Preparation of single-walled carbon nanotubes/polyvinylchloride membrane and its antibacterial property
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ABSTRACT
Polyvinylchloride (PVC) ultrafiltration membranes were modified by blending with single-walled carbon nanotubes (SWCNTs) to improve the membranes’ antibacterial property. Both modified and control samples were characterized for pore structure, roughness, hydrophilicity, permeability and mechanical properties. The membranes’ antibacterial property was accessed with Escherichia coli as the model microbes by several methods. It was found that, after being blended with SWCNTs, the surface roughness of the modified membrane increased. Also, the surface hydrophilicity was improved. The membrane flux increased accordingly. But the membrane elongation decreased obviously with the SWCNTs addition. The modified membranes did not show the antibacterial property as expected in this research. There was no bacterial inhibition circle around the SWCNTs/PVC membrane coupons in the culture plates. There were no morphological differences of the cells on the control and the modified membranes. Hoechst 33342/propidium iodide stain test showed that there were more than 90% living bacterial cells which could grow on the SWCNTs/PVC membranes. This study suggests that the polymer wrapping may reduce the SWCNTs’ antibacterial property greatly.

Key words | antibacterial property, membrane, polymer, polyvinylchloride (PVC), single-walled carbon nanotubes (SWCNTs)

INTRODUCTION
Membrane fouling is a major problem of membrane separation technology for water treatment, which results in the loss of filtration flux, increased energy cost, deterioration of effluent quality and frequent membrane cleaning. It has greatly restrained the membrane engineering applications. Membrane fouling consists of inorganic fouling, organic fouling and bio-fouling in terms of the foulant property. Among the three kinds of membrane fouling, bio-fouling is the hardest to control because it is caused by the microorganisms accumulating and attaching on the membrane surface or pores (Eshed et al. 2008; Malaisamy et al. 2010; Mauter et al. 2011). Microorganisms can produce metabolic products such as proteins and polysaccharides, which tend to accumulate on membrane surface and are apt to form a sticky film. They are proved as key membrane foulants by many researches (Kim et al. 2006; Ivnitsky et al. 2007). In addition, bacterial attachment on membrane surface, especially in membrane pores, may cause the possibility of bacterial penetration, i.e. bacterial leakage, which is also a serious problem for water purification.

Many countermeasures such as back washing and fluid disturbing have little effect on release of the membrane bio-fouling (Liu & Fang 2005; Kujundzic et al. 2007). Chemical cleaning with disinfection reagents such as hypochlorite may release the bio-fouling to some extent. But chemical cleaners may also damage membrane materials or lead to formation of disinfection by-products and secondary pollution of treated water. If the membrane materials can have the antibacterial property, hopefully, the attachment of microorganisms on it will be inhibited or restrained. Then the
anti-fouling performance of membranes will be developed. But few researches about this idea have been reported.

At present, some plastic products and textiles with proper modification methods have shown their special antibacterial functions to restrain microorganisms growing on them (Dastjerdi & Montazer 2010). But for membrane materials which are used for water purification, the additive for modification should be carefully selected. It is demonstrated that some nanomaterials have similar or superior antimicrobial activities compared with conventional chemical disinfectants (Li et al. 2008; Kunzmann et al. 2011). They can potentially be used as alternative disinfectants. The antibacterial properties of silver have been well-known for a long time, and silver nanoparticles (nAg) are now incorporated into a wide variety of consumer products for microbial control; nano-TiO2 and nano-ZnO are also commonly available antibacterial agents. Accordingly, there have been some Ag, TiO2 and ZnO-included membranes studied and applied (Kunzmann et al. 2011). But these nanoparticles are all inorganic particles. When they are blended with organic polymers, the nanoparticles will aggregate readily. So it is hard to make them highly dispersed in membrane casting solution. In addition, these nanoparticles are apt to come off from the membranes during application, which reduces the membranes’ antibacterial property. Also the loss of nano particles may cause toxicity concerns to the environment (Handy et al. 2008). Thus, we need to seek some alternative antibacterial materials which can be well-knitted with the polymers and are not lost easily from the membranes. In the present study we selected single-wall carbon nanotubes (SWCNTs) as the candidates to improve membrane antibacterial performance.

