Preparation of a novel positively charged nanofiltration composite membrane incorporated with silver nanoparticles for pharmaceuticals and personal care product rejection and antibacterial properties
Zhong-Hua Huang, Yan-Na Yin, Gu-li-mi-la Aikebaier and Yan Zhang

ABSTRACT
A novel positively charged N-(2-hydroxy-3-trimethylammonium)propyl) chloride chitosan (HTCC)-Ag/polyethersulfone (PES) composite nanofiltration membrane was easily prepared by coating the active layer, HTCC, onto PES as the support through epichlorohydrin as the cross-linking reagent and nano-Ag particles as the introduced inorganic components. Scanning election microscopy, X-ray photoelectron spectroscopy, atomic force microscopy, and X-ray diffraction were employed to characterize the morphology of the resultant membranes, of which the molecular weight cut-off was about 941 Da. At 25°C, the pure water permeability is 16.27 L/h·m²·MPa. Our results showed that the rejection of pharmaceuticals and personal care products (PPCPs) followed the sequence: atenolol > carbamazepine > ibuprofen, confirming that the membranes were positively charged. The antibacterial properties of the membranes were compared to elucidate the existence of Ag nanoparticles which help to improve antibacterial activity against Gram-negative Escherichia coli (DH5α, Rosetta) and Gram-positive Bacillus subtilis. The inhibition zone diameters of HTCC-Ag/PES membranes towards E. coli DH5α, E. coli Rosetta and Bacillus subtilis were 17.77, 16.18, and 15.44 mm, respectively. It was found that HTCC-Ag/PES membrane has a better antibacterial activity against E. coli than against Bacillus subtilis, especially for E. coli DH5α.

Key words | antibacterial activity, composite membrane, HTCC, nano-silver, PPCPs

INTRODUCTION
Pharmaceuticals and personal care products (PPCPs) are a diverse group of chemicals used extensively in medicines and personal hygiene products. To date, environmental pollution by PPCPs has been an increasing public concern due to their universal occurrence and potential adverse effects on non-targeted human beings and organisms (Kolpin et al. 2002; Dougherty et al. 2010). Especially in wastewater treatment plants, PPCPs cannot be removed completely during treatment so that considerable amounts of such chemicals remain in the treated wastewater and then enter the environment through disposal or reuse of treated wastewater and biosolids (Kinney et al. 2006). Therefore, how to improve the removal efficiency of PPCPs has attracted much attention.

Membrane technology has been widely used in many fields, including water treatment, protein separation and reverse osmosis (RO) pretreatment (Halpern et al. 2005). Nanofiltration (NF) is a liquid-phase pressure-driven separation process employing semipermeable membranes with the pore size in the nanometre range, between that of the ultrafiltration (UF) and RO membranes. The main separation and transport mechanism of the NF process involves size exclusion effect, convection and Donnan exclusion between the membrane and the treated solution. Due to its unique ability of separating and fractionating ionic and small molecule organic species, NF has a smaller occupation area and a better rejection of PPCPs with a molecular weight of 150–1,000 Da. In this study, we focused on the development of a positively charged membrane for the removal of PPCPs through easy modification of a UF membrane using a dip-coating and photoreduction technique. Compared with interfacial polymerization (Fang...
et al. 2013), the self-assembly process (Ouyang et al. 2008) and UV-assisted grafting polymerization (Deng et al. 2011; Zhong et al. 2012) that have been adopted for preparing positively charged NF membranes, the approach adopted in our study is simple and avoids using hazardous materials. In our early study, we reported a method to prepare a pot blending of biopolymer-TiO$_2$ composite membranes which had enhanced mechanical strength (Huang et al. 2015).

To date, NF technology has emerged as a valuable tool in purifying PPCPs. However, one of its disadvantages is the contamination on the membrane surface that decreases the fluid flux and reduces its efficacy. Membrane fouling leads to the rise in manufacturing and energy costs, damage or destruction of membranes, reduction in operating lifetimes, and secondary contamination of water by the metabolic products of microorganisms. Different strategies have been taken to address this issue, e.g., feed water pre-treatment, managing operational performance and development of modified antibacterial membranes (Gao et al. 2011). However, it still remains a challenge to realize the antibacterial property of the NF membrane surface by using a simple strategy.

