Preparation and characterization of an antibacterial ultrafiltration membrane with N-chloramine functional groups

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Abstract: In this study, a cardo poly(aryl ether ketone) ultrafiltration membrane containing an N-chloramine functional group (PEK-N-Cl membrane) was easily obtained via exposure of a cardo poly(aryl ether ketone) ultrafiltration membrane (PEK-NH membrane) to dilute sodium hypochlorite solution. The chlorination process did not harm membrane performance. In addition, the PEK-N-Cl membrane was stable in both air and water. The PEK-N-Cl membrane exhibited excellent antimicrobial properties against both Gram-negative and Gram-positive bacteria (i.e. *E. coli* and *Bacillus subtilis*, respectively). The PEK-N-Cl membrane provided 94.2% and 100% reduction of *E. coli* and *Bacillus subtilis*, respectively, within 30 min of contact times. Moreover, nearly 100% of the *E. coli* was killed after 2 h during the filtration process for the PEK-N-Cl membrane. In addition, the water flux decreased by 42% for the PEK-N-Cl membrane compared to 77.6% for the PEK-NH membrane after filtration of the *E. coli* solution and incubation on LB nutrient agar plate, indicating that the PEK-N-Cl membrane exhibits antifouling. Furthermore, the PEK-N-Cl membrane is recyclable via subsequent exposure to a sodium hypochlorite solution.

Keywords: ultrafiltration membrane, cardo poly(aryl ether ketone), N-chloramine, anti-bacterial, recyclable

1. Introduction

Currently, people in developing countries suffer from diseases caused by unsafe drinking water contains bacteria. The World Health Organization reported that approximately 1.8 million people including children died from diarrheal diseases each year due to the poor quality of drinking-water, and it is also reported that 500 million people are at risk for trachoma due to the absence of nearby sources of safe water [1]. Ultrafiltration (UF) is widely used in water treatment because it is highly selective and cheap. However, most of the ultrafiltration membranes are easily fouled by microorganisms, which can harm both membrane performance and human health. Therefore, numerous studies have been performed to meet the increasing need for antimicrobial agents that can effectively limit the growth of microorganisms on membrane surface. Several antimicrobial agents are widely used including nanoparticles consisting of metals and metal oxide [2-5], chitosan [6, 7], quaternary ammonium compound [8-11] and N-halamine compounds [12-18]. Among these agents, the N-halamine compounds have attracted much attention due to their effectiveness against a broad spectrum of microorganisms, long-term stabilities in both water and dry air, and ability to be regenerated by rechlorination as well as being relatively cheap. Moreover, N-halamines can inactivate various bacteria without causing any resistance, and are non-toxicity to humans or the environment.

Three types of N-halamines exist including amine (RR'-NX), amide (-C(O)-NX-R), and imide (-C(O)-NX-C(O)-X), which can be easily obtained by reaction of RR'NH with diluted sodium

hypochlorite. Antimicrobial N-halamine functional groups have been attached to surfaces of β -cyclodextrin, chitin and cotton by grafting [17, 19, 20], coating [21, 22] and copolymerization [15]. However, the introduction of N-halamines to the commonly used ultrafiltration membranes, such as polyethersulfone (PES) membrane, polysulfone (PSf) membrane, poly(vinylidene fluoride) (PVDF) membrane, is relatively difficult due to there are no presence of amine, amide or imide on those membranes. Fortunately, cardo poly(aryl ether ketone) (PEK-NH) (Fig. 1) was developed. It is an engineering thermoplastics that exhibits a variety of applications including as an ultrafiltration membrane, coating, film, gas separation membrane along with high-performance functions due to its excellent properties such as high glass transition temperature, excellent thermoxidative stability, and good membrane-formation property [23]. Moreover, the presence of an amide group ($-\text{C}(\text{O})-\text{NH}-\text{R}$) make it easy to transfer into the N-chloramine compound without a laborious grafting process. Therefore, N-chloramine cardo poly(aryl ether ketone) is a promising material for the fabrication of an antibacterial ultrafiltration membrane.