Numerous applications of CNTs have been proposed and reported (Mauter & Elimelech 2008). Also their potential biological applications create great attention in the field. Recently, some studies have reported that CNTs show antimicrobial property (Jia et al. 2005). Kang et al. (2007, 2008) studied the death of Escherichia coli on filtration membrane covered with CNT layer, which showed the bodies of E. coli were seriously damaged and the SWCNTs presented stronger toxicity than multi-walled carbon nanotubes (MWCNTs). Akasaka & Watari (2004) reported the bacteria could be captured by addition of the CNTs. Srivastava et al. (2004) showed that hollow fibers impregnated with CNTs could present effective disinfection of E. coli and poliovirus. At present, it’s still hard to predict whether it’s a promising method to couple CNTs’ reactivity with membrane filtration for water treatment. The ability of bacterial disinfection by CNT is relatively lower than conventional disinfectants. But it may suggest that CNTs can be blended in organic materials and can be used to restrain microbial attachment and the subsequent biofilm formation on membrane surfaces.

In this research, SWCNTs were selected as the antibacterial additives to modify the membrane material, because of their relatively stronger toxicity than MWCNTs (Kang et al. 2007, 2008). PVC was chosen as the main material to synthesize ultrafiltration membranes because, in China, PVC resin is a kind of material which is well studied for decades and is much cheaper than other membrane materials e.g. PVDF. However, until now, there has been little research reported about the properties of PVC membrane or PVC composite membrane. Our aim is to study whether the SWCNTs/PVC ultrafiltration membrane has obvious antibacterial property. Also, the structure, surface, and filtration properties of the membrane will be analyzed to evaluate the performance of this kind of membrane.

MATERIALS AND METHODS

Materials and reagents

The powder polyvinylchloride (PVC, Sanaifu Co. Ltd, China) resin was used as the membrane fabrication material. N,N-Dimethylacetamide (DMAC, Bodi Co. Ltd, China) was employed as the PVC solvent. Sodium hexametaphosphate (SHMP, Fuchen Co. Ltd, China) was used as the surfactant. And polyvinylpyrrolidone (PVP, Lanji Co. Ltd) was used as the porogen. The SWCNTs were purchased from Shenzhen Nanotech Port Co. Ltd, China. As claimed by the manufacturer, they are synthesized with a chemical vapor deposition method using iron, cobalt and nickel as the catalysts. The purity of the SWCNTs is more than 95%. The diameters of the SWCNTs are mostly less than 2 nm. The length of the SWCNTs is in the range of 5–15 μm. They are semi-conductivity materials. The amorphous carbon content is no more than 3%. Hoechst 33342 / propidium iodide (PI) double staining kit was bought from Biouniquer Technology Co., Ltd, China. All the other reagents used for the experiments were of analytical grade. Ultrapure water (Millipore Q Biocel system) or de-ionized water was used for all the experiments.

Purification and characterization of SWCNTs

SWCNTs were refluxed in 37% HCl for 8 h to remove the residual catalysts. After refluxing, the mixture was cooled...
to room temperature, and was washed with ultrapure water through a membrane with 0.2 μm pore size. The washing was repeated until the amounts of all the metal residuals could not be detected by inductively coupled plasma mass spectrometry (ICP-MS). The morphology of SWCNTs before and after purification was characterized by transmission electron microscope (TEM, FEI Tecnai G², The Netherlands).

**SWCNTs/PVC membrane preparation**

Membranes were made using the wet phase inversion process. Firstly, the SWCNTs were mixed in DMAC with sonication mixer at 40 kHz for 15 min at 22 °C, in order to make SWCNTs well dispersed in the organic solvent. Next, PVC was added to the polymer solution and mixed with thermostatic magnetic homogenizer at 800 rpm at 22 °C over 20 h as well as polyvinylpyrrolidone (PVP, Lanji Co. Ltd) to obtain a homogeneous solution. According to different membrane casting solutions, 9% w/w of PVC, 1.5% w/w sodium hexametaphosphate (SHMP, Fuchen Co. Ltd) and 1.5% w/w of PVP were added. The total amount of DMAC and SWCNTs was 88% w/w for all the SWCNTs/PVC membrane. The amount of SWCNTs and DMAC could be adjusted for the desired composite membrane.