Chitosan (CTS) is a kind of polysaccharide with several properties such as hemostatic activity (Bonfaroni et al. 2009), non-toxicity (Bhattarai et al. 2005), biodegradability, and antibacterial activity (Deng et al. 2011). N-[(2-hydroxy-3-trimethylammonium)propyl] chloride chitosan (HTCC), a water-soluble derivative of CTS, was found with better bacterial inhibition properties than CTS. The introduction of positively charged quaternary ammonium groups significantly enhanced the interaction between the CTS molecule and the bacterial cell wall, inhibiting the bacterial growth and reproduction as well (Qin et al. 2004). To date, HTCC has been widely applied for the preparation of membranes. It is also well known that silver has an inherent antimicrobial property and is capable of causing a bacteriostatic or a bactericidal impact (Thiel et al. 2007). Moreover, silver is very effective in purification systems for disinfecting water or air (Sondi & Salopek-Sondi 2004; Zhang et al. 2004). Recently, the hybrids of silver nanoparticles with amphiphilic hyperbranched macromolecules have been reported as an effective antimicrobial surface coating. They may affect the cell integrity and metabolism through direct interaction with cell membranes, releasing dissolved silver species, and generating reactive oxygen species (Jin et al. 2010; Marambio-Jones & Hoek 2010). Nano-Ag particles have been incorporated into nanocomposite microfiltration (Gunawan et al. 2011; Diagne et al. 2012), UF (Zodrow et al. 2009), and RO (Huang et al. 2012) membranes with various methods including membrane surface modification (Diagne et al. 2012), phase-inversion (Zodrow et al. 2009; Huang et al. 2012) and interfacial polymerization.

In our study, we report a simple method to synthesize a kind of novel composite membrane with silver nanoparticles which act as an antibacterial agent. The HTCC-Ag/polyethersulfone (PES) membrane was prepared through photochemical reduction technique on the base of an HTCC/PES membrane. The obtained composite membranes were characterized by scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), and atomic force microscopy (AFM). Meanwhile, we studied the rejection of PPCPs by HTCC/PES positively charged membranes and investigated the antibacterial activities of the membranes.

**EXPERIMENT**

**Materials**

Chitosan (weight-averaged molecular weight $\approx$ 150 kDa, Sinopharm Chemical Reagent Co., Ltd) was dissolved in the solvent acetic acid (CH$_3$COOH $\geq$ 99.5%, Sinopharm Chemical Reagent Co., Ltd). Glycidyl trimethylammonium chloride (GTMAC, 96% purity) was from Dongying Guofeng Fine Chemical Co., Ltd. Epichlorohydrin (ECH) (Sinopharm Chemical Reagent Co., Ltd) was used as a cross-linking agent. Ethanol, sodium chloride, silver nitrate, nutrient agar, glucose, sucrose and polyethylene glycol (PEG) (analytical grade) were used as received without any further purification; the PES UF membrane with molecular weight cut-off (MWCO) of 100 kDa was kindly provided by Ande Membrane Separation Technology & Engineering (Beijing) Co., Ltd. All aqueous solutions were prepared using purified water with a resistance of 18.2 M$\Omega$.cm.

**HTCC preparation**

Two grams of CTS was dissolved in 100 mL of 2% (v/v) acetic acid. The solution was then adjusted to pH = 9.0 by 6 mg/mL NaOH, and stirred for 2 h to allow CTS alkalinization. The precipitate obtained was transferred into a flask, followed by the addition of isopropyl (30 mL). After the mixture was stirred at 60°C for 4 h, 7.53 of GTMAC was added and stirred at 80°C for 8 h. Finally, the viscous solution was cooled to ambient temperature and poured into acetone. The HTCC obtained was washed three times by 500 mL acetone and dried at 80°C for 12 h. The degree of quaternization of HTCC was 88.7% and the solubility in water was above 250 mg/mL.
HTCC-Ag/PES membrane preparation

A novel positively charged composite NF membrane was prepared by coating HTCC onto a PES UF membrane. Briefly, a casting solution was prepared by dissolving 0.10 g of HTCC in 10 mL deionized water and then coated on a surface-dried PES UF membrane. The solvent was removed by keeping the composite membrane at 50 °C for 2 h. After being treated with 1.9 wt% ECH solution at 50 °C for 21 h, the HTCC/PES membrane was immersed in a 0.5 mmol/L AgNO₃ solution for 0.5 h and then dried under vacuum. The HTCC-Ag/PES composite membrane was finally obtained under 200 W UV lamp irradiation for 15 min. This synthesis route of HTCC-Ag/PES membrane can be represented as shown in a schematic form in Figure 1.