In this study, cardo poly(aryl ether ketone) was employed to fabricate an ultrafiltration membrane. The N-chloramine functional membrane was easily obtained since the membrane contained an N-halamine precursor moiety ($-\text{C}(\text{O})-\text{NH}$) which was transferred to $-\text{C}(\text{O})-\text{N}-\text{Cl}$ by exposure to a dilute sodium hypochlorite solution. The chlorinated membrane had been observed exhibited biocidal efficacy against both Gram-negative and Gram-positive bacteria (i.e. *E. coli* and *Bacillus subtilis*, respectively). The surface characterization and stability of the chlorinated membrane were also investigated.

Fig. 1 Synthesis of PEK-NH and PEK-N-Cl

2. Materials and methods

2.1 Materials

Phenolphthalein (PPH) was obtained from the Beijing Chemical Reagent Company and purified by recrystallization from a mixture of water and ethanol. 3,3-Bis (4-hydroxyphenyl)isobenzofuran-1-one (PPH-NH) was prepared in our laboratory according to the method reported by Wang et al [24]. 4, 4'-Difluorobenzophenone (DFDPK) was purchased from Sigma. N, N-Dimethylacetamide (DMAc) was dried with CaH_2 and then distilled under reduced pressure prior to use. Anhydrous potassium carbonate was powdered carefully prior to use. All other chemicals were reagent grade and used as received. Bovine serum albumin (BSA, $\text{pI} = 4.8$, $\text{Mw} = 67$ kDa) was purchased from Sino-American Biotechnology Co., Ltd. *E. coli* MG1655 (ATCC 47076) was obtained from The Coli Genetic Stock Center (CGSC), and *Bacillus subtilis* (CGMCC 1.1390) was obtained from the China General Microbiological Culture Collection Center (CGMCC).

2.2 Synthesis of cardo poly(aryl ether ketone)

Cardo poly(aryl ether ketone) was prepared according to the reported references [23, 25-27]. The process is described below: PPH-NH (38.1 g, 0.12 mol), DFDPK (26.2 g, 0.12 mol), anhydrous K₂CO₃ (41.2 g, 0.3 mol), 340 ml DMAc and 170 ml toluene were added to a 1000 ml three-neck round-bottom flask equipped with a mechanical stirrer and N₂ inlet. The reaction mixture was heated with continuous stirring to 120 °C for 6 h to remove water from the condensation reaction. Next, the toluene and water were removed from the reaction system by distillation. Then, the temperature was increased to 160 °C, and the mixture was allowed to react at this temperature for 10 h. The system was cooled to room temperature and diluted with DMAc followed by centrifugation to remove the inorganic salts. Then, the solution was poured into water to obtain a white flake product, which was washed several times with hot water to remove any residual inorganic salts and extracted with ethanol to remove any residual organic solvent. The resulting product was dried under vacuum at 140 °C for 24 h. The yield was 97%. ¹H NMR (DMSO-d₆, 500 MHz) δ(ppm) 9.90 (s, 1H), 7.77 (m, 4H), 7.68-7.42 (m, 4H), 7.15-7.09 (m, 8H). The inherent viscosity of the polymer was determined at 30 ±0.1 °C using an Ubbelohde viscometer at a concentration of 0.5 dL/g concentration in DMAc. The viscosity of the polymer was 0.9 dL/g. The Mw of the polymer is 1.8 ×10⁵ measured by gel permeation chromatography experiments.

2.3 Membrane preparation

The PEK-NH ultrafiltration membrane of PEK-NH was prepared using a phase inversion method. A solution consisting of 18% PEK-NH in DMAc was cast onto clean glass plates with a casting knife in a 100 µm gate gape. Immediately after casting, the film with the glass was immediately dipped into deionized water, and the membrane formed immediately. Then, the membranes were maintained in deionized water prior to use.