**Membrane characterization**

Membrane surface and cross-section morphology were analyzed by a scanning electron microscope (SEM, S-4700, Hitachi, Japan) at 10 KV in a high vacuum mode after coating with approximately 10 nm of gold to observe membrane asymmetry and pore structure. The cross-section of the membrane was obtained by breaking the membrane in the liquid nitrogen. Membrane surface roughness was analyzed with an atomic force microscope (Dimension Vx 210/310, Veeco Metrology Group, Japan) in the tapping mode. The dried membranes were scanned at three random positions. The membrane roughness was quantified as the root mean-square Rms.

The contact angle between water and membrane was directly measured with a JYSP-360 contact angle goniometer (Jinshengxin Co. Ltd, China) to evaluate the membrane surface hydrophilicity. Ultrapure water was used as the probe liquid in all measurements. A 5 μm drop of deionized water was placed onto a dried membrane in air, and the contact angle was calculated with the instrument's software. Membrane permeability was analyzed by dead-end filtration of ultrapure water with a stirred cell unit (Model8200, Millipore, USA) at a pressure of 0.2 MPa. The filtration test was repeated three times. Membrane thickness was tested by film thickness analyzer (CH-1-S, Shanghai Liuling Co. Ltd, China) at 22 °C after the samples were dried at room temperature. Membrane tensile properties were measured with an electric elastic yarn strength analyzer (YG020B, Nantong Sansi Co. Ltd, China) at 22 °C with an extension rate of 2 mm/min. All of these characterization tests were carried out more than three times and the averages and data errors were calculated.

**Antibacterial property characterization of the modified membranes**

Gram-negative *E. coli* K12 were selected as model bacteria, which were obtained from Harbin Medical University. In this research, the *E. coli* cells were incubated in Luria-Bertani (LB) broth, LB agar broth and isotonic saline solution (0.9% NaCl) when necessary at 37 °C.

Conventional inhibition zone test was carried out for membranes and SWCNT clusters to access their antibacterial properties. The membrane samples were cut into round coupons with 10 mm diameter. Both SWCNTs/PVC membrane and pure PVC membrane coupons were put into the same LB agar plates deposited with *E. coli* cells. Mostly, if the coupons have bactericidal property, there should be a growth inhibition zone around the coupons. To analyze the antibacterial property of SWCNTs, the powder SWCNTs were directly dispersed on the LB agar plates deposited with *E. coli* cells to observe whether there were growth inhibition zones around the SWCNTs.

Also, a plate counting method was adopted to access the living bacteria cells attached on the SWCNT-modified membranes. Firstly, 50 μL *E. coli* stock solution was seeded in 5 mL LB broth, and was cultured in shaking incubator at 37 °C for 12 h. Then, 1 mL *E. coli* cultured solution was transferred to one conical flask containing 500 mL 0.9% NaCl solution (isotonic saline solution). Next, 100 mL *E. coli* solution was forced to pass through the membrane coupons. There was no doubt that there would be some living *E. coli* cells attaching to the coupons if the coupons didn’t present strong antibacterial property. Next, the coupons were washed twice to remove the attached isotonic saline solution with *E. coli* cells. Then the coupons with attached cells were cultured in the isotonic saline solution for 50 min. Further, the attached cells were rinsed to a chamber for colony-forming unit (CFU) counts as well as the following dyeing tests with Hoechst 33542 / PI kit.


Hoechst 33342 / PI dyeing tests were applied to characterize the proportion of the living and dead cells attached on the coupons. Hoechst 33342 (2'-[4-ethoxyphenyl]-5-[4-methyl-1-piperazinyl]-2,5'-bi-1H-benzimidazole trihydrochloride trihydrate) is a cell-permeable DNA stain that is excited by ultraviolet light and emits blue fluorescence at 460 to 490 nm. PI is another kind of fluorescent DNA stain. But it is cell membrane impermeable and generally excluded from viable cells. PI is commonly used for identifying dead cells in a population and as a fluorescence counterstain (emission wavelength of 630 nm and excitation wavelength of 488 nm). Of the above rinsed solution, 1 mL was transferred from the chamber into a centrifugal tube. Then 10 μL Hoechst 33342 was added into this solution. This mixture was cultured for 10 min at 37°C. Next, 5 μL PI stain solution was added to this solution after 15 min, then the cell suspension was dropped onto one glass slide and put on a fluorescence microscope (BX51TF, OLYMPUS, Japan).

