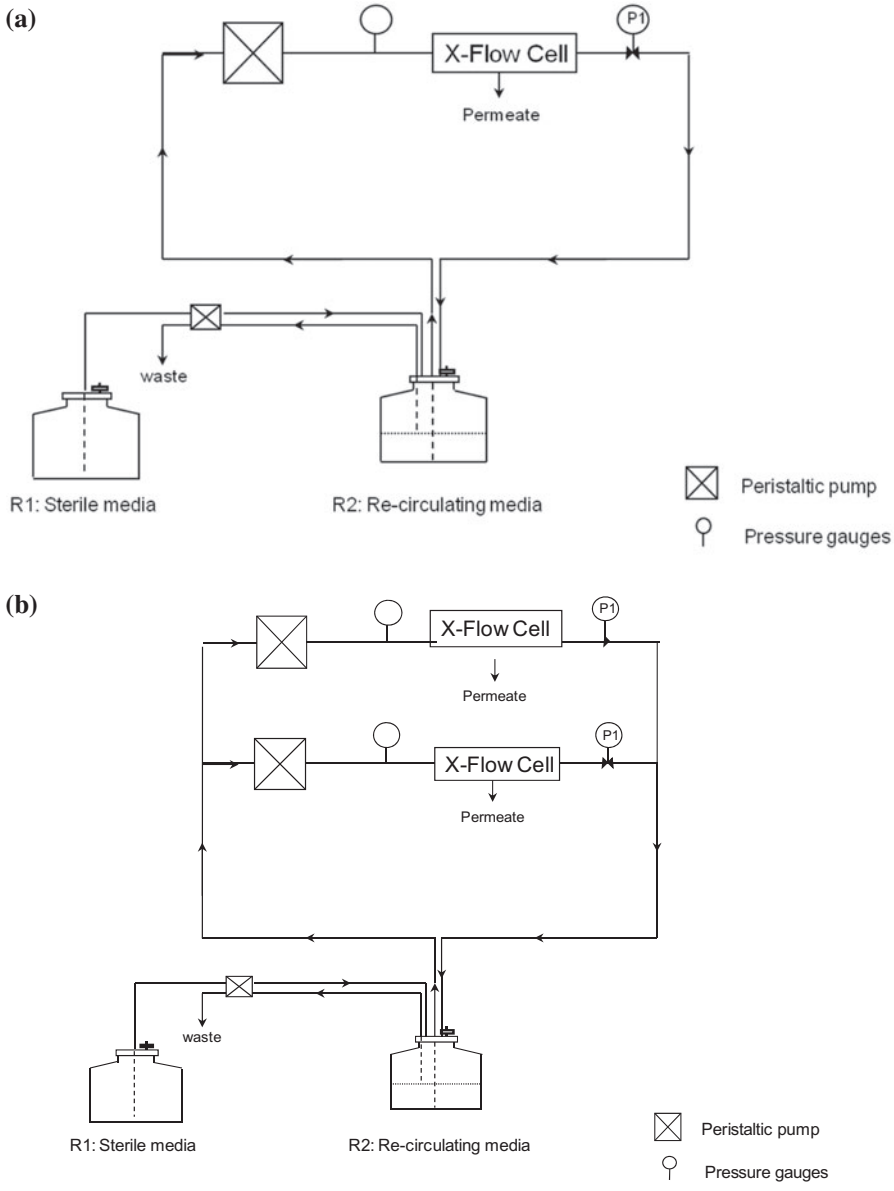

Fig. 2. Cross-flow filtration circuit for (a) single cell and (b) double cell rig.

maximum volume of media in the reservoir carboy (R1) at 5 L a tube was placed in the carboy to transfer excess media to waste via the peristaltic pump used for the nutrient media.

An alternative configuration with two cross-flow cells in parallel allowed two membranes to be tested with the same culture (Fig. 2(b)). In this configuration, the feed flow rate to each cross-flow cell was maintained with separate pumps. The flow rate of fresh media into the system was higher than the rate used for the single cell configuration because of the increased permeate flow from two cells rather than one. In all other respects the conditions were equivalent to the single cell system.

3. Results and discussion

3.1. Membranes with the theoretical level of 140 ppm silver concentration

PES composite membranes with theoretical level of 140 ppm silver from PES and Irgaguard B5000, Irgaguard B7000, Hygate 4000 and zinc-free silver zeolite were prepared. The latter was selected to compare its effectiveness in biofilm reduction to the one for Irgaguard B5000.

The permeability of membranes is given in Table 2 which shows that at this loading of silver additives there was no adverse effect on membrane permeability. Higher permeability of PES-B5000 membrane might be due to the sieving properties of zeolites.

The microstructural studies of membranes showed no significant difference between modified membranes and control. Asymmetric structure which consists of a dense skin layer and porous sublayer with a finger-like structure was observed for all membranes. However, the pores in the micro-void walls and the macro-void under the top layer seem slightly larger for blend membranes, especially for membrane modified with silver–glass additive; Irgaguard B7000 (Fig. 3(c)). The higher porosity might be due to the leaching of additives during the membrane formation; and the role of additives as pore former. The X-ray elemental mapping (not shown) of the membrane skin layer detected Si, Al, and Zn elements in membrane top layer. The signal from Ag was not detected due to low concentration of this element in the matrix.