2.4 Chlorination of the membrane

The PEK-NH membrane was immersed in a 5% of commercial aqueous sodium hypochlorite solution at ambient temperature for a predetermined period of time (i.e., 5 min, 30 min, 1 h, 2 h, 3 h). After N-chlorination, the membranes were washed with deionized water and the washing water was tested with potassium iodide/starch to remove any free chlorine from the surfaces of the membranes. Then, the membranes were dried at 45 °C for 1 h to ensure that the free chlorine was completely removed. To determine the active chlorine content on the membranes, a modified iodometric/thiosulfate titration was performed. In this procedure, a 2 cm ×2 cm membrane was solved in 5 ml of DMF, and then 1 ml of acetic acid (1 wt%) was added. Potassium iodide (0.5 g) was added and the mixture was stirred vigorously for 2 h at room temperature. Next, the solution was titrated against the titrant (sodium thiosulfate) until the yellow color disappeared. The active chlorine in the membrane was calculated using the following equation [28]:

$$Cl^+(%) = \frac{V \times N \times 35.45}{W \times 2} \times 100 \quad (1)$$

where Cl⁺(%) is the weight percent of active chlorine on the membrane, N is the normality (equiv/L) of sodium thiosulfate, V is the volume (L) of sodium thiosulfate used, and W is the weight of the membrane sample (g).

2.5 Membranes characterization

The ¹H NMR spectra were recorded on a Varian Unity spectrometer (500 MHz) with tetramethylsilane (TMS) as the internal standard and DMSO-d₆ as the solvent. The infrared spectra of the PEK-NH membrane as well as the chlorinated membrane were recorded on a FT-IR spectrophotometer (Bio-Rad digilab Division Fst-80) equipped with an ATR system. The thermal stability of the PEK-NH and chlorinated membrane was studied using a Perkin Elmer TGA-2

thermogravimetric analyzer (Inspiritech 2000 Ltd., UK) in N₂ at a heating rate of 10 °C/min up to 800 °C. XPS analysis was performed using a Thermo ESCAL 250 spectrometer with a monochromated Mg K α X-ray source (1253.6 eV photons). The survey scans were performed in the 0-1200 eV range at a take-off angle of 90°.

2.6 Membrane performance

Both the PEK-NH and chlorinated membranes were tested using a cross flow filtration cell, and the effective area of the membranes was 23.7 cm². First, each membrane was compacted at 0.2 MPa for 1 h. Then the operation pressure was lowered to 0.1 MPa for 1 h to obtain A pure water flux J (L/(m²h)), which was calculated using the following equation:

$$J = \frac{V}{A\Delta t} \quad (2)$$

where V is the volume of permeated water (L), A is the membrane area (m²) and Δt was permeation time (h).

The rejection of bovine serum albumin (BSA, pI = 4.8, Mw= 67 kDa) was calculated according to the feed and the permeate concentration using a UV spectrophotometer at 280 nm along with the following equation:

$$R = \left(1 - \frac{C_p}{C_f}\right) \times 100 \quad (3)$$

where C_p and C_f (mg/ml) are BSA concentrations in permeate and feed solutions, respectively.

2.7 Assessment of antibacterial efficacy

The bactericidal properties of the chlorinated membrane were evaluated using *E. coli* as model gram-negative bacteria and *Bacillus subtilis* as model gram-positive bacteria. *E. coli* and *Bacillus subtilis* were grown for 12 h at 37 °C under agitation (200 rpm) in Luria Bertani (LB) growth medium. Then the bacteria were harvested by centrifugation, washed three times with phosphate-buffered saline (PBS, pH 7.4), and then were resuspended in PBS (approximately 10⁶ CFU/ml). The “sandwich” system for antibacterial tests was employed [20]. The PEK-NH membrane and sodium hypochlorite treated PSf membrane were selected as control. In general, the membranes were cut into small pieces (2 cm×2 cm), and both sides of the PEK-NH membranes were sterilized for 30 min under UV light. Twenty microliters of an aqueous suspension containing approximate 10⁶ CFU/ml of *E. coli* or *Bacillus subtilis* was placed onto the surface of a membrane, and then another identical film was placed on the surface of the first membrane. The “sandwich” systems were held in place by sterile weights to ensure adequate contact with the bacteria. After contact times of 10, 30, and 60 min, the entire sandwich was transferred to sterile conical centrifuge tubes containing 4 ml of 0.2 mol/L sodium thiosulfate to quench any active free chlorine. Then, the mixture was vigorously shaken for 10 min. An aliquot of the solution was serially diluted, and 100 μ l of each dilution were placed onto an LB nutrient agar plate. The bacterial colonies were counted after incubation at 37 °C for 24 h.