RESULTS AND DISCUSSION

Preparation of SWCNTs/PVC membranes

Before the SWCNTs were blended in the PVC membrane casting solution, they were purified with a strong acid (37% HCl) to remove the residual catalyst metals such as Fe, Co and Ni, which are proved as matters that can influence the toxicity of SWCNTs. After the purification, all of these metals were under detection limit of ICP-MS. So, in this experiment, the influences from the catalyst metals were not considered. Both dispersed and aggregated SWCNTs (Figure 1) could be seen with SEM. The diameters of most SWCNTs were about 2 nm. The length should be several microns.

SWCNTs are hydrophobic materials. They were apt to aggregate in deionization water (Figure 2(a)). However, when SWCNTs were added into the PVC membrane casting solution, which is half-transparent (Figure 2(b)), with sonication, the solution turned into homogeneous black (Figure 2(c)) instantly. It is proved that SWCNTs can be more easily dispersed in PVC membrane casting solution. A series of SWCNTs/PVC membranes could be prepared with different amounts of SWCNTs addition. The prepared PVC membrane without SWCNTs was of white color (Figure 1(d)), while SWCNTs/PVC membrane was from light gray to thick gray (Figures 1(e)–1(g)) with SWCNTs addition. The above phenomena proved that the SWCNTs had been successfully blended into the membrane prepared. But, when the concentration of SWCNTs in SWCNTs/PVC reached 1.5%, it was observed that some SWCNTs began to settle down from the polymer casting solution due to the gravity if the sonication was stopped. So, in this research, no more than 1% SWCNTs was added in the modified membranes to get experimental samples with steady quality.

Membrane basic properties

The contact angles of SWCNTs/PVC membranes presented an obvious decreasing tendency with SWCNTs addition (Table 1), which suggested that the addition of SWCNTs to the membrane could improve the PVC membranes' hydrophobicity, while it was observed that, with the SWCNTs addition, the SWCNTs/PVC membrane's surface roughness was increased due to the increasing content of SWCNTs fabricated in the surface. Also, the increased membrane roughness might lead to the decreased contact angle of the membrane. In the membrane flux test, it was found that the SWCNTs/PVC membrane's flux slightly increased with SWCNTs addition. The flux of PVC membranes blended with 1% SWCNTs was about 5% higher than that.
of the pure PVC membrane. This phenomenon was also reported by Celik et al. (2011) when they studied the performance of MWCNTs/polysulfone blended membranes in their recent research. But we also noticed that Brunet et al. (2008) reported that there were no obvious changes in permeability and hydrophilicity of the blended membrane after MWCNTs addition. It should be noted that the membrane flux is influenced by many factors of membrane preparation and filtration. For our modified membranes, the increased flux might be due to the improvement of the membrane’s porosity and hydrophilicity with SWCNTs addition.

It was found that the prepared membrane’s thickness was not affected by SWCNTs addition (Table 1). But the greater data errors might be due to the fabrication condition which was not well controlled. From mechanical analysis, the tensile strength of SWCNTs/PVC membranes was slightly enhanced with SWCNTs addition, while the elongation for breaking of the modified membrane decreased obviously. The elongation ability of PVC membrane with 1% SWCNTs was just half that of the pure PVC membrane. This phenomenon revealed that the modified membrane lost part of its extension property when SWCNTs were added, which also suggested that the concentration of the SWCNTs should be controlled to an extent in consideration of the elongation property.