Table 2
Pure water flux for membranes

Sample	Flux (L/m^2 h)
PES-Irgaguard B7000	410 ± 30
PES-Hygate 4000	476 ± 33
PES-Irgaguard B5000	650 ± 56
PES-silver zeolite	440 ± 23
Control PES	445 ± 15

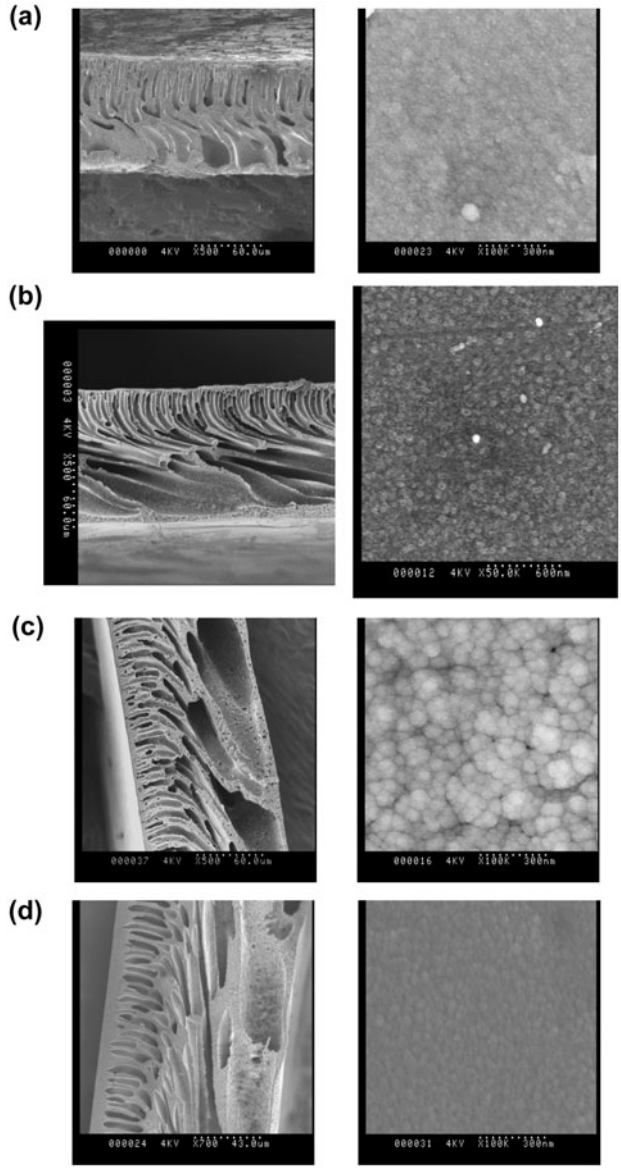

Fig. 3. SEM images of cross-section and surface of (a) control, (b) PES-Irgaguard B5000, (c) PES-Irgaguard B7000 and (d) PES-Hygate 4000 membranes.

Fig. 4 shows the AFM images of PES-Irgaguard B5000 membranes prepared at a scan size of 5 μm × 5 μm. The images show pores and the presence of silver particles on the top surface and also embedded in membrane below the surface. Phase image also distinguish between two phases of PES and silver additive. Smaller particles might be zinc oxide.