Membrane characterization

The surface morphologies of composite membranes were obtained by using SEM (JEOLJSM-6380LV; Japan). The existence of Ag nanoparticles was examined by X-ray diffraction (XRD, D8 Advance, Germany). AFM (Dimension310), operated in air atmosphere and in the tapping mode, was used to measure the surface images of the air-dried membranes. The root mean square roughness values in 4 μm × 4 μm area of the membrane surfaces were calculated at least three times for each independent membrane sample. The surface chemical compositions of the membranes were analyzed by XPS (PHI Quantera, Japan) using Mg Ka as radiation source.

Figure 1 | The synthesis route of HTCC-Ag/PES membrane.
Membrane performance evaluation

Pressurized filtration tests were performed employing a dead-end filtration setup to evaluate the permeation properties of the obtained HTCC-Ag/PES membranes in terms of pure water permeability (PWP), MWCO and rejections of different PPCPs. The NF membrane module used contains an O-shaped component with a diameter of 4 cm, resulting in an effective surface area of 12.56 cm². The pure water permeability was measured at temperature of 25 °C and feed pH of 7.0 ± 0.2, and all the samples were pressurized under 4.0 bar with deionized water for stable water flux before test. The PWP was calculated from the measured pure water permeation flux using deionized water. The permeate water flux (L/m²·h) was determined by direct measurement of the permeate volume collected over a certain period, using the following equation:

\[ J = \frac{V}{A \times \Delta t} \]

where \( J \) is the volumetric permeate water flux, \( A \) is the effective area of the membrane for permeation, and \( V \) is the volume of permeate over a time interval \( \Delta t \). The observed solute rejection, \( R_s \), was evaluated using the following equation:

\[ R_s = \left( 1 - \frac{C_p}{C_f} \right) \times 100\% \]

where \( C_p \) and \( C_f \) represent solute concentrations in permeate and feed, respectively.

The influences of ionic strength on rejection performance towards PPCPs was also investigated with a series of sodium chloride solutions mixed in 100 mL samples of pH 7.0 at 37 °C. All results presented are average data with standard deviation from at least three samples of each membrane type.

Antibacterial assay

We investigated the antibacterial property of HTCC-Ag/PES membrane using the Gram-negative Escherichia coli Rosetta and E. coli DH5α and Gram-positive Bacillus subtilis with the inhibition zone method. Nanofibrous mats were cut onto disks with a diameter of 1 cm and then sterilized with a UV lamp for 30 min. The diluted bacterial suspension (50 μl) with bacterial colonies of 5–10 × 10⁵ CFU/ml (CFU: colony forming units) was added into the agar culture medium uniformly (CLSI 2012a). The membranes mentioned above were sequentially placed on the plate. After the active layer was affixed to the surface of the medium, the plates were inverted and incubated at 37 °C for 24 h. CFU results were obtained from three measurements of three independent membranes based on triplicates (CLSI 2012b).

RESULTS AND DISCUSSION

SEM and AFM analysis

The surface morphologies of the original PES substrate and the modified composite membranes were measured by SEM, as shown in Figure 2(a)–(d). The pores of the HTCC/PES membrane nearly disappeared after HTCC was coated onto the PES UF membrane, indicating that the membrane was covered with a layer of relatively dense NF surface. As can be seen from Figure 2(c), on the surface layer was distributed a denser particulate matter compared to Figure 2(b), which proved the existence of silver nanoparticles on the membrane. Therefore, the above results illustrated that the morphology was greatly affected by the coating substance. AFM images, shown in Figure 2(e) and 2(f), illustrate that thickness of the coated HTCC/PES membrane was around 82 nm. After being coated with a layer of nano-Ag, the membrane has a relatively smooth surface with a roughness of 68 nm owing to the conjugation and accumulation of nanoparticle aggregates. The induced HTCC coating slightly increased the surface roughness, which might be caused by insoluble impurities in the HTCC solution. However, the resulting membrane still remained quite smooth and visibly shiny.

XPS and XRD analysis

XPS and XRD analyses with respect to the silver sub-peak pattern of HTCC-Ag/PES NF membranes are presented in Figure 3.