A respirometric method [29] was also used to measure the antibacterial properties. An *E. coli* suspension (10 μ l, 10⁶ CFU/ml) was placed onto the PEK-NH and chlorinated membrane surfaces in a 96-well plate, and membrane with the same size was employed to cover the first membrane for 1 h. Then, 100 μ l of LB and 10 μ l of Alamarblue were added to each cell. 100 μ l of LB plus 10 μ l of Alamarblue was used as control wells. The plate was incubated at 37 °C for 4 h with gently shaking. Then, the absorbances at 570 nm and 600 nm were obtained using a plate reader (Tecan Sunrise, Swiss). The Alamarblue reduction percentage was calculated as the following equation:

$$\text{Alamarblue reduction (\%)} = \frac{\varepsilon_{600} \times A_{570} - \varepsilon_{500} \times A_{600}}{\varepsilon'_{570} \times A'_{600} - \varepsilon'_{600} \times A'_{570}} \times 100 \quad (4)$$

where ϵ_{600} is the molar extinction coefficient of the oxidized form of Alamarblue at 600 nm and ϵ'_{600} is molar extinction coefficient of the reduced form of Alamarblue at 600 nm. In addition, ϵ_{570} and ϵ'_{570} are the molar extinction coefficients of oxidized and reduced form of Alamarblue at 570 nm, respectively. A is the absorbance of the test wells, and A' is the absorbance of the control wells.

To observe the morphology of the bacteria after treatment with the PEK-N-Cl membrane, the membrane surfaces were placed on the bottom of 24-well plates and covered with 2 ml of the 10^6 CFU/ml *E. coli* suspension at 37 °C for 24 h. Then, the bacterial suspensions were removed, and the membranes were gently washed with PBS to remove the unattached cells, fixed with a 3% glutaraldehyde solution for 0.5 h, and dehydrated in a series of ethanol solutions (30, 50, 70, 90, 100 wt%). The membrane surfaces were examined using a scanning electron microscope (SEM).

2.8 Stability

To study the stability of the chlorinated PEK-NH membrane, the membrane was stored under ambient light conditions or in sterile water. Then, the active chlorine content was tested periodically using the iodometric/thiosulfate titration method for a 10 day storage period. The stability of the chlorinated PEK-NH membrane was measured for 60 days. The membranes were also stored at different pH conditions (3, 5, 7, 9, 11, 13) for a 10 day storage period and the active chlorine content was measured.

2.9 Bacterial suspension filtration

To evaluate the durability of the antimicrobial effect and the biofouling of both the unmodified and chlorinated membranes, 1 L of *E. coli* with a concentration of 10^6 CFU/ml in a 0.85% NaCl solution was filtered through the membranes in a cross flow filtration cell. Prior to the filtration process, the cross flow filtration cell was first washed with a 75% ethanol solutions and then with sterile water for six times to ensure sterile conditions. The pressure was maintained at 0.1 MPa in all the experiments. The pure water flux (J_a) of all the membranes was measured according to Eq. (1). The samples were removed from the rejection filtering solutions at 0, 1, 2, 4, 6, 8 h and inoculated separately on LB nutrient agar plates using diluted solutions. The colonies were counted after 24 h at 37 °C. After filtration of the *E. coli* solution after 12 h, each membrane was incubated on an LB agar plate to maximize the *E. coli* growth. Next, the membranes were then washed three times with pure water to remove the unattached bacteria. Then, the pure water flux (J_b) was measured to evaluate biofouling. In addition, the membrane surface was examined using SEM to observe the biofouling directly.