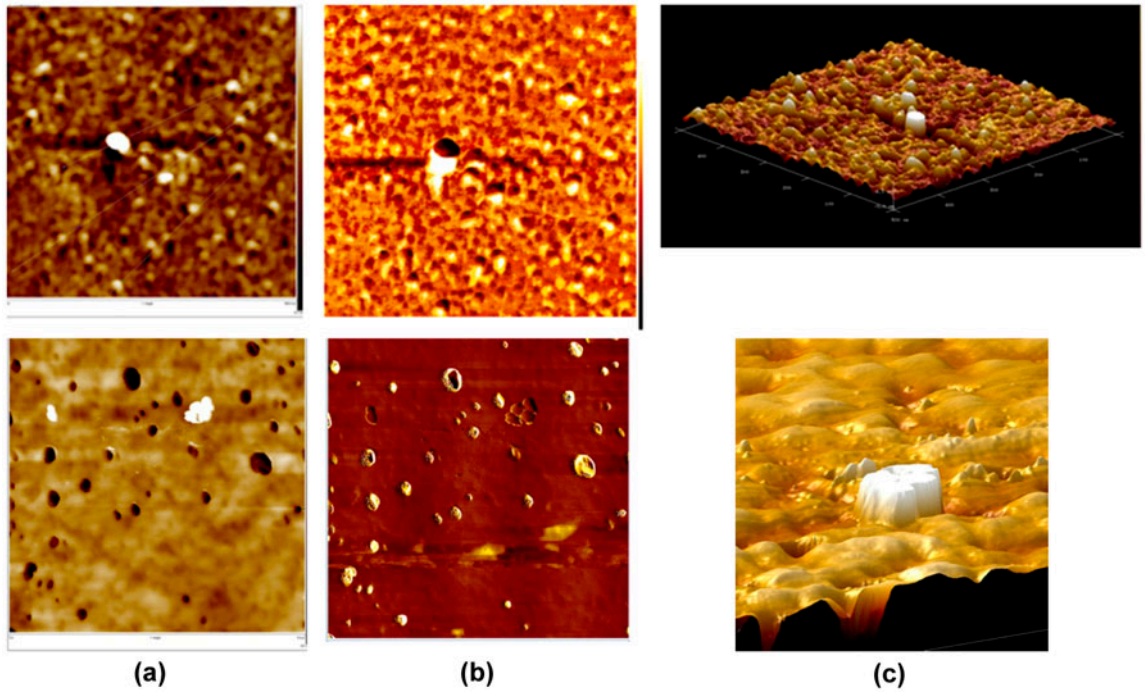

Fig. 4. AFM image of top (top row) and back side (bottom row) of PES-Irgaguard B5000 membrane: (a) topographic, (b) phase and (c) three-dimensional images at two different magnifications. Scan size: 5 μm by 5 μm.

Fouling tests of membranes using BSA as fouling agent were performed at constant flux of 41 ± 3 l/m^2 h. Initial water rinsing achieved a flux recovery of 0.3–0.75 for all membranes. The flux recovery after chemical cleaning is expectedly higher since sodium hydroxide solubilises protein to some extent and aids cleaning through its pH adjusting properties. The recovery after three cycles of cleaning was around 0.6–0.7 for control, and Irgaguard-modified membranes. These results show that the fouling behaviour with BSA is not significantly different for control and membrane modified with silver additives. On the other hand, the resistance removal at the end of the third cycle of chemical cleaning was 60% higher for PES-Irgaguard B5000 membrane as compared to control PES membranes.

During the filtration, cleaning, and pure water flux measurement, samples from permeate and retentate were taken and analysed for the silver content. Samples taken at different cycles from permeate show that the level of silver remains in the range of 0.2–0.4 ppb, as is shown in Table 3.

As previously outlined, one important aspect of modified membranes with silver containing additive is the rate of silver release. There are two significant issues for long-term performance of silver-based membranes: loss of silver due to leaching of silver from the membrane (lifetime) and also the toxicity of silver in water. The leaching of additives from a membrane should be lower than regulatory threshold value for drinking water. There is no specific regulation regarding silver and zinc threshold in EU drinking water guidelines, however in two reports this threshold for silver was assigned at around 100 ppb although no supporting legal documentations were found [15,16]. Furthermore, the leaching of silver should be slow so that the remaining amount in membrane is sufficient

Table 3
Release of silver (ppb) during water flux and cleaning cycles

	Initial water flux measurement	
Permeate	0.356	
Retentate	0.315	
	After water rinsing	After chemical cleaning
Cycle 2		
Retentate	0.157	0.171
Permeate	0.255	0.125
Cycle 3		
Retentate	0.237	0.242
Permeate	0.214	0.312

for the lifespan of the membrane and at the same time adequate for sustained antibacterial effect. The excessive leaching of silver or zinc may be toxic; however, since membranes are used in continuous and not batch (static) operation, this issue should not be too critical.

Other important issue is that the effectiveness of silver-based membranes is influenced by the composition of the water to which they are exposed. For example, the presence of some ions such as chloride, phosphates and sulphides in water can alter the solubility and bioactivity of silver. Bactericidal action of silver ions also increases with increasing temperature and pH which are two important factors in water treatment process control [31]. It was therefore important to investigate the leaching behaviour of membranes in different environments.

Fig. 5 shows the silver release from modified membranes. Comparing the leaching from modified membranes with Irgaguard B5000 and Irgaguard B7000, the initial leaching of silver was higher for the latter, however, the leaching rate decreased with time for this membrane. The low leaching rate might be partly due to the lower level of the residual silver in membranes. Silver zeolite had the highest leaching rate, probably due to its smaller particle size. The average leaching of the last 10 days of test as indication of the constant leaching is given in Table 4. Leaching of silver is much lower for membranes modified with Irgaguard B5000 and Irgaguard B7000 in comparison with the membranes modified with zinc-free silver zeolite, which may be the result of the stabilising effect of zeolite and glass on silver retention in membranes and smaller particle size.

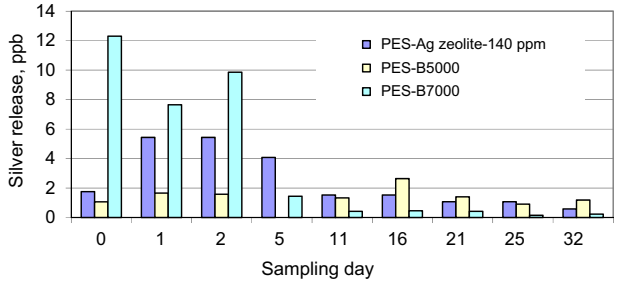

Fig. 5. Semi-dynamic leaching of silver from modified membrane as a function of immersion time.