Membranes morphology

Bare PVC membrane and the PVC membrane with 1% SWCNTs were selected as representative samples for membrane morphology analysis. SEM images (Figures 3(a) and 3(b)) of the two membranes revealed that the membranes prepared with and without SWCNTs showed similar pore structure. The pores were of asymmetric shapes (Figures 3(c) and 3(d)), similar to the present research, where there was a very thin skin layer supported by a thick porous layer composed of pores and macrovoids. Also, from the atomic force microscopy analysis (Figure 4), it could be seen that the surface of modified membrane with SWCNTs was rougher than

Table 1 | Properties of SWCNTs/PVC with different concentration of SWCNTs

<table>
<thead>
<tr>
<th>Properties</th>
<th>No SWCNTs</th>
<th>0.1% SWCNTs</th>
<th>0.5% SWCNTs</th>
<th>1% SWCNTs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact angle (°)</td>
<td>74.6 ± 3.8</td>
<td>67.6 ± 3.2</td>
<td>65.2 ± 2.2</td>
<td>65.2 ± 2.2</td>
</tr>
<tr>
<td>Roughness (nm)</td>
<td>94 ± 24</td>
<td>112 ± 28</td>
<td>135 ± 32</td>
<td>152 ± 26</td>
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<tr>
<td>Membrane flux (L/(m² h))</td>
<td>347 ± 12</td>
<td>355 ± 16</td>
<td>362 ± 12</td>
<td>364 ± 14</td>
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<tr>
<td>Thickness (μm)</td>
<td>349 ± 25</td>
<td>352 ± 32</td>
<td>348 ± 26</td>
<td>356 ± 28</td>
</tr>
<tr>
<td>Tensile strength (cN)</td>
<td>246 ± 14</td>
<td>252 ± 16</td>
<td>258 ± 18</td>
<td>264 ± 16</td>
</tr>
<tr>
<td>Elongation at break (%)</td>
<td>12.8 ± 3.2</td>
<td>10.2 ± 1.2</td>
<td>8.6 ± 2.4</td>
<td>6.6 ± 1.4</td>
</tr>
</tbody>
</table>

Figure 2 | SWCNTs dispersion in PVC casting matrix: (a) SWCNTs in DI water; (b) PVC casting solution without SWCNTs; (c) 1% SWCNTs/PVC casting solution; (d) PVC membrane prepared; (e) PVC membrane with 0.1% SWCNTs; (f) PVC membrane with 0.5% SWCNTs; (g) PVC membrane with 1% SWCNTs.
PVC membrane because the SWCNTs were fabricated in the membrane surface.

**Growth inhibition for bacteria near SWCNTs/PVC membrane**

There will be a circular inhibition zone around the disc containing antibiotic, where the growth of bacteria is restricted (Zodrow et al. 2009). In this research, the inhibition zone test was also applied to analyze whether the SWCNTs/PVC membrane could restrain the *E. coli* growth. It was found that the bacterial colonies could grow well at the edge of both the PVC membranes and the SWCNTs/PVC membranes (Figure 4). The obvious inhibition zone near the SWCNTs/PVC membrane was not observed. Also, in the SWCNTs zone (Figure 4), tiny bacterial colonies could grow among the SWCNT clusters. Some previous researches reported that several kinds of carbon nano materials, such as fullerene and fullerenol, revealed bio-toxicity due to their chemical-electrical performance under light (Lee et al. 2010). So the inhibition zone tests were also carried out under both dark and bright conditions (with sunlight). It is hard to tell any evidence of inhibition of *E. coli* growth near the SWCNTs/PVC membrane.

**Colony-forming unit counting for living *E. coli* growth on the SWCNTs/PVC membrane**

The suspension with *E. coli* cells was forced to pass through both the PVC and PVC membranes with 1% SWCNTs. There is no doubt that part of *E. coli* will attach to the membranes. With SEM scanning (Figure 5), it could be seen that many *E. coli* did grow on the membrane with 1% SWCNTs, which revealed that their growth had not been inhibited effectively. In addition, the shapes of *E. coli* on both PVC membrane and SWCNTs/PVC membrane didn’t show obvious differences as mentioned in the articles of Kang et al. (2007). Also, some researches were carried out to restrain bacteria with nano particles. But the biotoxicity of SWCNTs/PVC membrane to attached bacteria was questioned by this research. With CFU counts, there were...
93.5 ± 7.7 × 10^4 CFU on the control sample and 125 ± 25.5 × 10^4 CFU on the membrane with 1% SWCNTs. These results further revealed that there were plenty of living cells growing on the SWCNTs/PVC membrane surface (Figure 6).