To observe the morphology of attached bacteria, the membrane surfaces were placed on the bottom of 24-well plates and covered with 2 ml 10^6 CFU/ml *E. coli* suspension at 37 °C for 24 h. Then the bacterial suspensions were removed and the membranes were gently washed with PBS to remove the unattached cells, fixed by a 3% glutaraldehyde solution for 0.5 h, dehydrated in a series of ethanol solutions (30, 50, 70, 90, 100 wt%). The membrane surfaces were then examined using SEM.

2.10 Chlorination/de-chlorination cycles of the membrane

For the recycling test, five chlorination/de-chlorination cycles were performed on the PEK-NH membrane. In brief, the membrane was chlorinated with a 5% of commercial aqueous sodium hypochlorite solution, followed by removal of excess aqueous hypochlorite by washing with deionized water. Three samples were removed and dried at 45 °C for 1 h to determine the active Cl^+ content on the membrane via an iodometric/thiosulfate titration. The remaining membrane was soaked in a 0.3% sodium thiosulfate aqueous solution at room temperature for 0.5 h to quench the active chlorine and then chlorinated again according to the previously mentioned chlorination method with 5% sodium hypochlorite. This chlorination/de-chlorination process was repeated an additional four times.

3. Results and discussion

3.1 Membrane preparation

The PEK-NH ultrafiltration membrane was prepared using a the conventional phase inversion method. Then, the membrane was exposed to a 5% dilute sodium hypochlorite solution to obtain the chlorinated membrane. To evaluate the effect of the chlorination process on the membrane structure, the surface and cross-section morphologies of the PEK-NH and grafted N-chloramine membranes were investigated using SEM as shown in Fig. 2. The morphology of the membrane was maintained after the chlorination process.

Fig. 2 SEM images of a) PEK-NH membrane and b) the chlorinated membrane with 3.2% active chlorine content.

Before and after the chlorination process, the pure water flux (J) and rejections for BSA ($R\%$) were also studied to evaluate the effect of the chlorination process on the membrane performance, and the results are shown in Table 1. The pure water flux was calculated using Eq. (1), and the rejection was calculated using the Eq. (2). According to Table 1, the chlorination process had a minor effect on pure water flux and BSA rejection, which may be due to the morphologies being unchanged during the chlorination process.

Table 1 Pure water flux and BSA rejection

Membrane	J (L/m ² h)	R (%)
Original membrane	244 ± 2	99.9 ± 0.05
Chlorinated membrane	239 ± 5	99.8 ± 0.08

3.2 Active chlorine content of the N-chloramine membrane

The PEK-NH ultrafiltration membrane was treated with a sodium hypochlorite solution to introduce chlorine to the amide group at the cardo copoly(aryl ether ketone) (Fig. 1). Fig. 3 shows the effect of the chlorination time on the active chlorine content introduced to the PEK-NH membrane. The active chlorine content rapidly increased to 2.4% after only 5 min of chlorination time and reached 3.2% after 2 h. However, chlorination times of more than 2 h did not effectively increase the chlorine content. These results were due to the asymmetric structure of the membrane. First, the dense surface was preferentially chlorinated in a higher reaction speed, and then, the inside wall of the finger-like pores gradually reacted. As shown in Fig. 3, the saturated active chlorine content corresponded to

approximately 50% of the degree of substitution due to the dead porosity in the asymmetric membrane.

Fig. 3 Effect of chlorination time on the Cl content of the ultrafiltration membrane

3.3 Characterization of the N-chloramine membrane

The chemical structure of the PEK-NH and chlorinated membranes were analyzed by ¹H NMR using DMSO-d₆ as the solvent as shown in Fig. 4. The chemical shift at 9.8 ppm can be assigned to –NH of amide group, and the chemical shifts at 7~8 ppm was assigned to the phenyl protons. The degree of chlorination (XX) can be calculated from the intensity ratio of the proton peaks for the –NH and all of the phenyl protons followed the equation:

$$XX = (1 - [I_{\delta=9.8}] / [(I_{\delta=7-8}) / 20]) \times 100$$

Where $I_{\delta=9.8}$ and $I_{\delta=7-8}$ are proton peak intensities of –NH and all of the phenyl protons, respectively. Based on the ¹H NMR data, a chlorine substitution of 55% was determined, indicating that the Cl was successfully grafted onto the cardo poly(aryl ether ketone) backbone.