Table 4
Leaching of silver for the last 10 d of test

Sample	Leaching (ppb)	Leaching (mg Ag/kg of membrane per day)
PES-B5000	0.40	0.87
PES-B7000	0.05	0.08
PES-Ag zeolite-140 ppm	1.02	1.87

3.1.1. "Cumulative" leaching of PES-Irgaguard B5000 membrane

3.1.1.1. Milli-Q water. Cumulative long-term release of silver and zinc from PES-Irgaguard B5000 in Milli-Q water and seawater was investigated. For these studies, a piece of membrane (3 × 3 cm) was immersed in 500 ml of Milli-Q water in a glass bottle. At different times after immersion of membranes, 5–10 ml water samples were taken and 2 drops of 50/50 (v/v) nitric acid/Milli-Q water were added for stabilisation of silver.

The results presented in Fig. 6(a) demonstrate high leaching rate for the first 60 d of test, followed by leaching at lower rates. A plateau at around 0.07 ppb/m^2 of membrane was reached after 3 months test. It is worth noting that the silver saturation threshold in water is around 20–25 ppm. It seems that at an earlier

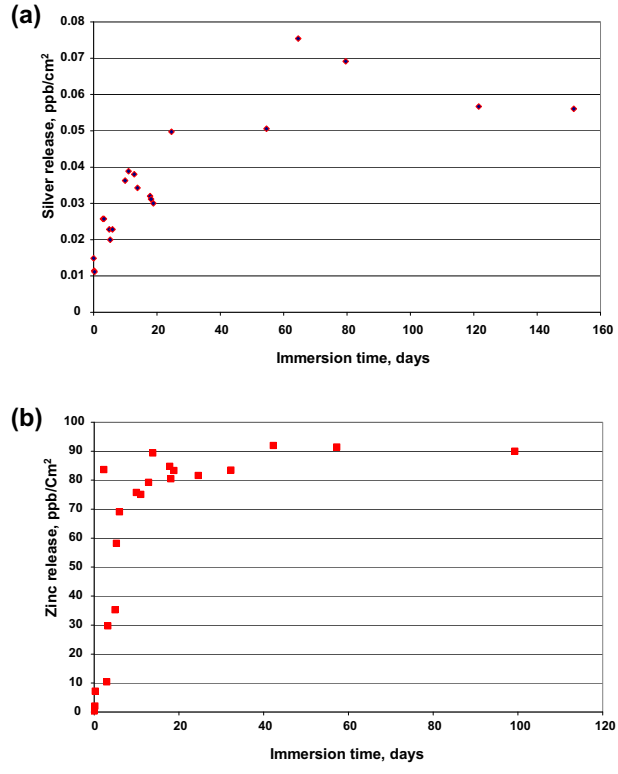

Fig. 6. Cumulative leaching of (a) silver and (b) zinc from PES-Irgaguard B5000 blend membranes as a function of immersion time.

time of immersion, most of the silver zeolite particles at the top surface of membrane and close to the pore walls were depleted and the particles which are embedded in the bulk of membrane leached at a slower rate. Fig. 6(b) presents release of zinc in Milli-Q water. As was expected the level of leaching was significantly higher than the one for silver. XPS and ICP-MS analysis of Irgaguard B5000 showed that it contains about 55–75 wt.% of zinc and also the EDAX analysis showed that the zinc particles are smaller.

To examine the effectiveness of the membranes after the initial silver release, two large pieces of membranes (27.5 cm^2) were immersed in Milli-Q water and after 42 d they were removed and immersed in fresh water. After nine months of immersion in fresh water, the membranes still presented antimicrobial activities when tested in bacteria filtration in a cross-flow rig.

3.1.1.2. Sea water. Feed in desalination plants and in the RO process involves sea water. In order to study the effect of the presence of salt on the release of silver and zinc, membranes were immersed in sea water and water samples were analysed by ICP-MS. For this work, the seawater from Sigma–Aldrich (S9148, natural seawater) was used.

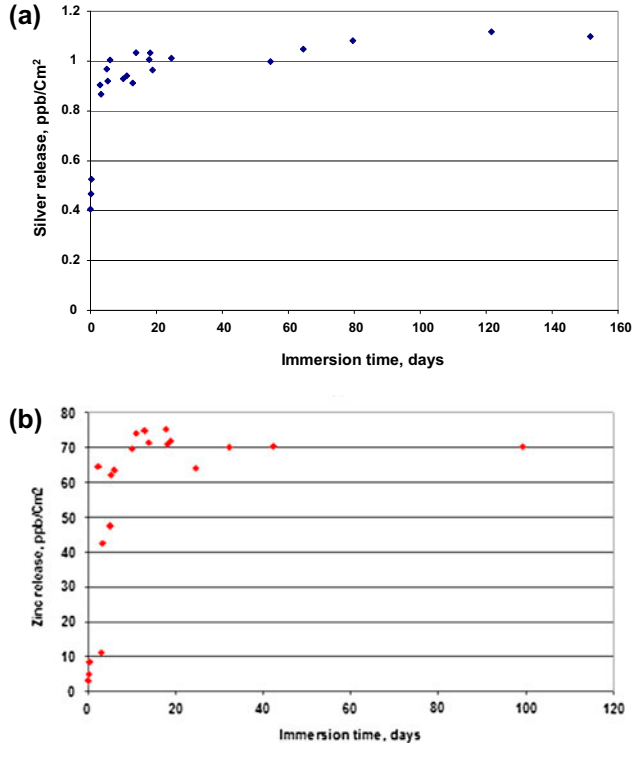

Fig. 7. Cumulative leaching of (a) silver and (b) zinc from PES-Irgaguard B5000 membrane in *sea water* as a function of immersion time.