**Fluorescence stain for living and dead cells on SWCNTs/PVC membrane**

A series of fluorescence stain experiments with Hoechst 33342/PI iodide kits for characterization of the living and dead cells attached to the membranes were carried out to further access the antibacterial properties of SWCNTs/PVC membranes. Similar methods had been applied to directly present the ratio of the dead and living bacteria cells on modified membrane or materials in some previous studies (Kang et al. 2007, 2008). The representative images for control membrane and PVC membrane with 1% SWCNTs are shown in Figure 7. The numbers of both dead cells (red) and living cells (blue) can be compared in one image. It is found that most cells are living ones (Hoechst 33342 stained) in each sample. The percentages of living cells are up to 90% of the total on average (shown in Figure 8) for different membranes. It's shown that, even though some dead cells can be found on the membrane, the living ones are predominant. From these results, it's hard to predict that SWCNTs/PVC membranes can kill living cells.

**Mechanism of the loss of antibacterial ability of SWCNTs/PVC membranes**

The results of this research may help to further understand the antibacterial ability and mechanism of SWCNTs and their composites.

There are two hypotheses for the mechanism of CNTs exerting toxicity to bacteria. One is the mechanical disruption, that the nanotubes can physically pierce or otherwise perturb the bacterial membrane. The other is oxidative stress, that the nano-carbon materials can act to directly or indirectly (e.g. through formation of radical species) damage the cell membrane (Aslan et al. 2010). Based on both hypotheses, we propose that the contact between CNTs and bacteria plays an important role in CNTs' antibacterial properties. This idea can be supported by this research as well as some previous studies. In the earlier research of Kang et al. (2007, 2008) the E. coli were forced to pass through the layer of SWCNTs or MWCNTs, that is, the E. coli contacted the CNTs directly. Then the bacterial cells were damaged seriously on the CNT layers. Schiffman & Elimelech (2011) incorporated the SWCNTs into the polysulfone fibers; polysulfone the small SWCNTs could be found at the tiny fiber surface with SEM analysis. When the fibers contacted bacteria, the chances of SWCNTs contacting bacteria could be increased. Aslan et al. (2010) further proved the shorter SWCNTs exert stronger toxicity to bacteria, which offered more chances for open ends of SWCNTs contacting microbes.
In this research, the loss of antibacterial ability of SWCNTs/PVC membranes may be due to the polymer burying or wrapping the SWCNTs in the modified membranes, which reduced the contact chances between SWCNTs and bacteria or changed the bio-effect of the SWCNTs. Also, the SWCNTs’ concentration in the surface layer may not be high enough to present antibacterial property with the membrane preparation method mentioned in this research. Tiraferri et al. (2011) enhanced the membrane’s antibacterial property by coating functional SWCNTs on the membrane surface with a covalent binding method. A similar method should be considered in further research.

CONCLUSIONS

It was shown that SWCNTs could be well dispersed in organic membrane casting solution. After being blended with SWCNTs, the modified membranes’ surface roughness increased. Moreover, the surface hydrophilicity and DI water flux of the modified membrane were slightly improved by SWCNTs addition. But the elongation of the modified membrane decreased obviously. The SWCNTs/PVC blended membranes did not exhibit obvious antibacterial properties in this research. There was no bacterial inhibition zone near the membrane samples on culture plate. With SEM scanning, it was found that there were no morphological differences between E. coli attached to both pure PVC membrane and PVC membrane with 1 wt% SWCNTs. Hoechst 33342/PI stain test showed that there were more than 90% living bacterial cells growing on the SWCNTs/PVC membranes. This study suggests that the polymer wrapping may have reduced the SWCNTs’ antibacterial property by reducing the contact chances with microbes. Further study should focus on how to incorporate SWCNTs on the
polymer membrane surface to enhance the membrane’s antibacterial property.

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