Fig. 4 ¹H NMR spectra of PEK-NH membrane and chlorinated membrane

The ATR-FTIR spectra of the original and N-halamine modified PEK-NH membrane were recorded (Fig. 5). After the chlorination process with the sodium hypochlorite solution, the N-H bond

in PEK-NH was transformed to N-Cl bond. Therefore, the N-H stretching vibration at approximately 3286 cm^{-1} decreased in intensity, and a new band at 813 cm^{-1} was detected, and assigned to the N-Cl group. Moreover, a distinctive characteristic vibrational band was detected at 1731 cm^{-1} and the band at 1698 cm^{-1} decreased in intensity after the chlorination process, which corresponds to the C=O stretching vibrations of the amide group. In agreement with previous study [30], the stretching vibration of C=O shifted to a higher wavenumber upon chlorination.

Fig. 5 ATR-IR spectra of original and chlorinated membranes

The XPS spectra were analyzed to identify the chemical composition of the original and modified surface structures on the ultrafiltration membranes as shown in Fig. 6. Two strong peaks were observed at 285.0 and 532.0 eV, which were due to the binding energies of C 1s and O 1s, respectively. The peak located at 399.2 eV before and after chlorination was associated with the electronic binding energy of N 1s. For the chlorinated membrane, the peak located at 201.0 eV corresponded to the electronic binding energy of Cl 2p, indicating that the chlorination process was successful.

Fig. 6 XPS spectra of PEK-NH membrane and chlorinated membrane [15]

3.4 Thermal analysis

The results from the TGA thermogravimetric analyses of the PEK-NH membrane before and after chlorination are shown in Fig. 7. The weight loss temperature of the PEK-NH membrane before chlorination was at 498.6 °C, due to the decomposition of the PEK-NH backbone. After chlorination, the N-H bond of PEK-NH was transformed into an N-Cl bond. Two decomposition steps were observed. The first weight loss temperature was 242.6 °C, corresponding to cleavage of N-Cl bond. The second decomposition step was located at 488.3 °C, which is slightly lower than that of unchlorinated membrane and corresponds to the degradation of the polymer backbone. The results shown in Fig.7 indicated that chlorination does not affect the thermal properties.

Fig. 7 TGA curves for PEK-NH membranes before and after chlorination.

3.5 Stability of the chlorinated membrane

The stability of the antibacterial membranes is important for their widespread application. Therefore, the stability of the PEK-N-Cl membrane was evaluated by exposure to environmental conditions and sterile water, as described in the experimental section. The results in Fig. 8 indicates that no significant active chlorine loss was observed during a 2-month time period under environmental conditions and in sterile water. The active chlorine content remained at approximately 96% of the initial bound active chlorine content, indicating that the N-Cl bond exhibits relatively high stability. The membranes were also exposed to different pH conditions (3, 5, 7, 9, 11, 13) for 10 days. The chlorine content remained the same as the initial content, indicating that the N-Cl bond is stable over a wide pH range.

Fig. 8 Active chlorine content of chlorinated membrane stored for different periods of time under laboratory light and sterile water.

3.6 Antimicrobial efficacy

The antimicrobial activities of the chlorinated and unchlorinated membranes were evaluated based on the killing kinetics. In general, the membranes were challenged with *E. coli* K12 and *Bacillus subtilis*, representing Gram-negative and Gram-positive bacteria respectively. The results are summarized in Fig. 9. The PEK-N-Cl membrane provided 94.6% reduction of *E. coli* within 30 min of contact, and completely inactivation of *E. coli* after 60 min of contact. However, the PEK-NH control membrane and PSf membrane treated by sodium hypochlorite did not exhibit a reduction in *E. coli*. A similar result was obtained for *Bacillus subtilis*. The PEK-NH membrane and PSf membrane treated by sodium hypochlorite treated exhibited no bacterial reduction. However, the PEK-N-Cl membrane provided 95.2% and 100% reduction within 10 min and 30 min of contact, respectively. These results showed that *E. coli* was more difficult to be killed than *Bacillus subtilis*, which may be due to the difference in the cell walls of *E. coli* and *Bacillus subtilis*. In addition, the results suggested that the chlorinated membrane could effectively destroy and inactivate the microorganisms, which is useful in the separation process.