Figure 3(a) illustrates the XPS peak fitting curve of the samples. The two remarkable peaks around 372.5 eV and 366.7 eV correspond to Ag3d₃/₂ and Ag 3d₅/₂, respectively, according to Sawada et al. (2012), which confirmed that nano-Ag was indeed deposited on the surface of composite membranes. XRD analysis was employed to investigate the architecture of the
HTCC-Ag/PES membrane so as to illustrate the existence of the nano-Ag particles deposited on the film surface. As can be seen in Figure 3(b), in the XRD pattern of the HTCC-Ag/PES composites exists a peak with a maximum intensity at $2\theta = 38^\circ$, which corresponds to the zero valence silver (Nguyen et al. 2011). From the XRD and XPS analysis, it is clear that Ag nanoparticles exist in composite membranes.

**PWP and MWCO of the membranes**

The PWP tests were conducted by varying the pressure from 0.2 to 1.0 MPa. Figure 4(a) shows the changes of water flux with operating pressure for the composite membranes. Pure water flux of each membrane shot up linearly with operation pressure. The linear behavior is described by a slope close to PWP according to the Spiegler-Kedem
model (Huang et al. 2006):

\[ J_v = L_p(\Delta P - \sigma\Delta \pi) \]

where \( J_v \) is the water flux, \( L_p \) is the PWP, \( P \) is the operation pressure, \( \sigma \) is the reflection factor of the membrane, and \( \Delta \pi \) is osmosis pressure. Given that pure water is the permeating feed, \( \sigma\Delta \pi \) can be ignored. Herein, from the linear fit of experiment data, the PWP obtained was 20.00 L/m²·h·MPa for the HTCC/PES membrane and 16.27 L/m²·h·MPa for HTCC-Ag/PES. It obvious that the PWP does not change very much between the two membranes.

Figure 4(b) shows the MWCO of the composite membrane, which was determined through permeation tests using 1 g/L glucose (198 Da), sucrose (342 Da) and three kinds of standard PEG with molecular weights of 600, 800, and 1,000 Da, respectively. The MWCO was the molecular weight of organic substance with a retention of 90% (Afonso et al. 2001). Figure 4(b) illustrates the observed rejections of the studied membrane towards five different fractions at \( \Delta P = 4.0 \) bar. From the rejection behavior, the MWCO of the composite membrane was found to be around 941 Da.

Silver nitrate adsorption behavior on composite membrane surface

The influence of deposition time on the composite membrane was investigated by varying deposition time from 0 to 50 min with the temperature fixed at 25 °C and the silver content being 0.5 mmol/L. As shown in Figure 5, with the deposition time changing from 0 to 30 min, the absorbance of silver nitrate dramatically decreased from 2.02 to 1.76. The might be because, after soaking, silver ions would bind to the membrane layer via a complexation reaction (Thomas et al. 2009). In contrast, from 30 to 50 min, the absorbance of silver nitrate was not affected significantly. The reason was that with the prolonged reaction time, the loaded silver ions would reach saturation on the membrane surface. Thus, in our study, the deposition time was limited to within 30 min in order to ensure the amount of silver ions absorbed on the membrane surface was maximized.

![Figure 4](image_url) | Flux for pure water against operating pressure (a) and molecular weight cut-off curves for the composite membranes (b) operation: at 25 °C and cycling flow of 30 L/h.

![Figure 5](image_url) | Absorbance curve of silver nitrate against membrane soaking time.
Impact of the silver content towards NF separation performance

The membranes were prepared with different silver concentration (in the range of 0–0.7 mmol/L) for rejection of carbamazepine through a dead-end filtration setup. From the results shown in Figure 6, we can see that when the silver content changes from 0 to 0.7 mmol/L, the rejection increases by 5% and the flux decreases by a factor of 60% from 10 to 4 L/m²·h. It is known that a higher silver content leads to smaller membrane pores, thus resulting in higher rejection and lower flux. Our rejection and pure water flux results were in agreement with those previously reported (Deng et al. 2011; Zhong et al. 2012). However, the rejection increases and the flux changes a little when the silver content is 0.5 mmol/L. Considering both the flux and rejection integrally, we selected the concentration of AgNO₃ solution as 0.5 mmol/L in our study.