Fig. 7 shows the significantly higher silver leaching rate in seawater than that in Milli-Q water due to the ion-exchange nature of silver release, while the level of leaching of zinc in seawater was comparable with its level in Milli-Q water. Silver ion is released from silver zeolite by ionic exchange with other cations (in this case Na^+) and the level of silver ion depends on the concentration of cations in the solution. So, it is expected that silver zeolite have shorter term activity in solutions with high ionic strength.

3.2. Membranes with higher concentration of antimicrobial additives

Based on the results of leaching of silver for Irgaguard B5000, Irgaguard B7000 and silver zeolite, with assumption that the dynamic leaching rates for membranes are similar to the results obtained in equilibrium conditions, it was estimated that the minimum required level of silver to sustain antimicrobial properties for three years (assumed lifetime of membrane) is about 2,000 ppm. In order to achieve this level of silver in membrane, higher loadings of additives were incorporated in membranes.

For additives with silver content between 0.4 and 1 wt.%, the additive to polymer ratio of up to one was necessary. Clearly, the addition of this level of additive is expected to lead to highly viscous dope solution and membranes with poor mechanical properties, especially in the case of additive with larger particle size. Irgaguard B5000 (silver zeolite and zinc oxide) with silver content of about 0.35 wt.% and large particle size was therefore not considered for these studies.

Table 5 shows the silver additives used and their silver content as well as their amount in membrane solution containing 18 g PES and 6 g PVP K 30.

Table 5
Silver content of additives and amount in membrane solutions

Additives	Silver content (wt.%)	Additive content in membrane solution (g)
Irgaguard B7000	0.35	10.3
Silver zeolite	35	0.1
Silver p-toluene sulhonate	38.6	0.09
Silver lactate	54.8	0.06
Silver benzoate	47.1	0.07
Hygate	100	0.36

Some organic silver additives were also tested due to their high level of silver content. As it is shown in Fig. 8, except for membranes with Irgaguard B7000, the permeability of composite membranes are the same or higher than control membranes. Increase in permeability might be due to the increase in porosity of membranes as a result of leaching of additives during membrane fabrication. In case of membrane modified with Irgaguard 7000, the addition of large amount of additive has led to the lower membrane permeability.

Although the permeability of membrane modified with Irgaguard 7000 was lower, chemical analysis of membrane showed that this membrane could retain more than half of its initially incorporated silver content after fabrication, while there was significant loss of silver for membrane modified with organic silver containing additives (Fig. 9).

3.3. Antimicrobial performance

3.3.1. Static conditions

Control membrane and membranes modified with Irgaguard B7000 were tested by TTP method for evaluation of their antimicrobial performance. Four replicates were assessed for each membrane. Results showed an inhibition effect of the PES-Irgaguard B7000 membrane on the cells in the bulk media and the cells attached to the membrane. For the control membrane, cells in the bulk media had a density of 2–9 × 10^7 cells/ml, whereas density in media with the PES-Irgaguard B7000 membranes was only 600–700 cells/ml. Cell densities on the control PES membrane were around 1 × 10^6 cells/membrane, whereas on the PES-Irgaguard B7000 membrane they dropped down one order-of-magnitude to 1 × 10^5 cells. These results showed the antimicrobial activity of modified membranes.

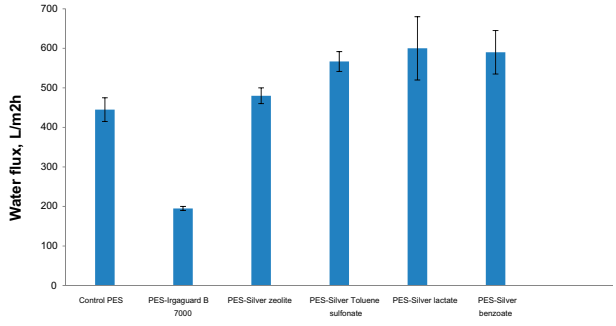

Fig. 8. Pure water flux for silver-modified composite membranes and control membrane.

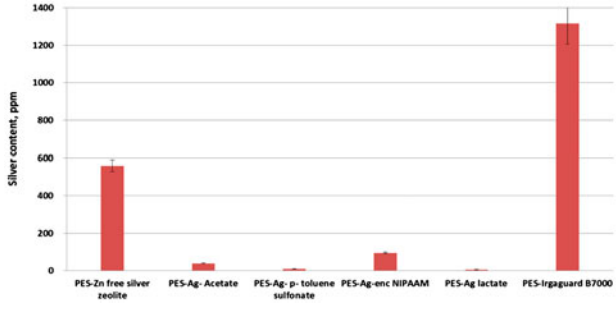

Fig. 9. Silver content of modified membranes with different types of silver additives.