Fig. 9 Biocidal test results of chlorinated and unchlorinated membrane

The antibacterial activity was also investigated using the Alamarblue cell viability assay. *E. coli* was selected as a model. The PEK-NH and PEK-N-Cl membranes were placed in contact with an *E. coli* suspension (10 μ l, 10^6 CFU/ml) for 1 h in a 96-well plate. LB and Alamarblue were added to each cell. LB plus Alamarblue were used as the control wells. The plate was incubated at 37 °C. After 4 h, the color of the cell that contained the PEK-NH membrane changed to pink but the color of the cell that contained the PEK-N-Cl membrane and the control cell remained blue (Fig. 10a). The blue color indicated a low *E. coli* activity. The reduction of Alamarblue was also determined using a Tecan Sunrise plate reader (Fig. 10b). The cell that contained the PEK-NH membrane exhibited an Alamarblue reduction of 15.2%. In comparison, the cell contained PEK-N-Cl membrane exhibited an Alamarblue reduction of only 6.2%. From all results above, it can be concluded that the PEK-N-Cl membrane has high antibacterial activity.

Fig.10 (a) The color of Alamarblue for *E.coli* treated with PEK-NH and PEK-N-Cl membrane
(b) Percent reduction of Alamarblue in 4 h for *E.coli* treated with PEK-NH and PEK-N-Cl membrane

To gain more insight into the biocidal activity, the original and chlorinated membrane surfaces were tested using *E. coli* as a model. The membranes were incubated in an *E. coli* suspension (2 ml, 10^6 CFU/ml) at 37 °C for 24 h to allow the bacteria to attach to the membrane surface. The viability of *E. coli* was determined by SEM (Fig. 11). As shown in Fig. 11, *E. coli* that attached to the original membrane exhibited an intact morphology that was similar to that of the untreated ones. However, the *E. coli* attached to the chlorinated membrane surface exhibited a loss of cellular integrity, indicating serious damage to the cellular membrane. The proposed mechanism of bacterial killing involves the release of active chlorine. Although the N-chloramine structure is stable in an aqueous solution due to its low dissociation constant (approximately 10^{-8}), the active chlorine from the N-chloramine may be transferred to the microorganisms in aqueous at a moderate rate to destroy the cellular membrane followed by the leakage of cell components, leading to cell death [31-33].

Fig. 11 SEM images of *E. coli* adhesion on a) original membrane and b) chlorinated membrane.

3.7 Bacterial suspension filtration

To evaluate the durability of the antimicrobial effect as well as the biofouling of chlorinated membranes, 1 L of *E. coli* at a concentration of 10^6 CFU/ml in a 0.85% NaCl solution was filtered through the membranes in a cross flow filtration cell. The concentrations of living *E. coli* cells in the reject water at different time (i.e. 0, 1, 2, 4, 6 and 8 h) during the filtration process were determined using a counting method for both the PEK-N-Cl membrane and the PEK-N-Cl membrane stored in sterile water for 60 days. As shown in Fig. 12, nearly 100% of *E. coli* was killed after 2 h with the PEK-N-Cl membrane. Furthermore, no significant decrease in the antibacterial efficiency was observed for the PEK-N-Cl membrane even after storage in sterile water for 60 days.