Separation performance of PPCPs

Figure 7 shows the rejections of atenolol, ibuprofen, and carbamazepine by HTCC/PES and HTCC-Ag/PES membranes, together with the physical–chemical properties of PPCPs. The rejection of carbamazepine by HTCC-Ag/PES membranes could reach 75.0%, whereas the rejection of ibuprofen was 71.2%. Moreover, the membranes exhibited a superior rejection of up to 83.6% for cationic atenolol. At pH 7.0, atenolol, ibuprofen, and carbamazepine were considered

<table>
<thead>
<tr>
<th>PPCPs</th>
<th>logK_{ow}</th>
<th>pK_{a}</th>
<th>Charge at pH 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atenolol</td>
<td>0.16</td>
<td>9.4</td>
<td>+</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>3.97</td>
<td>4.4</td>
<td>-</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>2.45</td>
<td>7.0</td>
<td>Neutral</td>
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to be positive, neutral, and negatively charged, respectively. Figure 7(a) shows that the rejection of PPCPs by membranes follows the decreasing order of atenolol (pKₐ = 9.4), carbamazepine (pKₐ = 7.0) and ibuprofen (pKₐ = 4.5) at pH 7.0, due to electrostatic repulsion, revealing the positive charge characteristics of the composite membranes. For neutral PPCPs (carbamazepine), the major separation mechanism by membranes is adsorption, which is closely related to hydrophobic interactions between the solute and the membranes. Normally, the hydrophobicity of the solute can be quantified by the octanol–water partition coefficient (logKₗₒ), since when logKₗₒ is above 2, the organic molecules are hydrophobic (Liu et al. 2012). However, studies have shown that adsorption only had a positive effect on the initial stages of membrane separation (Afonso et al. 2001). When the adsorption reached equilibrium, the adsorbed hydrophobic organic molecules would also penetrate the membrane, leading to a further decline on the rejections. Thus, it can be concluded that due to the inherent charged characteristic, the separation performance is mainly influenced by the electrostatic effect rather than adsorption of PPCPs.

As shown in Figure 7(b), the rejection of atenolol and carbamazepine decreases while the rejection of ibuprofen increases with an increasing concentration of Na⁺. The reason is that there exists an interaction between the dissolved salts such as sodium chloride and the membrane surfaces. Furthermore, the existence of Na⁺ would reduce the thickness of the electric double layer, thus weakening the electrostatic repulsion. When the ionic content increased, the rejection of the ibuprofen and atenolol by the composite membranes changed steeply while that of carbamazepine changed very little, which was not only characteristic of a charged NF membrane but also explained by electrostatic effect with the dissolved salts.

**Antimicrobial performance test**

Figure 8 shows that the HTCC-Ag/PES membranes had an enhanced antimicrobial activity, compared to HTCC/PES membrane. In another study, the mechanism of reduction of silver ions to silver nanoparticles by UV irradiation in solution of AgNO₃ and chitosan solution was also studied, and the resulting chitosan–nanosilver films exhibited...
excellent antimicrobial activity (Thomas et al. 2009). Silver nanoparticles are effective against various aquatic microorganisms including fungi, bacteria, and algae (Sondi & Salopek-Sondi 2004; Li et al. 2008; Choi et al. 2010), as they can destroy the cell wall of the bacteria easily. Furthermore, the cell wall of Gram-negative E. coli is made up of a thin membrane of peptidoglycan and an outer membrane consisting of lipopolysaccharide, lipoprotein and phospholipids (Liu et al. 2004). Hence, the NH$_3^-$ of HTCC and negative charged bilayer structure of the bacterial surface can attract each other. Another reason was that the lipopolysaccharide layer of Gram-positive Bacillus subtilis was thicker than that of Gram-negative E. coli so as to protect the cell wall from being destroyed. From Figure 8(b), the inhibition zone diameters of HTCC-Ag/PES membranes towards DH5α, Rosetta and Bacillus subtilis were 17.77, 16.18, and 15.44 mm, respectively. It is obvious that HTCC-Ag/PES membrane has a better antibacterial performance towards E. coli than Bacillus subtilis, especially for E. coli DH5α.

As mentioned above, the order in which the three strains were inhibited by the HTCC-Ag/PES membranes is as follows: E. coli DH5α > E. coli Rosetta > Bacillus subtilis. Overall, the inhibition effect of Gram-positive bacteria is weaker than that of Gram-negative bacteria.

CONCLUSION

In summary, the positively charged HTCC-Ag/PES membranes were successfully prepared via photoreduction technique. Our results showed that silver nanoparticles assembled on the surface of the membranes. The PPCP rejection by the membranes followed the sequence: atenolol > carbamazepine > ibuprofen, revealing the characteristic of positively charged NF membranes. We found that the PPCP rejection was mainly influenced by the electrostatic repulsion. It was also proved that the existence of nano-Ag particles helped to improve antibacterial activity against both Gram-negative and Gram-positive bacteria. Our method could provide new ideas and theoretical support for removal of emerging trace contaminants by CTS-based NF membranes.

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REFERENCES


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