3.3.2. Cross-flow filtration (dynamic condition)

3.3.2.1. Single-cell cross-flow filtration. Initial tests of the PES-Irgaguard B5000 (140 ppm silver content) and PES membranes were performed in a single-cell cross-flow filtration rig (Fig. 2(a)). Direct observation of the biofilm on the two membranes indicated that there was significantly less biofilm on the PES-Irgaguard B5000 membrane than the PES membrane (Fig. 10). Results from the carbohydrate assay were consistent with these observations. For modified and control membranes, overall mean absorbance was 30% that of the PES membrane i.e. 0.06 ± 0.027 and 0.197 ± 0.02, respectively. CSLM images of the biofilm also indicated significantly less biofilm present on the PES-Irgaguard B5000 membrane compared to the PES control membrane as shown in Fig. 11.

3.3.2.2. Double-cell cross-flow filtration. A more direct comparison of the two membranes was undertaken using the double cross-flow configuration. Two replicate experiments were performed in which the PES-Irgaguard B5000 and PES membranes were exposed to the same media. An additional experiment was conducted in which two PES membranes were tested to determine if a different result was obtained when the modified membrane was not included in the circuit. Experimental method was similar to the single cell filtration rig.

On opening the filtration cells similar levels of biofilm were observed on casual inspection between both membranes. For experiment in which PES-Irgaguard-B5000 membrane was included, the biofilm was also observed to be easily dislodged on opening the cells from both membranes. After cutting the membranes lengthwise into two sections, the "total biofilm" was assayed on one section. The other half was placed into a cell-free solution of media from the cross-flow circuit

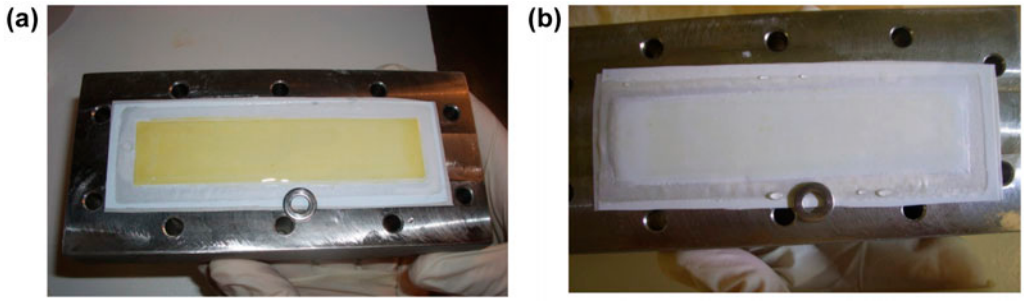

Fig. 10. Picture of membranes after 6 d cross flow test (a) control PES membrane and (b) PES-Irgaguard B5000 antimicrobial membrane.

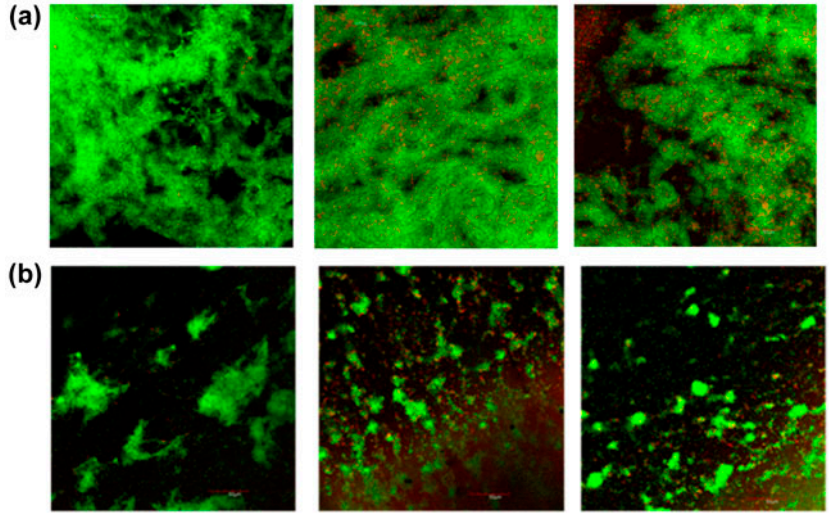

Fig. 11. Representative CSLM images of stained biofilm from the (a) PES control and (b) PES-Irgaguard B5000 modified membranes after 6 d in the single-cell cross-flow filtration test. Images on the left correspond to the inflow, images in the centre correspond to the middle and images on the right correspond to outflow areas of the membrane in the cross flow cell.

and gently agitated to generate the "residual biofilm" sample.

Measured "total biofilm" for membranes in the PES-Irgaguard B5000-PES tests were found to be less than those where the modified membrane was excluded (Table 6). In the PES-Irgaguard B5000-PES tests carbohydrate was less than 50% the amount in the PES-only test and protein was less than 70%. Also consistent with these results is the observation that permeate flow rate was lower for the PES-PES filtration circuit as a result of more fouling.

The single-cell cross-flow experiments and the double-cell experiments indicate that the antifouling activity of the PES-Irgaguard B5000 membrane is likely to be due to leachate from the membrane. Significant differences were observed between the cross-flow circuits that contained the PES-Irgaguard B5000 membrane and those that did not. When PES-Irgaguard B5000 membrane was compared with the PES membrane in the same circuit the differ-

Table 6
"Total biofilm" with and without the PES-Irgaguard B5000 membrane in the double cross-flow cell

	Cross-flow filtration membranes (double cell)	
Total biofilm	PES-Irgaguard B5000 and PES	PES–PES
Carbohydrate (μg/cm^2)	457 ± 41	1,147 ± 57
Protein (μg/cm^2)	931 ± 53	1,441 ± 32
Permeate flux (ml/min)[a]	4.1 ± 1.0	0.3 ± 0.1

[a]Average for the days 1–6.

ences were insignificant, with the amount of biofilm on the PES membrane being significantly reduced compared to the amounts of biofilm measured when the PES membrane was tested separately.