Fig. 12 Bacterial counts CFU at various time for PEK-N-Cl membrane and PEK-N-Cl membrane stored in sterile water for 60 days

After the solutions containing *E. coli* were separately filtered through the PEK-NH membrane, PEK-N-Cl membrane, and PEK-N-Cl membrane stored in sterile water for 60 days, the membranes were exposed to an LB nutrient agar plate for 24 h to maximize the *E. coli* growth. After removal of the unattached bacteria, decreases in the water flux were measured (Fig. 13). As shown in Fig. 13, the water flux decreased 77.6%, 42% and 42.9% for the PEK-NH membrane, PEK-N-Cl membrane and PEK-N-Cl membrane stored in sterile water for 60 days, respectively. In comparison to the PEK-NH membrane, PEK-N-Cl membrane exhibited a smaller decrease in the water flux, suggesting that the PEK-N-Cl membrane exhibited a high antibiofouling property. Fig. 14 shows the SEM images of the membrane surface after biofouling. The *E. coli* colonies were formed on the PEK-NH membrane surface. The *E. coli* can't be removed by washing three times with water, indicating strongly adhesion. In comparison, almost no *E. coli* was observed on the membrane surface of either the PEK-N-Cl membrane or the PEK-N-Cl membrane stored in sterile water for 60 days. The enhanced antibiofouling property of PEK-N-Cl membrane was primarily due to the good antibacterial function of the

N-chloramine group which killed the bacterial colonies and prevented biofouling formation. Based on these results, the chlorinated membrane exhibited excellent antifouling performance.

Fig. 13 Flux decline after filtration of *E.coli* suspension and incubation on LB agar. a) PEK-NH membrane b) PEK-N-Cl membrane c) PEK-N-Cl membrane stored in sterile water for 60 days.

Fig. 14 SEM images of membrane surfaces after filtration of *E. coli* solution and incubation on LB agar for 24 h. (a) PEK-NH membrane (b) PEK-N-Cl membrane (c) PEK-N-Cl membrane stored in sterile water for 60 days.

3.8 Recyclability of chlorinated membrane

The antibacterial properties of a membrane, such as those modified by metals and metal oxide nanoparticles, typically decrease or are completely lost completely during the separation process. Thus, the recyclability of the antibacterial properties is very important for widespread application of ultrafiltration. The recyclability of the PEK-N-Cl ultrafiltration membrane was evaluated for five cycles, as described in the experimental section. First, the PEK-N-Cl membrane was quenched with a 0.3% aqueous sodium thiosulfate solution to form the PEK-NH membrane, simulating the state of the membrane after the bacterial cells were killed. Then, the PEK-NH membrane was chlorinated again by exposure to a sodium hypochlorite solution, and the active chlorinate was measured. The results in Fig. 15 indicate that the bound Cl content of the membrane at each cycle was similar to that in the first cycle after completion of the chlorination step. This result indicates the effective recyclability of the N-halamine membrane, which can be widely used widely in ultrafiltration processes.

Fig. 15 Chlorination/de-chlorination cycles of PEK-N-Cl ultrafiltration membrane

4 Conclusions

In this study, cardo poly(aryl ether ketone) was synthesized and used to fabricate an ultrafiltration membrane. The N-chloramine antibacterial membrane can be easily obtained by exposure of the cardo poly(aryl ether ketone) membrane to a sodium hypochlorite solution for 2 h. The chlorination process does not affect the membrane performance, and the PEK-N-Cl membrane was stable in both air and water. In addition, the PEK-N-Cl membrane exhibited excellent antimicrobial properties against both Gram-negative and Gram-positive bacteria (i.e., *E. coli* and *Bacillus subtilis*, respectively). The PEK-N-Cl membrane provided 94.2% and 100% reduction of *E. coli* and *Bacillus subtilis*, respectively, within 30 min of contact. Moreover, nearly 100% of the *E. coli* was killed after 2 h during the filtration process using the PEK-N-Cl membrane. Furthermore, in comparison to the membrane modified with metal and metal oxide nanoparticles [2-5], the PEK-N-Cl membrane is recyclable via exposure to a sodium hypochlorite solution. Therefore, the chlorinated membrane is an attractive candidate for industrial applications due to its excellent antimicrobial activity, recharging capacity, and safety to humans.

5 Acknowledgments

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PEK-NH membrane

PEK-N-Cl membrane

Healthy bacterial cell

Dead bacterial cell