Although the cross-flow design included the inflow of nutrient into the circuit for the whole test period, the level of media turnover was not great enough to reduce the level of leachate from the PES-Irgaguard B5000 membrane down to a concentration where the biofilm on the PES membrane was unaffected. A similar problem was experienced with this membrane in the static bioadhesion experiment. In this test, the effect on the control membranes was only resolved and a significant difference between control and test membranes only established when the number of PES-Irgaguard B5000 membrane samples was reduced.

As biofilm was observed to be established within 1 d of inoculation, it may be possible to test the membrane under filtration conditions and low leachate concentration by increasing the rate of fresh media pumped into the circuit and reducing the test period down to less than 24 h.

3.3.3. Dynamic silver release

Silver release from membrane was also measured during the bacteria filtration in a cross-flow filtration rig shown. The aim of these studies was to investigate the effect of shear and filtration on the release of silver. Filtration was carried out for 6 d and during this period samples were taken from permeate, feed and retentate streams. Fig. 12 shows the result of the ICP-MS analysis when silver-contained membranes are tested in a single-membrane cell configuration. The silver release in all streams is below 0.5 ppb and in average is around 0.25 ppb.

Similar test was run in a double cross-flow rig where both control and modified membranes were tested at the same time for 6 d using a 1 mg/L *P. Aeroginosa* strain as feed. Results are shown in Fig. 13 for leaching of silver and zinc, the maximum concentration were 0.01 and 1.8 ppb/cm^2 of membrane for silver and zinc, respectively.

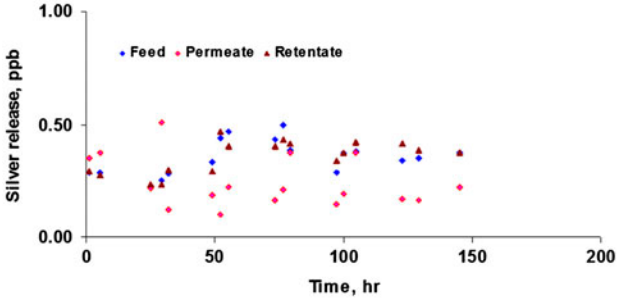

Fig. 12. Release of silver (ppb) during the bacteria filtration in a cross flow filtration rig (single-cell configuration).

3.4. Issues of silver and zinc loss during membrane fabrication

As was described earlier, the analysis of antimicrobial membranes by ICP-MS showed that significant level of biocidal active agents (silver and zinc) was lost in the process of membrane fabrication. In order to optimise the membrane composition/fabrication method, it is important to investigate the loss amount of additive at each stage of the fabrication process. Membranes modified with Irgaguard B5000 were selected for these studies with the composition of PES (18 g), PVP (6 g) and Irgaguard B5000 (2 g) in 74 g NMP. Assuming a silver content of 0.55 wt.% and zinc content of 58 wt.% for additive, the theoretical silver content and zinc content of membrane were expected at 550 and 58,000 ppm, respectively.

Membranes were dried at room temperature for 2 d and in oven of 70˚C overnight. The membranes taken from glycerol solution were further dried in

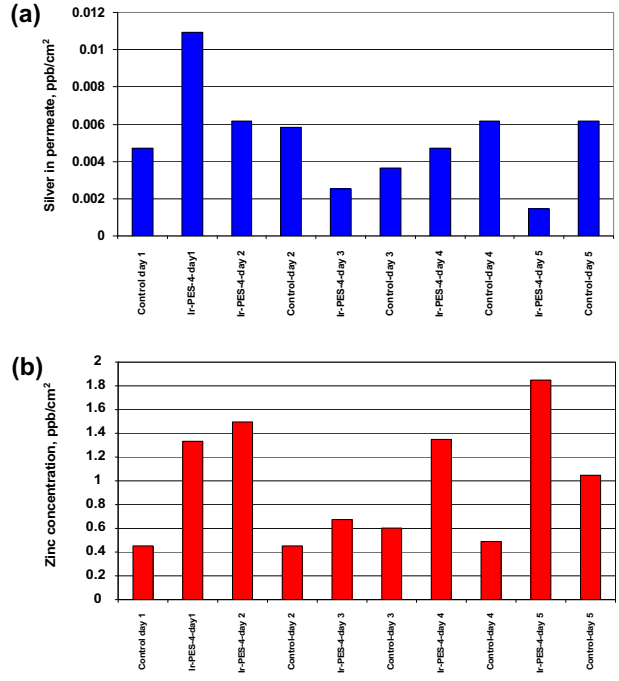

Fig. 13. (a) Silver and (b) zinc in leachate from control-PES and PES-Irgaguard B5000 blend membranes in a double cell cross-flow filtration rig. Control: PES membrane; Ir-PES-4 stands for PES—B5000 membrane with 140 ppm theoretical silver content.

Table 7
Summary of leaching results from ICP analysis[a]

Fabrication Stage	Vol. (l)	Time (min)	Ag (μg/l)	Zn (μg/l)
Non-solvent immersion	2	10	6.63 ± 0.97	162.34 ± 22.7
Hot water	1	30	6.98 ± 0.65	302 ± 33
Water (day 1)	1	1,440	0.748 ± 0.88	558.4 ± 21.31
Water (day 2)	1	2,880	1.137 ± 0.12	958 ± 53
NaOCl-1	0.5	30	26.2 ± 6.3	49.7 ± 3.44
NaOCl-2	0.5	30	19.5	30.6
NaOCl-3	0.5	30	84.9	102
NaOCl-4	0.5	30	36.4 ± 1.13	50.6 ± 1.26
Glycerol	0.5	240	8.35 ± 1.48	377 ± 38

[a]Between 2 and 12 replicates were used for each test.

110˚C oven for 5 h. TGA showed insignificant residues of solvent or glycerol for membranes after these drying regimes.

The following tests were carried out to evaluate the membranes and to investigate the leaching rate at different stages of membrane fabrication:

(1) ICP analysis of water (Ag, Zn).
(2) ICP-OES analysis of membrane after digestion in HNO_3 (Ag, Zn).

Table 7 shows the level of silver and zinc in the water in which membranes were immersed at different stages of fabrications. As can be seen, the leaching rate of silver was the highest at the NaOCl treatment stage. The values probably are underestimated due to the formation of NaCl from reaction of sodium hypochlorite with additive. On the other hand, the lowest leaching rates were observed for the water immersion steps.

On the other hand, the leaching rate of zinc was higher at casting and hot water treatment stages. This is due to high solubility of zinc oxide in water. Unlike silver in which its leaching is also influenced by the presence of NaOCl, the leaching of zinc was mainly through diffusion of particles out of the membranes. ICP-MS analysis of membranes (5 replicates) showed about 311 ± 12 ppm silver after hot water treatment and 204 ± 11 ppm after sodium hypochlorite treatments. Zinc content of membranes at all stages of treatment was about 4.6 wt.%.

4. Conclusions

One of the major issues for antimicrobial membranes has been the silver loss during membrane fabrication and application. To investigate the rate of silver/zinc loss, membranes modified by the addition of Irgaguard B5000 were extensively analysed at each step of the fabrication. Results showed that silver was mainly leached during sodium hypochlorite treatment stage of membrane preparation while zinc was mainly lost at water immersion stages.

Control of biofilm fouling by inhibition of growth and biofilm release by antimicrobial membranes were assessed by filtration of a single strain bacteria (*P. aeruginosa*; *ATCC* 25619), and 24-well Titre Plate methods.

The results showed the good antifouling performance under cross flow filtration and in the Titre Plate test conditions of PES-Irgaguard B5000 membranes.

Initial tests of membranes were performed in single-cell cross-flow filtration circuits. Direct observation of the biofilm on the two membranes indicated that there was less biofilm on the blend membrane than the PES control membrane. Results from the carbohydrate assay were consistent with these observations with overall mean absorbance 30% of the PES membrane. Also it was found that for both nutrient and bacteria filtration the permeate flux was higher for the modified membrane. An alternative configuration with two cross-flow cells in parallel was designed to test two membranes with the same culture. When PES-Irgaguard B5000 blend membrane was compared with the control PES membrane in the same circuit the differences between membranes were insignificant, while the amount of biofilm on the PES membrane was significantly reduced compared to the amounts of biofilm measured when the PES membrane was tested separately. The result supports the idea that the antimicrobial activity of the PES-Irgaguard B5000 blend membrane is due to leachate from the membrane. Silver concentration in the permeate lines was up to 0.01 ppb/cm^2 of membrane during the course of the test.

The studies with ionic silver salts with organic anions as antimicrobial additives showed much lower

stability of these membranes. There was significant loss of silver during membrane fabrication for these membranes with initial silver content of 2,000 ppm. On the other hand, the residual silver in membrane modified with Irgaguard B7000 was in the range of 1,000–1,400 ppm for initial loading of 2,000 ppm.

One important aspect for the antimicrobial membranes is the level of leaching of active components and subsequent loss of activity during the lifetime of membrane. To study this effect, the long-term leaching of silver and zinc from PES-Irgaguard B5000 was investigated. After five months of testing, the leaching in Milli-Q water from Irgaguard blended membrane was around 0.08 and 90 ppb/cm^2 of membrane for silver and zinc, respectively. The silver leaching in sea water was 10 times greater due to the ion exchange of silver with the salt present in the sea water. The leaching of active components is expected to slow down when particles at the surface or pore walls are completely depleted. Static tests results showed that slow leaching rate after about 60 d of test.

Antimicrobial activity assays of the PES-Irgaguard B5000 membranes aged for nine months in water and tested in bacteria filtration demonstrated the biocidal properties, which shows the long-term stability of these membranes.

The release of silver ions may be further controlled by selecting the suitable copolymers to immobilise/encapsulate the silver in membrane. Such copolymers may have block segments with high affinity with silver and membrane material.

Acknowledgement

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